CONTENTS

Customized Rigid Gas Permeable lens fitting for keratoconus

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Abstract: Keratoconus, a non-inflammatoryectasic disorder of the cornea, with corneal thinning and distortion resulting high degree of irregular myopic astigmatism. To best correct patients' vision with keartoconus, one would require Rigid Gas Permeable contact lens. Due to the distorted shape of cornea, the correction of keratoconus remains one of the most difficult challenges in the art of contact lens fitting. In this paper, we trialed and fitted a newly diagnosed bilateral keatoconus patient with many different parameters in order to reach a final appropriate lens design for maintaining the patient's minimum binocularity and improving his visual acuity as much as possible. The patient was in his early 30's with no previous knowledge of kearotoconus and came in to our center for a pre-Lasik evaluation. Diagnosis of the disorder was confirmed by axial map and ring verification of corneal topography. The final treatment of course included special designs of rigid gas permeable lenses for both eyes. Patient was very impressed with result which allows his vision to improve 4 lines in both OD and OS on the vision test chart.
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Keywords: Keratoconusmulticurvedesign,Rigid gas-permeablelens, Rigid lens edge lift, Customized contact lenses

1. Introduction

Keratoconus is a slowly progressive non-inflammatory disease of the central cornea which causes central stromal thinning, apical protrusion, and irregular astigmatism.[1] The loss in vision can range from mild to severe. The management for keratoconus can be spectacle correction, rigid gas permeable contact lens fitting or penetrating keratoplasty. The mode of management depends on the severity of the condition. The spectacle correction is for early stage of the condition when the protrusion of the cornea is still insignificant, whereas penetrating keratoplasty is the final option when rigid gas permeable contact lens can no longer provide adequate vision or tolerated by the patient. With the advanced technology in contact lens design today, we can now fit successfully for most of keratoconic eyes.

This report discusses the management of rigid gas permeable lens fitting for a newly diagnosed bilateral keratoconus patient.

2. Material and Methods

Best corrected visual acuity was done by using Topcon phoropter. Documenting ectatic condition was done by Dicon topographer (Paradigm Medical, USA) and keratometry reading via Topcon keratometer. Contact lenses used are keratoconus lens and traditional 4-curve spherical rigid gas-permeable lens, both from Hiline Optical Company in Taiwan.

3. Results

A 30 year old Asian male with unremarkable health condition presented for a pre-Lasik evaluation. The patient never wore any spectacle or contact lens. There was no chief complain except that he noticed his vision is not as good as it used to be. The test result was as follows:

Manifest refraction:
OD: -1.25-3.00X090 20/400 Pinhole: 20/60-
OS: -3.25-3.50 X100 20/40- Pinhole: 20/30+
HVID 12.2, OU, Palpebral Aperture 8.5 mm, OU
Keratometry:
OD: 56.75 @039/62.75@129 corneal astigmatism: 6.00 D.
OS: 38.50@104/41.87@014 corneal astigmatism: 3.37 D.

Corneal topography were done to both eyes and the maps revealed inferior protrusion in the lower 1/3 of both corneas, with OD being much worse than OS (Fig. 1).

Keratoconus were diagnosed for both eyes. After several trail lens fittings, the following lens specifications were determined.

• OD:
6.15/-13.75/8.7 20/50- Hine Keratoconus lens, with peripheral curve loosen by 0.1mm in radius (Fig. 2).
• OS:
7.55/-7.00/9.0 20/30 Hine 4-curve spherical lens, with peripheral curve loosen by 0.4 mm in radius (Fig. 3).

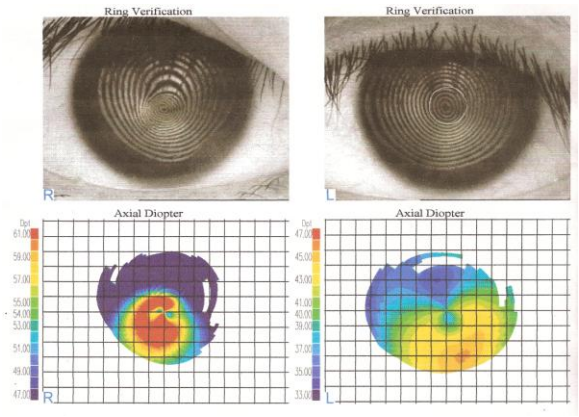

Fig. 1 keratoconus OU, OD worse than OS.

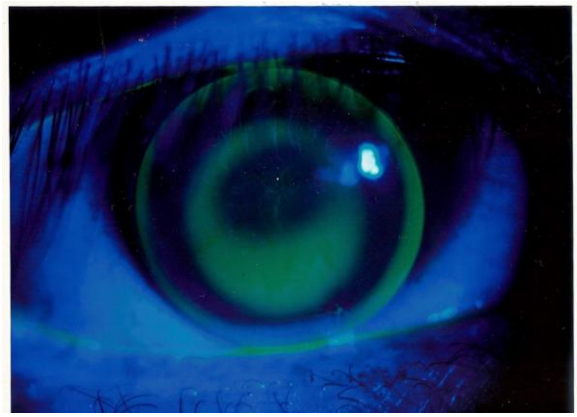

Fig. 2 OD with lens parameter of 6.15/-13.75/8.7 PC -0.1mm

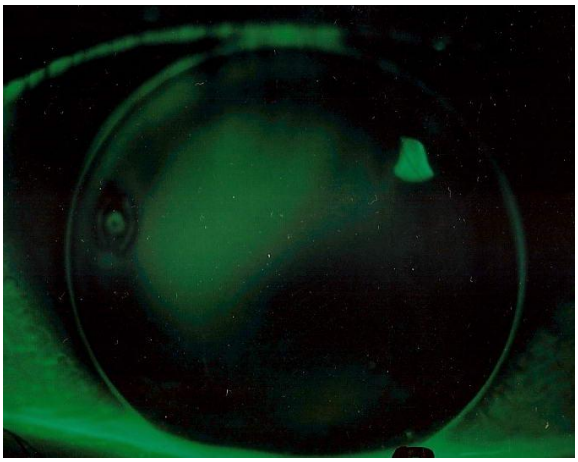

Fig. 3 OS with lens parameter 7.55/-7.00/9.0, PC -0.4mm

First follow up visit:

3 days after the initial lens fitting, the patient returned for the 1st follow up. The fluorescein pattern of OD showed too much apical compression, whereas OS had very narrow edge lift. Both the fit were unacceptable to the examiner, although the patient was satisfied with the corrected visual acuities, 20/50 for OD and 20/30+ for OS. The designs of the lenses were changed to

• OD:

6.05/-14.00/8.5 20/50+ Hine Keratoconus lens, with peripheral curve loosen by 0.1mm in radius (Fig. 4).

• OS:

7.55/-7.00/9.0 20/30 Hine 4-curve spherical lens, with peripheral curve loosen by 0.7 mm in radius (Fig. 5).

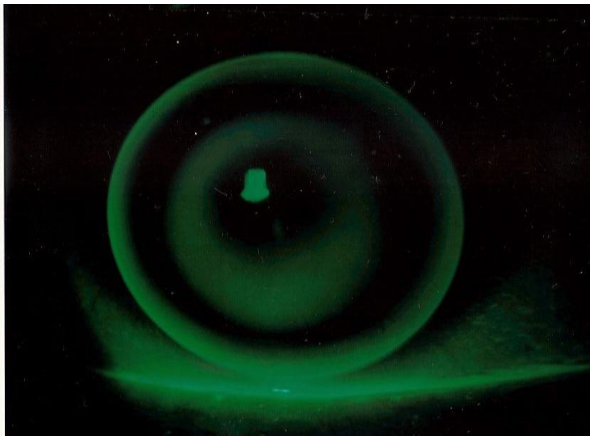

Fig. 4 OD with lens parameter 6.05/-14.00/8.5. PC -0.1mm

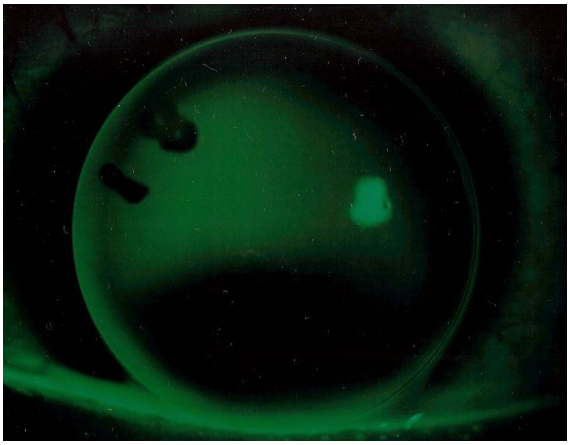

Fig. 5 OS with lens parameter 7.55/-7.00/9.0, PC -0.7mm.

Upon the second follow up visit, both the fit and visual acuity of OD lens was acceptable. The OS fit, however, continued to show an insufficient edge lift at the peripheral curves. A bigger diameter, slightly flatter base curve and much flatter peripheral curves of rigid lens was redesigned to increase the edge lift as much as possible. The new OS lens parameter was 7.65/-7.00/9.2 Hine 4-curve spherical lens, with peripheral curve loosen by 0.9 mm in radius (Fig. 6).

At the 3rd follow up visit, both the OD and OS revealed acceptable fit, with adequate movement, minordecentration, and sufficient edge lift. The rule of three-point-touch was used to finalize the best fit for both eyes. The visual acuities were further improved to 20/40- for OD and 20/25 for the OS. The patient was instructed to continue the lens wear.

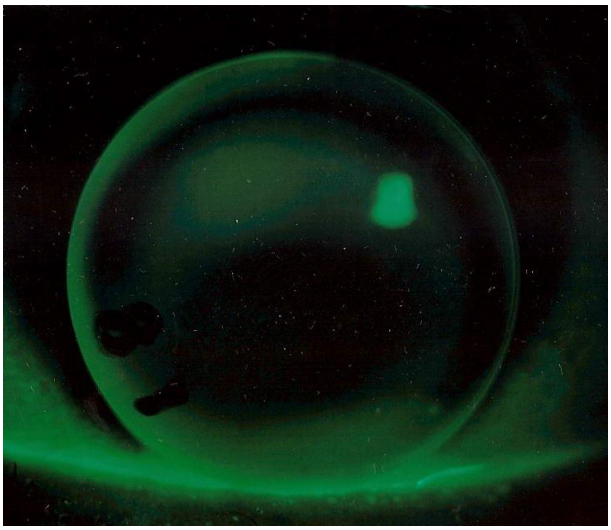

Fig. 6 OS with lens parameter 7.65/-7.00/9.2, PC -0.9mm.

4. Discussion

Keratoconus is a progressive non-inflammatory disease of cornea characterized by thinning, ectasia, distortion and increased curvature of the cornea.[2.3] The abnormal curvature affects the refractive power of the cornea, resulting in myopia or irregular astigmatism.[4] Individuals with keratoconus in the advanced stage require a rigid gas-permeable (RGP) contact lens to reduce distortion and provide better vision.[5.6]

Correction of Keratoconus by rigid lens remains one of the most difficult challenges in the art of contact lens fitting. Given the ectatic condition, to fit the lens on a keratoconic eye, one must overcome the challenges of lens decentration, insufficient or too much edge lift, vaulting of the lens and over-compression at the corneal apex. Any of the area mentioned above is crucial to the success of the lens fitting. For example, decentration of the lens would induce glare and poor visual acuity, insufficient or too much edge lift would cause corneal epithelial desiccation, inadequate tear exchange and air bubble trapped under the lens, over–compression at the corneal apex would no doubt lead to corneal scarring and loss of best corrected visual acuity. In this paper, we described the details the lens fitting parameters at different stages of time in order to show the reader how important one must try to achieve the best fit possible when it comes to keratoconus fitting.

5. Conclusion

Keratoconus, with incidence of approximately 50 to 230 per 100,000, is seen more often than most eye care practitioners can expect. Due to poor vision of the disorder by spectacle, rigid contact lens fitting remains one the most common mode of treatment for keratoconus. Although the fitting of such disease can be quite difficult, one should not be afraid to try and modify the lens wherever possible until the best fit can be determined.

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10/2/2012

Appraisal of Reader's Role in Revolutionary Potential of Hypertext Fiction

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Abstract: One of the outstanding effects of development in technology is on literature; times when novels were read just through printed books have passed. By introducing electronic literature readers can experience a virtual reality and additional aesthetic pleasure in compare to printed texts. This paper puts forward that electronic literature and specifically hypertext fiction encourages readers to see writing in a radical sense as connecting one text to another and form a new composite. The cybernetic environment endows readers different roles to engage in a story and incorporate their identities toward fictional characters. Literary creativity needs to converge with computer and through by this way readers encounter various layers of meaning and can interpret a story without the author's interference. However, the problem is that with this structure, it is impossible for the readers to respond in a realistic normal way as they did in print technology because, the electronic authors create several paths to be followed. Therefore, this study aims to analyze how hypertext novel has empowered readers to take some of the roles historically played by authors. Recognition that modern ways of reading caused by introduction of the Internet and also connection between literature and science illuminate literary texts, make this paper an inevitable area of research.

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Keywords: hypertext fiction, digital reader, interactivity, cyber genre.

1. Introduction

Readers are undergoing subtle, but important changes during postmodern digital literary era. These changes are directly related to the nature of hypertext and other electronic texts. Readers seem initially confused by the choices they are asked to make when they read hypertext literature, but their confusion quickly gives way to more animated mode of reading, where they read chunks of text for pleasure and seek links that would take them to explore much information. Thereby, this study has confronted the distinguishing position of the traditional and the contemporary reader in novels, which is a quite fresh argument. What is significant in this study is to provide the answer that the digital reader concretizes and reifies the story in a different manner from a traditional reader with the opportunity to enjoy multimedia elements in a hypertext fiction.

2. Hypertext fiction and readers' interactivity

Hypertext, especially hypertext fictions place readers in an environment where they have to read differently and perhaps more actively, than many of them have read before. It also permits levels of authorship without suggesting that one level is more important or worthy than others. Apparently, there seems to be a kind of inevitable change in the author's and reader's function and as Janet Murray points out that with electronic text, the "author" is procedural, like a choreographer, "who supplies the rhythms, the context, and the set of steps that will be performed" (Murray, 1997: 153). Therefore, it is considered that the reader alters the environment of the text through his or her participation by entering a digital environment and this kind of intervention in the reading of hypertext fiction is rather rare in more traditional narrative forms or printed novels. However, it is noteworthy that this study agrees with Murray's view that the reader of electronic text, and especially hypertext, is not experiencing authorship but the reader is experiencing agency (Murray, 1997: 126), and this is an intriguing point which shouldn't be ignored.

In fact, the tradition of printed novel privileges the author but the role of the reader is stronger in the majority of digital novels than it is in conventional fiction. The actualization of the work and utilizing the multimedia elements change the position of a reader to a role of a participant.

George Landow claims that in hypertext fiction, the functions of the reader and the writer become more deeply entwined with each other than ever before (Landow, 2006: 125). This is because this cyber genre promises a greater sense of "agency" and it allows the reader to challenge or contest the authorial role in a more immediate sense (Patterson, 2000: 76). Davis Bolter also asserts that hypertext fiction offers "a new literary experience in which the reader can share control of the text with the author" (Bolter, 2001: 122). This merging of the reader and the author effect on the

reader's response, and consequently, on a text. Furthermore, Stuart Moulthrop postulates that such texts create or at least aim for discursive intimacy and engagement, rather than the more traditional, modernist, mode of textual impersonality and entrancement (Moulthrop, 1997: 661).

Therefore, hypertext readers' experience a story of a novel differently as their fluidity in navigating events changes from reading to reading, from reader to reader. The hypertext novelists impulse readers to be independent users. Richard Lanham also has observed that digital media such as digitized novels and interactive narratives have no "final cut" (Lanham, 1989: 269). This indicates they have no singular, definitive beginnings, middles or endings, and no single, definite reading order is given priority over the others which exist alongside it. Contrary to our expectations based on reading print narratives, there is no single story and readings do not provide varying versions of this story or collection of stories. Similarly, Bolter has argued each reading generates or determines the story as it proceeds and there is no story at all but there are only readings, and the story is the sum of all its readings. Further, he states that each reading is a different turning within a universe of paths set up by the author (Bolter, 1991: 124-5).

2.1 Reader's response

In fact, what it means by analyzing the reader's role is to study how open to negotiation is reader's responses because hypertext fiction allows its readers moral and emotional judgments on the action of characters through their collaboration and thus offers a reader a particular pleasure. In addition, different genres result in different modes of text-reader interaction, and may result in different types of reader's involvement. In this regard, Louise Rosenblatt proposes, "Literature equals book plus reader" (Rosenblatt, 1960: 304). He adds that the reader, and the text are more analogous to a pianist and a musical score. But the instrument that the reader plays upon is he himself. His keyboard is the range of his own past experiences with life and literature, his own present concerns, anxieties, and aspirations. Under the stimulus and guidance of the text, the reader seeks to strike the appropriate key, to bring the relevant responses into consciousness. Out of the particular sensations, images, feelings, and ideas which have become linked for him with the verbal symbols, he creates a new organization. This is for him the story (Rosenblatt, 1960: 305). Therefore, readers are seen as breathing life into the texts they read in a creative effort nearly comparable with its author. The digital reader concretizes and reifies the story in a different manner from a traditional readers with the opportunity to enjoy multimedia elements in a hypertext fiction.

In this regard, Barthes also argues in *The Death of the Author*:

- a text is made of multiple writings, drawn from many cultures
- and entering into mutual relations of dialogue, parody,
- contestation, but there is one place where this multiplicity is
- focused and that place is the reader, not... the author. ...[T]o
- give writing its future... the birth of the reader must be at the
- cost of the death of the Author (Barthes, 1997: 148).

From the above discussion, this paper attains that readers of hypertext novels have an impressive story. They feel empathy for the characters, understand the causal chain they're tied, and along the reading, they tensely try to guess the next event in the novel. In fact, digital novels are more receiver-oriented. Murray defines the digital reader with ardent, or as she calls him/her, the "interactor."

The reader is a navigator, protagonist, explorer, or builder, [who] makes use of [a] repertoire of possible steps and rhythms to improvise a particular dance among the many, many possible dances the author has enabled. We could perhaps say that the interactor is the author of a particular performance within an electronic story system, or the architect of a particular part of the virtual world, but we must distinguish this derivative authorship from the original authorship of the system itself (Murray, 1997: 153).

Along these lines, Murray is reminding us that each time readers enter a hypertext Web, they create a "new" text, written by the choices they make as they travel through the Web. Landow also consistently reminds us that the text an interactor reads is not necessarily the text an author planned (Landow, 2006). This is an important concept for readers and writers because it reinforces the fact that readers and writers approach their tasks with purpose, and those purposes may not be the same. All this seems much like the ancient storyteller, who changes the text to fit the wishes of each audience. The audience and the storyteller (author) collaborate to create the narrative (Patterson, 2000: 78).

3. To be Conventional or Digital Reader

There is no clear distinction between text production and text reception in hypertext fiction, and the readers can decide where to begin their reading and where to end it. They choose their own path and thereby create their own story in the hypertext system. It should be repeated that an important functional feature of hypertext is hyper-reading with its ability to

grant reader a navigating mode and so as the name suggests the move structure of a text indicates a preferred way of organizing the text in order to realize a particular story/event in a plot. Therefore, the reader by creating a sequence of moves through which he/she read the novel will become a hyper-reader. On the contrary, readers of print narratives begin reading where the print begins on the first page of the book. Conventional readers, nonetheless, move more or less straightforwardly through printed novels like *Pride and Prejudice* or *Huckleberry Finn*. The reader's gradual progression from beginning to end follows a carefully scripted route which ensures that "the reader does indeed get from the beginning to the end in the way the writer wants him or her to get there" (Slatin, 1990: 871).

However, most hypertext narratives have no single beginning, for example, to read the hypertext novel like *10:01*, readers generally load the hypertext onto a PC or laptop and begin moving through the text by clicking on seat images or directional arrows or even by clicking on different times in the homepage which have been implemented by external links as well. Indeed, the most obvious difference between traditional reading and hyper-reading boils down to that of linearity; with hypertext reading being regarded as non-linear and traditional text reading being regarded as linear. In addition to reading mode which leaves the user in a traditional reader position with sequential reading, navigating mode allows the reader to navigate the narrative structure and actively construct his/her own reading path through one or several nodes.

Consequently, hypertext narratives encourage readers to shape the outcomes of the stories they read by the decisions they make in the reading process. Readers who read the digital narratives are totally engaged, and yet they are clicking on links and making choices as to how to proceed, and how to manipulate the story. In this way, the readers feel the power of the text. In order for that to happen, readers must write the text for them-selves, and in the reader's mind, the text shifts through previous experiences as the reader goes through the meaning-making process. In this sense, the reader is always central to the text.

As a result, this paper postulates that hypertext gives permission to readers to insert themselves into the meaning construction process and interpret or read a text in a way that is often different from what the author predicted. Readers engaging interactive narratives also have the option of limiting their textual experience to the pursuit of narrative strands, which intrigue them and the more links which each reader must confront in navigating through the narrative, the less singular and determinate the meaning of the hypertext narrative as a whole, since no single path through the text has priority over all others.

4. Conclusion:

Hypertext novel can be seen as involved in the construction of its readers. This new cyber-genre endows reader a different identity. In this position they would not accept the preferred readings which may be built into texts for them. For these readers optional ways of readings provide their responses to texts. Although readers can never hold a hypertext novel or feel its pages physically, they nonetheless, actually interact with the text far more tangibly than do readers of printed novels. By participating in the creation of the textual structure, the reader becomes both author and reader at the same time. And if the writing space is arranged in a way that the reader's choices can be fruitful, then the reader may give the new life to the text.

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12/8/2012

Monitoring and management of rapidly progressing monocular keratoconus over 5 years

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Abstract: Keratoconus is a slowly progressive non-inflammatory disease of the central cornea which causes central stromal thinning, apical protrusion, and irregular astigmatism.[1] I report the management of over 5 years of a 25 year old monocular keratoconic Asian male whose condition progressed remarkably rapid. During the 5 year period, the patient's flat K increased by 4.00 diopters and his steep K increased by 5.75 diopters in his keratoconic eye. The corneal topographies documenting the continuing of corneal protrusion and our lens design at different stages of the ectasia are detailed described in this paper. Patient is still under yearly monitoring for his lens treatment as his keratoconus has not stabilized. [Santos Shan-Yu Tseng, Kuo-Chen Su, John Ching-Jen Hsiao. **Monitoring and management of rapidly progressing monocular keratoconus over 5 years.** *Life Sci J.* 2012, 9(4):5247-5250] (ISSN:1097-8135). http://www.lifesciencesite.com.781

Keywords: Keratoconus, Rigid gas-permeable lens, Special lens, Rose- K, Irregular astigmatism

1. Introduction

Keratoconus, with incidence of approximately 50 to 230 per 100,000, is seen more often than most eye care practitioners can expect. The symptoms and clinical sings include distorted vision, monocular diplopia, frequent prescription change, increase myopia astigmatism, distorted keratometry mires, Fleisher's ring, Munson's sign, Vogt's lines, and most important of all, reduced best corrected visual acuity by spectacle. In other words, Keratoconus is a progressive, often asymmetric, non-inflammatory disease characterized by the thinning, protrusion, and scarring of the cornea.[2,3]

This report discusses a remarkably rapid progressing keratoconic male, whose ecatatic condition monitored by corneal topographies is detailed here in this paper. Special lens treatment at different stages of keratoconus is given to the patient for maintaining minimum binocularity and improving visual acuity.

2. Material and Methods

Best corrected visual acuity was done by using Topcon phoroptor. Documenting ectatic condition was done by Dicon topographer (Paradigm Medical, USA) and keratometry reading via Topcon keratometer. Contact lenses used are Rose K design rigid gas-permeable lens for keratoconic eye and traditional tri-curve rigid gas-permeable lens for non-keratoconic eye.

3. Results

July 2004

A 25 year old Asian male with unremarkable health condition visited our center for a complete ocular-visual examination. His chief complaint was seeing blur in his OD. The test result was as follows:

• Manifest refraction:
OD –7.25 –2.75 x 015 6/15^{-} (20/50^{-});
OS –5.75 –1.50 x 160 6/7.5 (20/25)
• Keratometry:
OD 42.25@035 /46.25@125; corneal cylinder: –4.00 DC x 035;
OS 40.25@165 /42.00@075; corneal cylinder: –1.75 DC x 165

The corneal topography showed an inferior area of protrusion of the cornea in the right eye (Fig. 1), and a regular with-the-rule astigmatic pattern in the left eye (Fig. 2). Keratoconus was hence diagnosed OD. After several trail lens fittings, the following lens specifications were finalized:

OD: 7.45/–7.00/8.90 6/7.5^{-} (20/25^{-}) Rose K rigid gas-permeable lens
OS: 8.25/–5.75/9.80 6/7.5^{+} (20/25^{+}) tri-curve lens

The parameters denote the lens' base curve, back vertex power, and total lens diameter, respectively. The lenses were ordered and dispensed one week later to the patient together with proper instruction on lens cares and wearing time.

Patient was pleased with corrected vision with contact lenses.

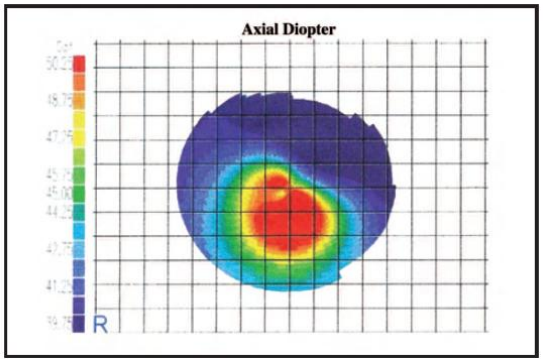

Fig. 1 July 2004: right eye topography at initial presentation, showing keratoconus.

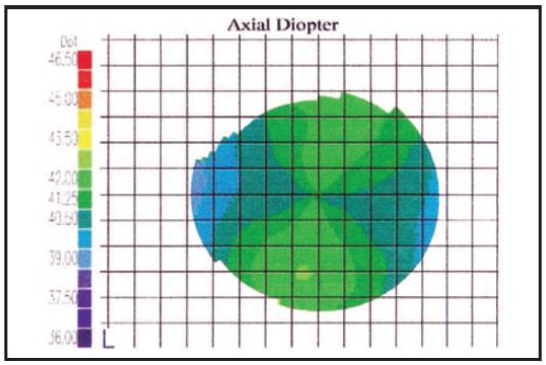

Fig. 2 July 2004: left eye topography at initial presentation.

June 2005

One year later, the patient came back for monitoring of his keraotoconus condition and lens refitting because he lost his OD lens. He noticed that prior to losing the lens, the lens does not feel as comfortable as before and it seems to fell out frequently during lens wear.

The examination data was as follows:

• Manifest refraction:

OD –8.25 –4.00 x 018 6/18– (20/60–);

OS –5.75 –1.25 x 158 6/7.5 (20/25)

• Keratometry:

OD 43.25@032 / 48.75@122; corneal cylinder: –5.50 DC x 032;

OS 40.25@162 /41.75@072; corneal cylinder: –1.50 DC x 162

The clinician was surprised to note that the patient's right eye flat-K was steeper by 1.00 D, and his steep-K was 2.50 D steeper as compared to his last visit 1 year ago.

Topography was performed OD (Fig. 3). The result was consistent with the keratometry findings which demonstrated a progressing ectasia in his right eye.

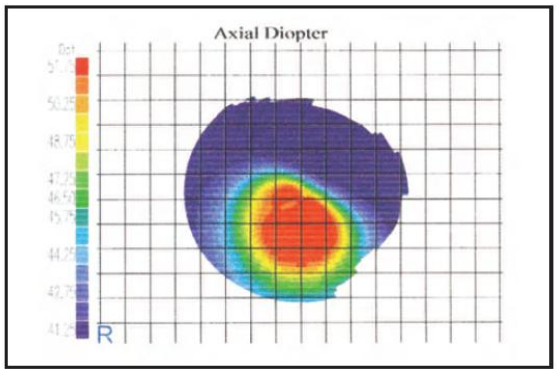

Fig. 3 June 2005: right eye topography showing keratoconus.

The following gas-permeable lens was refitted for his right eye:

• OD: 7.20/–8.50/8.90 6/7.5⁻ (20/25⁻) Rose K gas permeable lens

As one can see, the base curve radius was steeper by 0.25 mm and the back vertex power was higher by 1.25 D with respect to his previous lens in order to accommodate for the eye's steeper curvature. The left eye's lens was left unchanged.

October 2006

The patient returned to the clinic and complains of recent increasing discomfort with his right lens. The examination data was as follows:

• Manifest refraction:

OD –9.50 –3.75 x 013 6/24+ (20/80+);

OS –5.75 –1.50 x 160 6/7.5 (20/25)

• Keratometry:

OD 45.25@035 / 50.25@125; corneal cylinder: –5.00 DC x 035;

OS 40.50@165 /42.00@075; corneal cylinder: –1.50 DC x 165

Once again, the test result shows the progression of the patient's keratoconus.

Compared to the last visit, flat-K was steeper by 2.00 diopters and steep-K was steeper by 1.50 diopters. The topography map showed a stable with-the- rule astigmatic left cornea (Fig. 4), but in the right eye, we witnessed yet another increasing protrusion of the cornea (Fig. 5). The eye was thus needed to refit with the following lens:

• OD: 7.00/–12.25/8.90 6/7.5– (20/25–) Rose K gaspermeable lens.

The base curve radius was steeper by 0.2 mm and the back vertex power was higher by 3.75 D with respect to his previous lens. The left eye lens was left unchanged. The patient was told to not to rub the eye and making sure to keep up his yearly visit to our center.

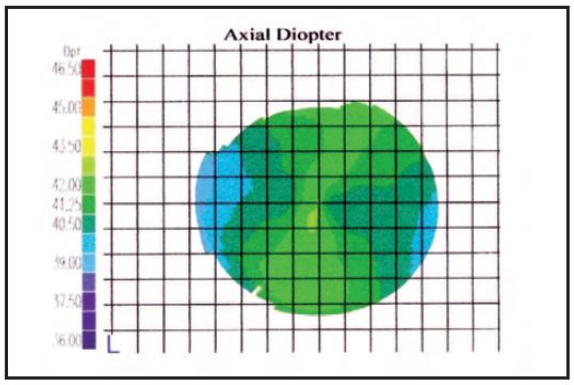

Fig. 4 October 2006: left eye topography.

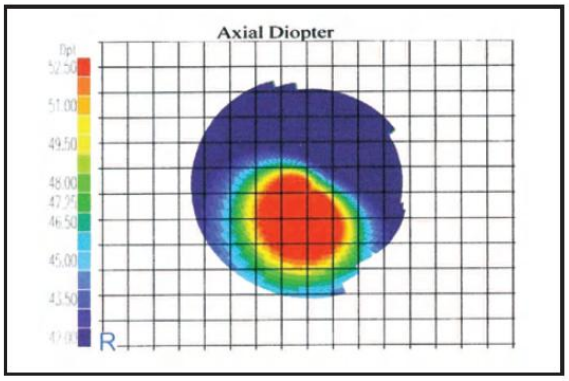

Fig. 5 October 2006: right eye topography showing keratoconus.

November 2008

The patient returned to the clinic 2 years later complaining of slightly reducing wearing time in this OD. The examination data was as follows:

• Manifest refraction:

OD –10.00 –3.75 x 013 6/30+(20/100+);

OS –5.75 –1.50 x 160 6/7.5 (20/25)

• Keratometry:

OD 45.50@040 / 51.00@130; corneal cylinder: –5.50DC x 040;

OS 40.50@165 /41.75@075; corneal cylinder: –1.25 DC x 165

This time the result continued to show the progression of the patient's keratoconus, but much less change was found. Compared to the last visit, flat-K was steeper by 0.25 diopters and steep-K was steeper by 0.75 diopters. The topography map continues to show a stable with-the- rule astigmatic left cornea. In the right eye, we witnessed another but smaller increasing protrusion of the cornea (Fig.6). The eye was again needed to refit with the following lens:

• OD: 6.95/–12.50/8.90 6/7.5⁻ (20/25⁻) Rose K gas permeable lens

The base curve radius was steeper by 0.05 mm and the back vertex power was higher by 0.25 D with respect to his previous lens. The left eye lens was left unchanged. The patient was told that the progression has slowed down and returns for follow up in 1 year time.

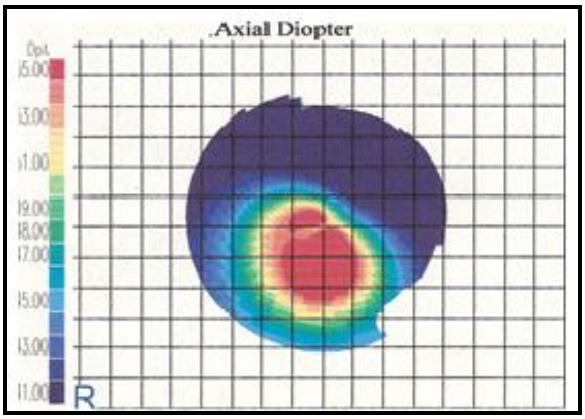

Fig. 6 November 2008: right eye topography showing keratoconus.

October 2009

The patient returned to the clinic the following year complaining of discomfort due to excessive lens movement upon blinking of his right lens. The examination data was as follows:

• Manifest refraction:

OD –11.00–4.25x 013 6/40-(20/200+);

OS –5.75 –1.50 x 160 6/7.5 (20/25)

• Keratometry:

OD 46.25@035 /52.00@125; corneal cylinder: –5.75 DC x 035;

OS 40.50@165 /41.75@075; corneal cylinder: –1.25 DC x 165

Again, the result continued to show the progression of the patient's keratoconic condition with more change than that of last year. Compared to the last visit, flat-K was steeper by 0.75 diopters and steep-K was steeper by 1.00 diopters. The topography map continues to show a regular astigmatic left cornea. In the right eye, we noticed another increasing area of protrusion of the cornea (Fig.7). The eye was thus again needed to refit with the following lens:

OD: 6.85/–13.25/8.90 6/7.5⁻ (20/25⁻) Rose K gaspermeable lens

The base curve radius was steeper by 0.10 mm and the back vertex power was higher by 0.75 D with respect to his previous lens. The left eye lens was left unchanged. The patient was made aware of his progressing condition.

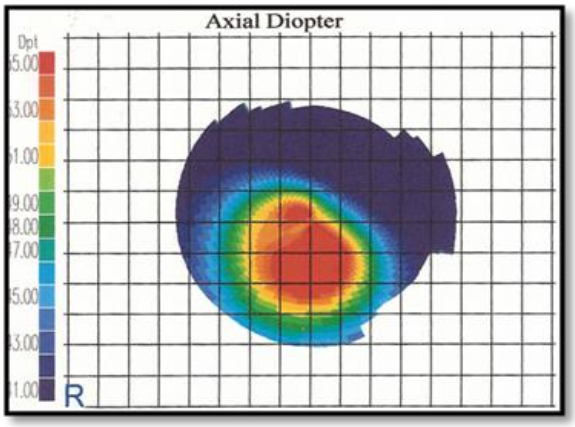

Fig. 7 August 2009: right eye topography showing keratoconus.

4. Discussion

It is not uncommon to see the disease progression of keratoconus, especially in young adults such as our patient. What is unusual about this case is that the patient's ectasia progression was remarkably fast. Fitting of an RGP lens improves visual acuity and many such lenses have been developed for management of keratoconus patients.[4] During the 5 year of monitoring, the patient's flat K increased by 4.00 diopters, from 42.25 D to 46.25 D, and his steep K increased by 5.75 diopters, from 46.25 D to 52.00 D, in his keratoconic eye. The significant changes in patient corneal shape required us to redesign the contact lens fitting from time to time. The design of lenses used is as follows:

The right eye:

In 2004, the keratoconic eye, the right eye, was fitted with a Rose K rigid gas permeable lens, which is specifically designed for kerotoconic cornea. After many trial lenses fitting, using fluorescein stain and 3 point touch method, the final base curve of the lens used was 7.45 mm with the diameter of 8.9 and the vertex power of -7.00 D.

As patient's keratoconic eye progressed and became steepen during the following visits, the base curve and vertex power of lens used was steeper and more myopic every time. For example, in 2005, the lens base curve and power used was 7.20 mm and -8.50 D. In 2006, the lens base curve and power used was 7.00 mm and –12.25 D. In 2008, it was 6.95 mm and –12.50 D and finally in 2009, it was 6.85 mm and –13.25 D.

The left eye:

The left eye, without presence of keratoconus, was easily fitted with standard Tri-curve rigid gas permeable lens. By compare the cornea's Flat K and corneal astigmatism, we fitted the eye with a lens that was 0.62 D steeper than flat K. Since flat K was 40.25 D, we fitted a lens with an 8.25 mm base curve which corresponds to 40.87 in diopter.

All these fittings of contact lenses were done with trial lenses on eye to determine the suitability. With the aids of fluorescein, corneal topography and proper trial lenses, we were able to design the proper lenses for patient for the minimum level of comfort and acuity for him. In the case of the keratoconic lens, the peripheral curves were specifically flattened to increase edge lift. This is because the original trial lenses did not offer the sufficient tear exchange due to relatively tight peripheral curves.

5. Conclusion

Due to fact that Patient's keratoconus condition is still under progression, we believe the management of this patient is not yet over. The corneal shape, the suitability of rigid gas permeable lens and the rate of keratoconus progression should all be carefully documented and monitored in following visits, and of course the redesign of contact lens will be done whenever necessary. We will also continue to observe his left eye for any signs of incipient keratoconus since the disease affects bilaterally.[5]

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10/19/2012

Proposed a Mathematical Based Applicable Method for Moho Depth Estimation by Use of Bouguer Anomaly Gravity Data- Case Study

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Abstract: At the present study, variation of crustal thickness by Bouguer anomaly gravity and topographic data for the southeast of Iran in the southern part of Kerman province has been investigated. At the first, 2D low-pass filters and upward frequently type analytical techniques were applied to the Bouguer gravity data of the region to inspect the regional gravity anomalies behavior. Then by application of a Moving Window Power Spectrum Method (MWPSM), probable structural depth variations between 32 to 46 km were determined. By applying, the Euler Deconvolution Method (EDM) on Bouguer anomaly gravity data, an investigation of changes in crustal thickness and the type of the anomaly resources was carried out. To validate the proposed method, a comparison between the obtained values with results of Inverted Parker-Oldenburg's Method (IPOM) was conducted. Generation and improvement of a probable 2D crust model by combination of obtained results and proposed seismic velocity models for this region in previous studies was the key factor of this paper.

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Keywords: Crustal thickness, Moving Window Power Spectrum Technique, Euler Deconvolution, Inverted Parker-Oldenburg's Method

INTRODUCTION

The Power Spectrum Method has used for the determination of crustal thickness using gravity data (Bhattacharyya, 1965 and 1966; Spector and Bhattacharyya, 1966; Jenkis and Watts, 1968; Spector and Grant, 1970; Cianciara and Marcak, 1976; Karner and Watts, 1983; Akgun et al., 1996). By developed MWPSM, a determined profile by windows, which move by certain intervals, can show the depth variations (Cianciara and Marcak, 1976). Euler Deconvolution Method is the other one for estimation of structural parameters. This technique determines the location of the structure, depth and the type of source by using horizontal and vertical derivatives of the anomaly (Thompson, 1982; Reid et al., 1990; Keeting, 1998; Barbosa et al., 1999; Beasley and Golden, 1993; Zhang et al., 2000; Roy et al., 2000; Ozyalin, 2003).

GEOLOGICAL BACKGROUND OF STUDIED AREA

Active tectonics: The northward motion of Arabian with respect to Eurasia, which is shown in figure1, dominates the active tectonics of Iran. At longitude 56° E, ~25 mm/yr of north-south shortening is accommodated across Iran (Sella et al., 2002; McClusky et al., 2003; Vernantet al., 2004a). Several large earthquakes have occurred on the right-lateral strike-slip fault systems along the western margin of the Dasht-e-Lut (Berberian et al., 2001), which accommodate right-lateral shear between central parts of Iran and Afghanistan. However, low rates of seismicity lead the parts of central Iran west of the Dasht-e Lut desert to be considered as relatively strong and non-deforming crustal blocks, in which relatively few active faults have been mapped and relatively few historical earthquakes are recorded (Ambraseys and Melville, 1982). GPS velocities also suggest that the rates of deformation across central Iran are low, at~3 mm/yr at the longitude of Tehran (Vernant et al., 2004a,b) and at rates of less than 2 mm/yr at the longitude of Kerman (Vernant et al., 2004b).

Geology of Kerman province: The northwest-southeast-trending Kuh-e Bahr Aseman (~3800 m), Kuh Hezar (~4400 m), Kuh-e Lalehzar (~4350 m) and Kuh-e Mamzar (~3100 m) mountain ranges form part of the Sanandaj-Sirjan Tertiary volcanic belt, formed during closure of the Neo-Tethys and subduction of oceanic material (e.g. Stocklin, 1968). The Sanadaj-Sirjan ranges dominate the central part of the study area and separate regions of low relief. We call the northern region the Rafsanjan plain (with an average elevation of ~2000 m to the south of Kuh-e Kalleh Gav and 1600-1700 m to the north of Kuh-e Kalleh Gav) and the southern region the Sirjan plain, again with an elevation of roughly 1700 m. Kuh-e Kalleh Gav and Kuh-e Jupar form isolated ranges within the Rafsanjan plain. Kuh-e Sekonj runs along the eastern boundary of the study area and borders the Gowk right-lateral strike-slip fault (Figs. 1; Berberian et al., 2001). Kuh-e Sekonj and Kuh-e Jupar in the east of the study region consist of conformable Mesozoic and Tertiary sediments. The bedrock geology of northern parts of the Sanandaj-Sirjan zone (south of Anar and in the Kuh-e Kalleh Gav) form a sequence of Cretaceous and early Tertiary turbitide basins (Dimitrijevic, 1973). Rocks exposed within the high ranges of Kuh-e Lalehzar and Kuh-e Hezar are a mixture of unconformable Tertiary sediments and volcanic complexes, with up to 7 km thickness of Eocene volcanics exposed in Kuh-e Bahr Aseman massif (Dimitrijevic, 1973). Thick deposits of Neogene and Quaternary sediment underlie the Rafsanjan and Sirjan plains. The Neogene deposits are typically fine-grained and light-colored marls and sandstones, which are likely to represent the sedimentation before the onset of uplift and faulting. The marls grade upwards into gravels shed from adjacent mountain ranges. The transition to

gravel deposition probably marks the onset of active faulting in the region. The Neogene and Quaternary basin deposits have subsequently been deformed by folding. No detailed information exists on either the stratigraphy or structures developed in the Neogene and Quaternary deposits. Large areas of eastern and central Iran are covered by large late Quaternary alluvial fan surfaces, which are often abandoned and incised by drainage. Although the patterns of river incision may be controlled at the local scale by fault movement, the repeated cycles of deposition and incision within the river system must ultimately be driven by climatic variation within the late Quaternary, as is the case in other parts of central Asia (e.g. Brown et al., 1998; Pan et al., 2003). There is little chronological data to date periods of alluvial fan deposition in Iran. Figure2 shows a simplified geological map of the studied area.

APPLIED METHODS

1. **Moving Window Power Spectrum Technique:** The accuracy of the spectrum estimation is a statistical approach that depends on the variance and average square error level. In this application, a division on 1D data into equal parts, by window function, is executed and spectrum is obtained separately for divisions. The values in different frequencies are then integrated and their arithmetic mean value is calculated to obtain the spectrum (Jenkins and Watts, 1968). In this method, power spectrum for each division ($\bar{S}(w)$) is given by Cianciara and Marcak (1976) as:

$$\bar{S}(w) = \frac{1}{R}\sum_{r=1}^{R}\sum_{P=1}^{P} b_r^P(w, \epsilon_1^{Pr}, \epsilon_2^{Pr}, \ldots \epsilon_n^{Pr}) \exp(-2wh), \qquad (1)$$

R: number of divisions, w: angular frequency, h: depth, ε: structure parameter and b: function of anomaly

If, in equation (1), the below inversion is made (Jenkins and Watts, 1968), the equation (2) will appear.

$$b_r^P(w, \epsilon_1^{Pr}, \epsilon_2^{Pr}, \ldots \epsilon_n^{Pr}) = c^{pr} = \text{constant} \qquad c = \sum_{r=1}^{R}\frac{1}{R}\sum_{P=1}^{P} c^{Pr}$$

$$S = c.\exp(-2wh). \qquad (2)$$

By taking the logarithm of equation (2), the average depth of the structure causing anomalies is found as equation (3).

$$\bar{h} = \frac{\ln S(w_{l+1}) - \ln S(w_l)}{2(w_{l+1} - w_l)} \qquad l = 1, 2, \ldots\ldots \pm N \qquad (3)$$

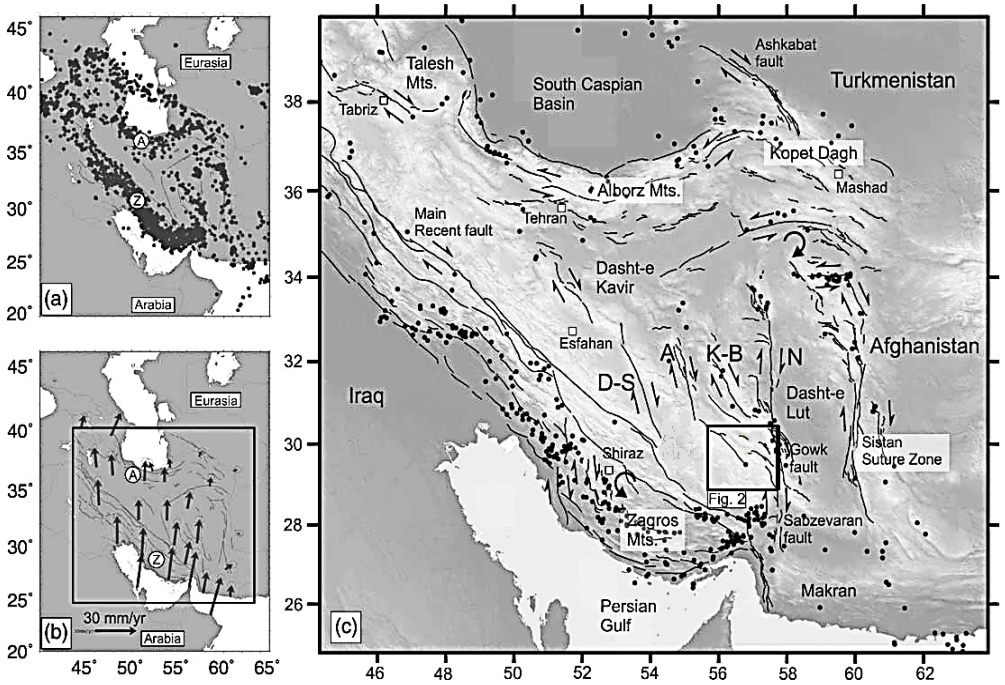

Figure1. (a) Instrumentally recorded earthquake epicenters in Iran from the catalogue of Engdahl et al. (1998). (b) A velocity field for Iran determined from repeated GPS measurements (Vernant et al., 2004). Both the seismicity and the deformation measured by GPS are concentrated in central parts of Iran including the selected region (c) Shaded SRTM topography of the studied area showing the major active faults (DehShir (D-S), Anar (A), Kuh-Banan (K-B) and Nayband (N)). The indicated box in geological map represents the studied region that has shown in later figures.

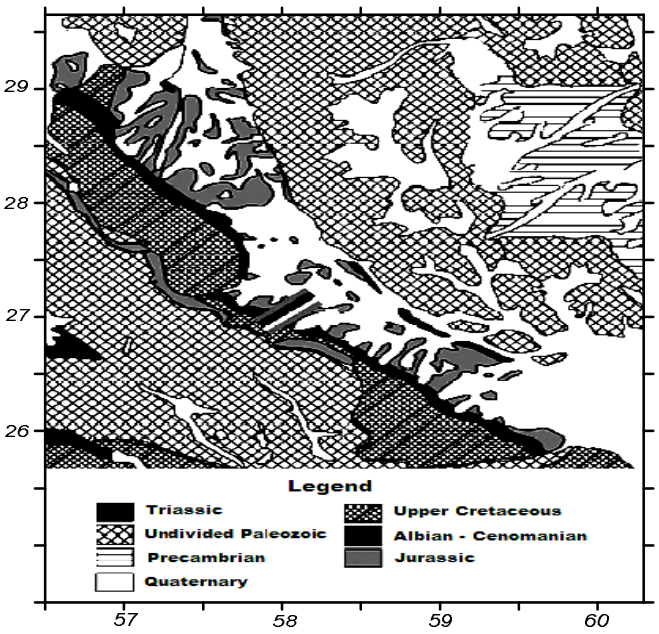

Figure2. Simplified geological map of Kerman province (Hukriede et al., 1962)

2. **Euler Deconvolution Technique:** This technique uses potential field derivatives to image subsurface depth of a magnetic or gravity source (Hsu, 2002). Mushayandebvu et al., (2001) described 2D space Euler's deconvolution equation as given in equation (4).

$$(x-x_0)\frac{\partial T}{\partial x}+(z-z_0)\frac{\partial T}{\partial x}=-N\Delta T \qquad (4)$$

(X_o, Z_o): coordinate position (top of the body), Z: measured depth as positive down, X: horizontal distance, ΔT: residual field value, and N: structural index

The structural index is a measure of the variation or fall off rate with field distance and therefore is a function of the causative bodies' geometry. Thus, the magnetic field of a dipole falls off as the inverse cube, giving an index of three, while a vertical line source gives an inverse square field fall off and an index of two. Extended bodies will form assemblages of dipoles and will therefore have indices ranging from zero to three. If ΔT_i is the residual field at the i^{th} point in a magnetic or gravity survey, with the point of measurement at (X, Z) and the coordinate position of the top of the body (X_0, Z_0), then equation (5) can be written as,

$$\left[\frac{\partial}{\partial x}\Delta T_i \frac{\partial}{\partial z}\Delta T_i\right]\begin{bmatrix}x-x_0\\ z-z_0\end{bmatrix}=N\Delta T_i, \qquad (5)$$

By calculating the horizontal and vertical gradients of the field, equation (5) has only three unknowns X_0, Z_0 and N, where the first two describe the location of the body. Many simultaneous equations can be obtained for various measurement locations, which can give rise to one matrix equation.

$$\begin{bmatrix}\frac{\partial}{\partial x}\Delta T_1 & \frac{\partial}{\partial z}\Delta T_1\\ \frac{\partial}{\partial x}\Delta T_2 & \frac{\partial}{\partial z}\Delta T_2\\ \vdots & \vdots\end{bmatrix}\begin{bmatrix}x-x_0\\ z-z_0\end{bmatrix}=N\begin{bmatrix}\Delta T_1\\ \Delta T_2\\ \vdots\end{bmatrix}, \qquad (6)$$

The least squares method can be used to obtain the unknowns X_0 and Z_0 if the structural index N is known (Thompson, 1982). Table (1) displays structural indices for different possible geological structures.

Table (1): Structural indices for different geological structures (after Reid et al., 1990)

Structural Index	Geological Structure
0	Contact
0.5	Thick Step
1	Sill / Dike
2	Vertical Pipe
3	Sphere

3. **Parker-Oldenburg's Inversion:** The inversion procedure uses the equation described by Parker (1973) to calculate the gravity anomaly caused by an uneven, uniform layer of material by means of a series of Fourier transforms. This expression, in its one-dimensional form, is defined as:

$$F(\Delta g)=-2\pi\rho G e^{(-kz_0)}\sum_{n=1}^{\infty}\frac{k^{n-1}}{n!}F[h^n(x)] \qquad (7)$$

where F(Δg) is the Fourier transform of the gravity anomaly, G is the gravitational constant, ρ is the density contrast across the interface, k is the wave number, h(x) is the depth to the interface (positive downwards) and z_0 is the mean depth of the horizontal interface. Oldenburg (1974) rearranged this equation to compute the depth to the undulating interface from the gravity anomaly profile by means of an iterative process and is given by:

$$F[h(x)] = -\frac{F[\Delta g(x)]e^{(+kz_0)}}{2\pi\rho G} - \sum_{n=2}^{\infty}\frac{k^{n-1}}{n!}F[h^n(x)] \qquad (8)$$

This expression allows us to determine the topography of the interface density by means of an iterative inversion procedure. In this procedure, we assume the mean depth of the interface, z_0, and the density contrast associated with two media, ρ. The gravity anomaly is first demeaned prior to the calculation of the Fourier transform. Then, the first term of equation (8) is computed by assigning h(x) = 0 (Oldenburg, 1974) and its inverse Fourier transform provides the first approximation of the topography interface, h(x). This value of h(x) is then used in the equation (8) to evaluate a new estimate of h(x). This process is continued until a reasonable solution is achieved. Following Oldenburg (1974), the process is convergent if the depth to the interface is greater than zero and it doesn't intercept the topography. Further, the amplitude of the interface relief should be less than the mean depth of the interface. As the inversion operation (equation 8) is unstable at high frequencies, a high-cut filter, HCF(k) is included in the inversion procedure to ensure convergence of series. This filter is defined by:

$$HCF = \frac{1}{2}\left[1 + \cos\left(\frac{k - 2\pi WH}{2(SH - WH)}\right)\right] \qquad (9)$$

$$WH \le k \le SH$$
$$HCF(K) = 0, k < WH$$
$$HCF(K) = 1, k > SH$$

The mentioned condition is used to restrict the high frequency contents in the Fourier spectrum of the observed gravity anomaly. The frequency, k can be expressed as 1/λ, being λ the wavelength in kilometres. The iterative process is terminated when a certain number of iterations has been accomplished or when the difference between two successive approximations to the topography is lower than a pre-assigned value as the convergence criteria. Once the topographic relief is computed from the inversion procedure, it is desirable to compute the gravity anomaly produced by this computed topography. In general, this modelled anomaly must be very similar to the one used as input at the first step of the inversion process. Estimations of crustal thickness in paper is described with the aid of the flow chart in figure 3.

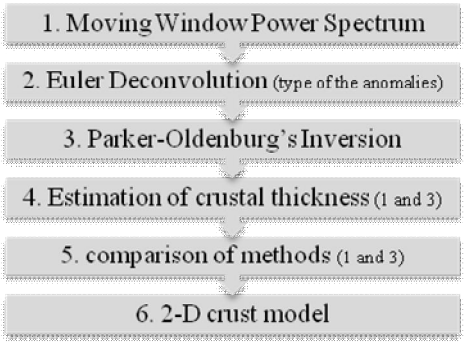

Figure3. Flow chart of various stages in study.

IMPLEMENTATION

Bouguer gravity and topographic anomaly maps of Kerman province extending approximately between 57°- 60° E longitudes and 27°- 30° N latitudes with 1-km sampling intervals, which were used at the application stage of this study, were provided by the contribution of the Geological Survey of Iran. When the Bouguer gravity map of Kerman province in figure4 is examined, it can be seen that a regional anomaly with high negative amplitude is dominant. In order to investigate the long-wavelength variation of the high-amplitude anomaly in the region, the two-dimensional low-pass filtering and upward analytic continuation methods were applied to the data as shown in figure5. According to the Airy compensational mechanism, negative anomalies on the bouguer gravity map are interpreted as regions with thick crust and excess mass (mountainous regions) or as hot regions with low density (Hofmann et al., 2006). For this purpose, preceding all, filtering and analytic continuation maps were compared with the topographic map, which has plotted in figure6.

Although the two types of maps seem to be similar in main features, close examination reveals that regions with the same elevations present different Bouguer gravity anomalies. For example, although the section in N-S direction on 58°E longitude on the topographic map in Figure 6 has a topography over 3000 meters, the section giving high amplitude negative anomaly on the filtering map has a boundary between 28.5°-29.5° N latitudes. This shows that the anomaly does not stem only from excess mass and it conveys the presence of another factor. For throughly investigating the depths in the region, moving window power spectrum was applied to Bouguer gravity data. The map for probable structural depths in the Kerman province is shown in figure7.

Examination of the obtained depth map (figure7) reveals that the depths in the region range from 32.5 to 45.5 km. In order to estimation the changes within borders of the windows; the Euler deconvolution method was applied to Bouguer gravity data (figure2, profiles A, B) in these profiles (figure8). Examination of the obtained depth map (figure9) reveals that the depths in the region range from 34 to 45.5 km.

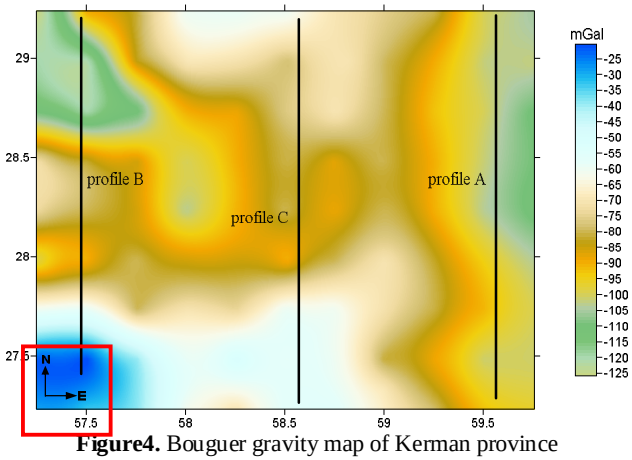

Figure4. Bouguer gravity map of Kerman province

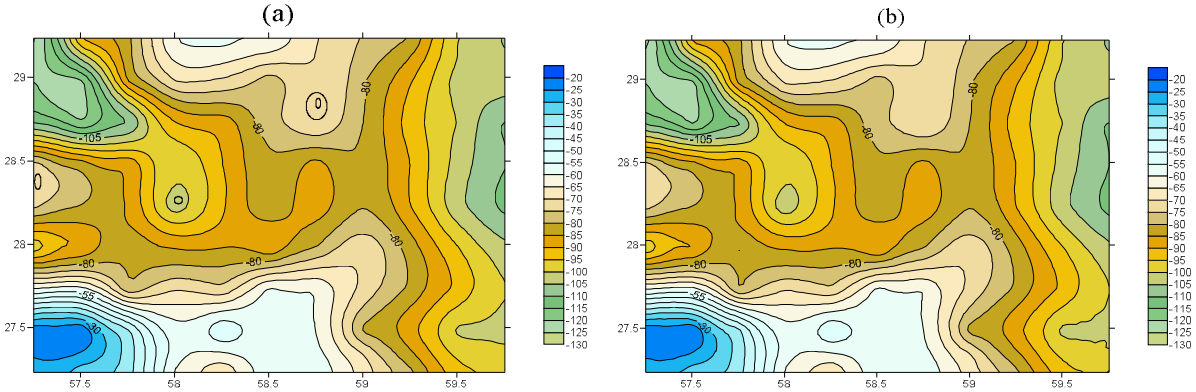

Figure5. a) Low-pass filter map of Bouguer gravity data in figure4 b) Upward analytic continuation map of Bouguer gravity data in figure4

Examination of the obtained depth map reveals that the depths in the region range from 34 to 45.5 km. From the application of moving windows power spectrum, it was determined that crustal thickness which is ~37.5 km in the south Zagros fault region extends up to ~44 km in the east Sistan suture Zone. And From the application of Parker-Oldenburg's inversion, it was determined that crustal thickness which is ~39 km in the south Zagros fault region extends up to ~44 km in the east Sistan suture Zone which is shown in Table (2).

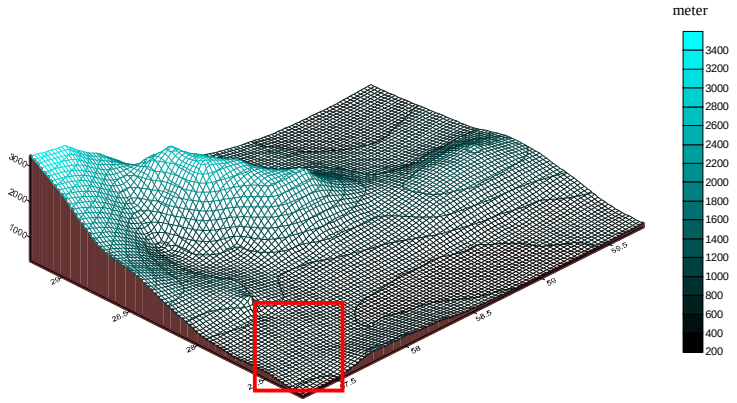

Figure6. Topographic maps of the field of study

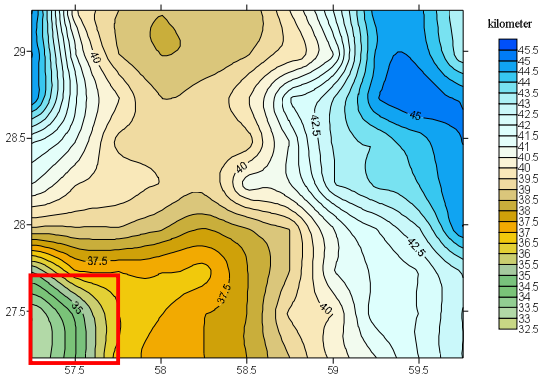

Figure7. The map of possible structural depths belonging to the Kerman province obtained from the application of moving window power spectrum method on Bouguer gravity data

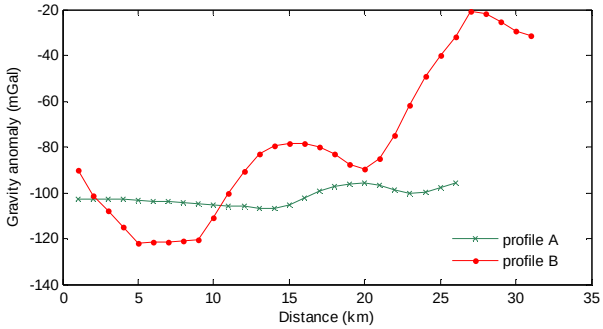

Figure8. Values of profiles A, B

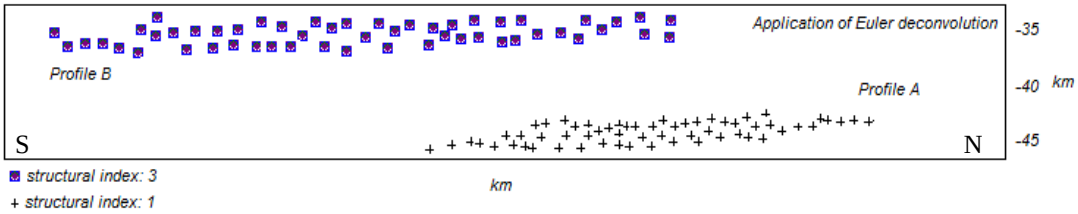

Figure9. Application of Euler deconvolution method on Bouguer gravity data of profile A, B in figure8

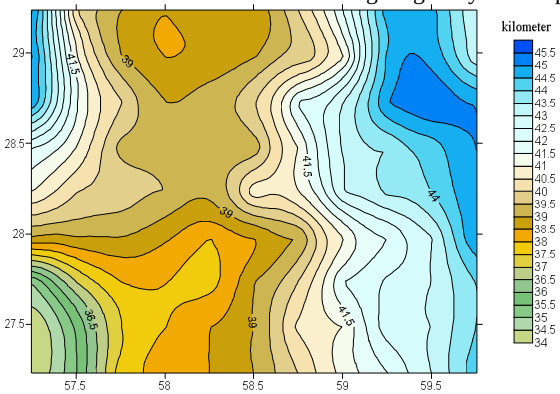

Figure10. The map of possible structural depths belonging to the Kerman province obtained from the application of Parker-Oldenburg's inversion on Bouguer gravity data

Table (2). Depth values determined from the application of power spectrum method and Parker-Oldenburg's inversion

	Kuh-Banan Fault	Zagros mountains	Gowk fault	Sabzevaran fault
moving window power spectrum	39 (km)	41 (km)	44 (km)	37 (km)
Parker-Oldenburg's inversion	39 (km)	40 (km)	44 (km)	38 (km)

DISCUSSION AND CONCLUSION

In this study, using gravity and topographic data from the Kerman province, applications and evaluations have been carried out to determine the variations in crustal thickness. In the first stage, techniques of two-dimensional low-pass filtering and upward analytical continuation were used and behavior of regional gravity anomalies with high negative amplitude in the Kerman province was investigated. After that the moving windows power spectrum method was applied to gravity data; depth estimates were obtained and changes in crustal thickness were examined. In other stage of the application, Euler deconvolution method was applied to Bouguer gravity data, and location and depth of the structure and the type of source were detected. In the last stage, findings obtained from this study, geodynamic processes in the region were examined together with the results of other geophysical and geological studies, and a probable crustal simple model was formed.

Determined thickness by applying the Euler deconvolution method (figure9) pointed the crust thinning begins at nearly 57.5E longitude and continue in the eastern and northern directions. This finding overlaps with those obtained from power spectrum application and Parker-Oldenburg's inversion (figurs7 and 10). Analysis of the Euler deconvolution application provides the solution of the sphere model for the source type causing the anomaly in the western part of the region (figure8, profiles B), and towards the east, it gives the solution of the dike model (figure8, profile A). By application of calculated densities of seismic velocities obtained in the region, Bouger gravity data belonging to profile C in figure4 were modeled using Talwani et al., (1959) method (figure11). The section where the gravity of the anomaly decreased corresponds to the section where the structural depth is less than expected in particular (The red boxes in figures 4, 6 and 7). Regional anomaly showing high negative amplitude and reaching up to – 125 mgal in the region stems from the low-density zone formed due to high temperatures. The presence of low-velocity layers in a region brings into discussion the concept of low density. Besides the fact that the lithosphere is becoming thin, and the asthenosphere is becoming high causes a decrease in density due to the factor of temperature. These approaches show the presence of low amplitude gravity anomaly. Average depth values determined from the application of power spectrum method and Parker-Oldenburg's inversion in south, north, east and west of Kerman province is shown in Table (2).

Regional anomaly showing high negative amplitude and reaching up to - 125 mgal in the region stems from the low-density zone formed due to high temperatures. Average depth values determined from the application of power spectrum begins from 38 km in the South and reaches 45 km in the East. Moho depth was determined to be changing from 35 to 38 km in sections that present direct correlation between gravity and topographic data according to the Euler deconvolution application. Examination of the lithosphere proposed for the region and the crust-thinning model that comes because of thinning in the lithosphere, support the results obtained from this study.

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Assessment of socio-demographic characteristics in Infertile men who Referred to Shariati Hospital in Iran

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Abstract: At least 50% of infertility is partly or completely attributable to a male factor. Some factors affecting infertility is related to genetic and environmental factors. This study examined demographic characteristics of men with infertility were referred to the Shariati Hospital Infertility Clinic. This is a cross-sectional study. 200 eligible patients were enrolled with written consent Face-to-face interviews were based on a questionnaire that included variables on socio-demographic characteristics and Sperm analysis test performed by all individuals. Their height and weight measurements were recorded. The mean age was 34.1 ± 5.7 the average year after marriage number was 3.6 ± 1.3 years. The majority of people were self-employed, and a high proportion of them were exposed to chemicals in their work environment. However, this study found that the risk of diabetes disease, mumps, varicocele is higher among workers .The sperm analysis showed that the most common Problem of inferile men was decrease in sperm motility. , So that more than 162 participants of the study were 40% lower sperm motility. In this study the relationship between sperm parameters in infertile men and factors such as obesity, smoking, BMI, occupational status and previous disease and was not significant.

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Key words: semen analysis parameters, infertility, socio-demographic characteristics

Background and Goal:

Infertility refers to inability have a child at least two years after marriage without using prevention devices (1). According to the World Health Organization, infertility affected about 80 million couples across the world (2) which 50% of reasons relates to male factors (3). In a research in USA, infertility prevalence was predicted as 10-15% and about 19% in Australia (2). Infertility prevalence in 2009 among couples between 21-26 years old in iran was predicted as 17.2% (4). Experience of infertility which some called it “Infertility Crisis” follows physical, economic, psychological and social stress (5). Due to Many differences which exists at different environmental conditions that is related to infertility behavior such as marriage age, multiple sex partner, environmental pollutions, Alcohol Consumption, smoking cigarette and infectious disease prevalence, so etiology of infertility incidence and frequency of different reasons of infertility In different regions are different (6). Some studies represented harmful impacts of environmental factors including toxic substances, pesticides and radiations on men’ infertility (7, 8). Drinking alcohol and smoking cigarette also can be related to men infertility (9, 10, 11, and 12). Impact of heavy physical work and sitting jobs also are discussed (13). Varicocele also is one of main effective environmental factor in men infertility but its treatment impact on infertility improvement is unclear (14, 15). There is paradox in this subject about celiac disease (16,

17). Increase body mass index is considered effective in some studies and affectless in others (19, 20, and 21).

Since that main and most logical approach for decreasing infertility problem is to decrease its incidence and improve fertility health in order to prevent infertility incidence, then awareness of frequency of different factors of infertility at each region enjoys health importance and can be effective on managers' decisions. This research was performed with the aim of assessing some factors including age, occupation and literacy level, medical treatment, smoking cigarette and disease history in infertile men whom refers to Shariati infertility Clinic.

Materials and Methods:

This is a cross-sectional study that was part of a larger study which was performed in March 2010 through May 2011 with aim of genetic assessment of infertility. All qualified infertile men whom referred to Shariati infertility clinic were included in the study. 200 patients were qualified to enter in this study after they presented written consent. In this study, men infertility refers to those cases that married more than one year ago and no fertility happened in their wives and the relative results showed infertility and Sperm disorder. The data were collected by questionnaire which included questions related to demographic features (age, occupation, height, weight, marriage duration, marriage age, fertility history, medication, history of heart disease, high blood pressure, diabetes, smoking cigarette history, and surgeries such as hernia surgery, prostate and varicocele and Sperm Test that the researcher directly referred to those clinics and interviewed men. Sperm Test was performed for all patients by one laboratory. Their heights and weights were measured by stadiometer and registered in questionnaire. According to the main purpose of the study, all of these items were designed as yes or no. including the number of cigarettes consumed and the amount and duration of exposure to chemicals QUESTION did not work.

Exclusion criteria were as following: 1- those whom were not ready to cooperate in this plan verbally or in written form. 2- Those whom their semen was just enough for their clinical tests. The variables were consistent with questionnaires then their incidence amount and average were assessed in infertile men. Protocol of this study was approved in ethic committee of Tehran University of medical science.

Data analysis was performed by SPSS software and subjects were assessed by using descriptive-statistical tests in terms of such features such as age, Body Mass Index, occupation, smoking cigarette, drugs and surgery. Relation between many variables such as cigarette, disease, drugs, the surgery on sperm parameters also were determined by linear regression. Confidence coefficient equals to 95% (a=0.05)

Findings:

The age mean was predicted as 34.1±5.7 in terms of demographic features among 200 assessed infertile men in this study. Average of years after marriage equals 3.6±1.3. Body Mass Index was calculated as 24.1±5.7 which represented normal weight among these peoples.

Most patients of this study were self-employed that most of them based on self report were dealing with chemicals Including lead, mercury, toxic gases, chemical colors. Their literacy level was less than Diploma (121(60.5%). Three-quarters rejected in terms of any history of smoking cigarette, disease, surgery, medication and sexual disorders. It was observed however that risk of some diseases such as diabetes, Orion, varicocele was higher among workers than clerks. Semen analysis test showed that most infertile men problem relates to decreased Sperm movement so that more than 162 patients had sperm movement less than 40% (81%). 136 (68%) respondents had no minimum normal sperms. The number of sperms was so that 84 patients (42%) suffered reduced sperm and of this, 39 (46%) had no sperm.

Peoples of sample society were divided to two groups by considering minimum border of 20 million sperms per 1 mL semen and there was no significant difference between two groups in terms of factors including age, years after marriage, Body Mass Index, literacy level, smoking cigarette, disease history, sperm movement and morphology indexes (table 1).

By considering smokers and nonsmokers, peoples were divided to two groups and there was no significant difference in terms of analysis indexes of sperm (table 2).

By dividing peoples to two groups of patients and non-patients, there was no significant difference between these two groups in term of sperm analysis index (table 3).

A relationship of many factors including cigarette,BMI,occupation, disease history, and medication history with sperm count was measured using linear regression and no significant difference was observed (table 4).

Discussion:

Samplem of infertility etiology is different across the world based on different human,health, geographical and cultural conditions.It is important to determine effective factors on infertility among developing countries.Aim of this study also was to perform this and relationship of some factors such as overweight,smoking cigarette and past disease with sperm indexes didn't exists in it.

In this study in term of sperm count (by considering minimum sperm border of 20 million sperms per liter for fertility, WHO 2010) peoples were divided to two groups and indexes had no significant difference among two groups. No significant impact was reported in term of BMI effect on the sperm quality. In contrast, Guner et al (11) and Poush et al (21) found in their researches that increased BMI negatively effects sperm quality. Douplesis et al (22) in addition to confirm effect of overweight on sperm quality addressed that weight loss had no effect on sperm quality improvement. Pauli et al (23) however also reported low effect. One of the reasons of conflict findings in this study can be definition of natural amount of analysis indexes including numbers, movement and its morphology and at the other hand definition of "infertility" term. At the other hand, effect of overweight on total sperm indexes was assessed in this research. Potential impact of overweight on infertility maybe exists in more specific biological and hormonal area that is hidden in this paper. No negative effect was reported in term of impact of smoking cigarette on sperm quality. Guner et al (11), Ghahremani & Ghaem (7) and Karimpoor et al (6) however, represented different finding. Harmful substances which exist in cigarette smoke impact on hypothalamic generation cycle and prevent enough and high-quality sperm generation (23). Dominated conditions on this study maybe the reason of conflict findings since that according to evidences, sample of infertility etiology can be different based on different human, health, cultural and geographical conditions across the world (6). At the other hand according to low number of smokers in this study (ratio of 50 smokers against 150 nonsmokers) it seems that this conclusion be at the level of this study.In term of impact of physical illness on sperm quality also no negative impact was reported in this study. In many studies (7, 26) this impact was observed. But Karimpoor et al (6) confirmed this finding. Although varicocele was identified as main known infertility factor in men in the past, but according to recent evidences and studies, we cannot certainly confirm relationship between varicocele and infertility, but at least we can point to relationship between varicocele and men infertility.The main evidence to confirm this relationship is more varicocele prevalence among infertile men in contrast with public population. Mechanism of varicocele on men fertility is not specified certainly however this mechanism can be multi-factors and total factors can be effective. By the way, in spite of mechanism of varicocele and based on findings in this study it can be said that this effect was started with Lesion formation at low ages and reaches stable state in fertility ages but after that, fertility condition will not be more serious under that lesion, progressively.

In this study among those who were self-employed, number of peoples who deal with chemicals was more than others that represented effect of these factors on men infertility. Such result also observed in case study by Mendiula et al (28) and review study by Boundi (27). Testicular sensitivity to chemicals effects men fertility process (27). But Inhorn et al didn't confirm such relationship (29). The reason is that environmental facing with chemical purified has not been at deleterious level and higher doses should be observed for men regeneration system to be at risk. At recent decade, there is high tendency of studies toward assessing environmental factors on men infertility. It seems that occupational and environmental factors and also geographical features can be effective on some parameters of infertility. However, knowing how to effect, can be helpful to prevent these factors. Short sample size could be considered as a limitation of the present study because genetic studies, sperm samples and limited laboratory facilities for researchers to consider a larger sample size were not possible.

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Table 1- Socio-demographic characteristics of the two groups of infertile men compared with zero and one (based on sperm count)

Socio-demographic characteristics*	Group 1 (based on sperm count < 20) (n=80)	Group 1 (based on sperm count < 20) (n=116)	†P.Value
Age	34.1±6.4	34.1±5.1	0.9
Marriage age	3.5±1.4	3.7±1.3	0.3
Weight	77.2±12.9	73.7±12.4	0.06
Height	1.75±6.8	1.75±7.4	0.6
BMI	24.1±5.09	23.7±4.06	0.6
Sperm analysis component			
Sperm morphology Sperm motility	22.9±18.2 21.8±13.3	22.6±19.1 19.3±12.3	0.9
Occupation (self-employed)	78(%65.52)	58(%67.28)	0.4
Smoking(yes)	19(%22.61)	31(%26.72)	0.3
History of disease Varicocele			
Orion Hernia Diabetes No	6(%7.14) 4(%4.76) 0(%0) 3(%3.57) 71(%84.52)	16(%13.79) 6(%5.17) 3(%2.58) 6(%5.17) 85(%73.27)	0.2
History of medication	13(%15.74)	24(%20.68)	0.2
History of Surgery	14(%16.66)	29(%25)	0.1
History of sexual disorders	23(%27.38)	41(%35.34)	0.1

*Values based on the mean ± SD and number (percent)

† T test for quantitative variables and the chi-square and Fisher's exact test was used for qualitative variables and the statistical level of less than 05/0P.Value <was considered significant.

Table 2- Comparison of semen analysis parameters in both zero and one(based on smoking or non-smoking)

Semen analysis parameters *	Group 1 (non smoker) (n=150)	Group 2 (smoker) (n=50)	†P.Value
Sperm count (million per Ml)	23.4±19.4	23.3±19.1	0.9
Sperm morphology (percent)	26.1±19.4	21.6±18.5	0.7
Sperm motility (percent)	20.3±12.02	20.9±13.3	0.1

* The values are based on the mean and standard deviation.

Table 3- Comparison of semen analysis parameters in both zero and one (based on patient or Healthy)

semen analysis parameters *	Group 1 (Healthy) (n=156)	Group 2 (patient) (n=44)	†P.Value
Sperm count (million per Ml)	22.4±19.5	26.6±17.5	0.1
Sperm morphology (percent)	21.7±17.8	26.2±21.5	0.5
Sperm motility (percent)	21.1±13.01	19.6±12.9	0.1

*Values based on the mean ± SD and number (percent)
†Based on t test and the statistical level of less than 05/0P.Value <was considered significant.

Table 4- Socio-demographic factors associated with sperm

Confounding factors	B‡	Confidence interval (95%)	P.Value†
Occupation (self-employed)(yes)	3/83	(-0/4 ، 8/09)	0/07
BMI (>25)	0/8	(-3/4 ، 5/2)	0/6
Smoking (yes)	-2/2	(-6/8 ، 2/2)	0/3
Disease (yes)	-0/41	(-5/1 ، 4/2)	0/8
Drug (yes)	-7/1	(-6/61 ، 2/2)	4/0

‡ Regression coefficient that represents the effect of the factor in model.

The beneficial property of hydroalcoholic extract of Annab on burn healing

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Abstract: It has been suggested that Annab (*Zizipus vulgaris* L.) might be beneficial on burn healing, but there is not any evidence in literature for this effect of the plant. This study was carried out to evaluate the effect of hydroalcoholic extract of Annab on burn healing. In this study wounds were made by placing a hot plate with a surface area of 1.5 cm^2 on the back of animals, for 10s. Forty mice were designated in four groups and treated with vehicle or test substance two times per day for 21 days. The first group received nothing, the second group received Vaseline, the third and fourth groups received 1% and 10% Annab ointment two times/day, respectively. The percentage of burn healing and the total time required for complete healing was evaluated. Results showed that 1% dose of Annab extract had significant burn healing compared to control group ($P<0.01$). Annab has considerable effect on burn wounds and its usage might be beneficial in these patients.

Key words: Balb/c mice, Burn, *Zizipus vulgaris* L.

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Keywords: history, Timur, Timurid architecture, architectural style, Khorasan, Transoxiana

Introduction:

Burns and their resulted damages are among the commonest causes of mortality and inability across the world. About 1200000 people are afflicted with burns in America every year that are in need of treatment (1). Healing dermal burns is a process that is conducted by the harmony of different tissues, cells, and factors (2). Among the most important reasons of delay in wound healing, it can refer to remaining inflation or the insufficiency of veins secretions (3).

Although with the introduction of synthetic drugs, the usage of medicinal plants decreased (4, 5), however, the multiple side effects of these drugs caused people to return to medicinal plants (6-8). The present synthetic drugs for burn healing have low efficacy and high adverse effects. Medicinal plants are recently used for different diseases and have made good hope for various conditions (9). Recently, some herbal medicines have shown hopeful activities against burn wounds (10, 11). Traditional use of Ziziphus vulgaris as a therapeutic agent is common among the people in Chaharmahal & Bakhtiari province, and also the anti-inflammatory effects of this plant have been reported in recent years (12). Ziziphus valgaris is a shrubby plant with stretched egg like leaves, large fruits in 1.5 × 2.5 centimeter size, ellipse or almost sphere formed, sweet, and edible that grows in broad areas of Iran (13, 14). Ziziphus vulgaris fruit contains fat acids, beta-carotene, alpha tocopherol, and seven phenolic combinations including catechin, cafeic acid, epicatchin, ferolic acid, rutin, p-hydroxic benzoic acid and chlorogenic acid (15). There is about 69 milligrams of vitamin C in each 100 gram edible part of raw Ziziphus vulgaris (16). In addition, different studies have frequently proved the effects of fat acids and such antioxidants as vitamin C in accelerating wound healing (16, 17). However, no studies have been carried out about the effects of this fruit on the process of burn wound healing. Considering the point that wounds resulting from burns are among the late healable ones and finding natural substances accelerating wound healing with little side effects, can be regarded as a remarkable revolution in treating burn wounds. Consequently, this study was carried out to investigate the effects of hydro-alcoholic extract of Ziziphus vulgaris on the process of burn wound healing in Balb/c mice.

Materials and Methods:

In this experimental study, 40 Balb/c mice with approximate weights of 30±3 grams were chosen. Having made burn wounds of 1.5 square centimeter area on the mice's backs by a hot round metal and got sure of type two burning, they were divided into four groups of control, sham and under treatment by two ointments containing extracts of one and ten percent Ziziphus vulgarises on Vaseline basis. The animals were kept in the conditions of 22-25 ^{0}C temperature, 50% moisture, 12 hour darkness lightness cycle, and usual feeding in separate cages (18).

In order to mark wound, first the animal was anesthetized by injection of Ketamine (50 mg/kg) and Zilazine (50 mg/kg), and then the hair on the back of the animal was removed and the skin thereof was completely cleaned by cotton and alcohol. Afterwards, surface burn wound of type two was made through putting a hot round metal of 1.5 square centimeter area on the fifth vertebra of thoracic for ten seconds. In the groups under treatment by vaseline or 1% and 10% Ziziphus vulgaris ointments, 1 gram of Vaseline or these ointments were respectively rubbed on the wounds twice a day, in such a way that the whole area of wound and some parts of its edges were covered. No substance was rubbed on the control wounds. All wounds were remained undressed and open. Microbiological experiments conducted on the utilized ointments showed that they were devoid of any microbial agents (19).

Having made the wound until the complete betterment, wounds were taken photos on 1st, 7th, 14th and 21st days after the animals' anesthesia. Photo taking conditions were the same during the whole experiment period. Using the taken pictures and also video image analysis software, wound area, and percentage of betterment on different days were calculated based on the following formula:

Wound percentage= wound area on the intended day/ wound area on the first day×100

And also, betterment percentage=100- wound percentage.

In order to compare the groups generally, first Kruskal wallis test and then Dan test were carried out by SPSS software. The level of $p<0.05$ was considered statistically significant in the study.

Results:

The percentage of wound betterment on 7th, 14th, and 21st days in groups of control, were 8.06%, 36.47%, and.92.97%, Vaseline 12.57%, 13.90%, and 97.69%, 1% Ziziphus vulgaris ointment 30.52%, 96.32%, and 99.96%, and in 10 % Zizaphus vulgaris oimtment group12.86%, 75.06%, and 99.38%, respectively.

According to the test of Kruskal wallis, the wound betterment percent was significant among the groups ($p<0.001$). Using the test of Dan, the percentage of wound betterment in the group of 1% Zizaphus vulgaris was higher than the control ($p<0.01$) and Vaseline groups ($p<0.05$) (Table 1).

Table 1: Comparison of the betterment percentage the under study groups on the basis of the test of Dan

Groups	Rank Difference	P Value
Control with Vaseline	-33.3	p>0.05
Control with 1% Zizaphus vulgaris	-36.67	p<0.001
Control with 10% Zizaphus vulgaris	-20	p>0.05
Vasaline with 1% Zizaphus vulgaris	-33.33	p<0.001
Vaseline with 10% Zizaphus vulgaris	-16.67	p>0.05

Discussion:

Healing results in this study were indicator of the superiority of the under treatment groups with 1% Zizaphus vulgaris over the control and 10% Zizaphus vulgaris groups.

Factors that bring forth inflammation reduction and disinfection have positive effects on burn betterment and healing (20-21). Disinfectant and anti-inflammatory effects of Zizaphus vulgaris have been observed in the other studies, so it may be said that the disinfectant and anti-inflammatory effects of Zizaphus vulgaris have positive effects on burn betterment and healing.(22-23)

Studies conducted on Zizaphus vulgaris fruit have shown 9 fat acids, 2 Saponins, lots of vitamin C, alpha tocopherol, and 7 phenolic compounds including catechine, cafeic acid, epicatechine, ferolic acid, Rutin, p-hydroxicbenzoic acid, and chlorogenic acid (9,19). On the other hand, it was known based on the conducted studies that fat acids cause the increase in collagen synthesis and also acceleration in wound healing through increasing the level of interleukin 6 (24). Accordingly, the existence of such fat acids in Zizaphus vulgaris is considered a positive agent, and also as the cause of the anti-inflammatory property of olive oil is known due to the existence of fat acids inside that can be replaced by arachidonic acid in cells membranes and decrease the substrate needed for the inflammatory enzymes, some parts of its anti-inflammatory effects can be justified for Ziziphus vulgaris plant, too. Miscellaneous studies have

frequently proved the strong antioxidant effects of vitamins A and C in accelerating the wound healing (16,25), and as it was mentioned there are such other antioxidants besides vitamin C in Ziziphus vulgaris as fat acids, alpha tocopherol, b-caroten, and phenolic compounds like ferolic acid. On the other hand, the study carried out by John et al. shows that the existence of antioxidant vitamins C and E as well as ferolic acid together have better protective effects against the dermal damages resulting from the ultra-violet ray (26). Perhaps, it can also be said in our study that the adjacency of vitamin C and ferolic acid in Ziziphus vulgaris fruit has been effective in wound healing acceleration. In addition, the existence of alpha tocopherol, vitamin C, and polyphenols together have synergistic effects on their antioxidant properties (27), and it is perhaps the existence of these three substances together in Ziziphus vulgaris has been effective on wound healing acceleration. Ivoone et al. stated in their study that such antioxidants as vitamins C and E, carotenoids, and felarenoids_take peroxidant property under special conditions and high dosages (28). Since Ziziphus vulgaris consists also of these vitamins, carotenoids, and the other antioxidants, the better effect of 1% Zizaphus vulgaris than the 10% one may be justified. Meanwhile, some studies have revealed vitamin C has antioxidant and peroxidant properties in the concentrations of 60 to 100 micromollars as well as lower or higher than this range, respectively (29). Furthermore, it has been expressed in studies that the effects of α-tocopherol_antioxidants, scorbic acid, and beta- caroten decrease and their peroxidant properties increase under the circumstances they contain a lot of oxygen (30-31).

Conclusion:

Considering the fact that Ziziphus vulgaris fruit contains high volumes of unsaturated faty acids, vitamins A and C, ferolic acid, α-tocopherol, and betacaroten, as well as the remarkable treating effects of such combinations in improving inflammation, the theory of synergistic effects of vitamins C and E antioxidants and polyphenols together, and also the wide availability of Ziziphus vulgaris in Iran with cheap prices, it was attempted in the current study to investigate and examine the ointment taken out of Ziziphus vulgaris to be used as an ointment effective on burn healing. However, this important point should be taken into consideration that the healing effect of Ziziphus vulgaris decreases in the case the dosage of its extract increases. Accordingly, it seems the other concentrations of this extract should be studied to reach better effects and results.

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An Investigation of the Role of Continuous Improvement Excellence Model

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Abstract: The current survey investigates the continuous improvement excellence model view points of staff in this company. The research method was a descriptive survey and the statistical population consisted of all the staff of Company. (2584 people) out of which 334 were selected as statistical sample, using random clustered sampling and considering the number of population. The research instrument was a researcher made questionnaire using 5-scale Likert and including 32 statements. The validity of the questionnaire was substantiated by specialists and also a number of the participants. The reliability of the questionnaire was substantiated by specialists and also a number of the participants. The reliability of the questionnaire proved to be 0.96 using Cronbach's Alpha coefficients. To analyze the research data, the descriptive statistics consist of abundance, percent, average, standard deviation and the deductive statistics include single variable test and manova analysis, are used. The findings revealed that role of continuous improvement excellence model the leadership, strategy, people, partnerships resources, processes products services customer results, people results, key results, society results were more than average ($p<0.05$), in regard with demographic data amount, no significant difference was seen in staffs points of view (gender, professional background, field of study and position).

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Keywords: Management system, Continuous improvement, Model of Excellence, Staff

1- Introduction

Ultimate goal of each human system is moving toward development, evolution and excellence, and its evolution and excellence depend on growth, development, expansion and evolution of its components (Jafari Ghoushchi, 2005). In today world, profound changes in innovative management systems and methods have occurred and completely different attitudes have governed on managing organizations along with dramatic changes which have taken place in social, economic and technological dimensions. Such concepts which find a significant place in international organizations are quality, continuous improvement and model of excellence of organizations (Riahi, 2005). In the literature, the word "quality" has different meanings. Piterzo & Waterman (1982) defined quality as excellence. Fagnbam (1983) defined it as value, Grinal & Juran (1988) defined it as applicability, and Gronorous defined it as customer satisfaction. These definitions demonstrate different aspects of quality. Some famous thinkers such as Doming, Piterzo and . . . claim that quality is the basis of excellence (Sharma & Talwar, 2007). Effort to propose a total definition was started from 1982 by publishing a book called *seeking excellence* by Piterzo & Waterman (Park & Dahlgaard, 2007). Excellence was a word which replaced some words such as quality and quality management. The reason of this replacement was the existence of a lot of ambiguity in the word "quality management' (Adbanjo, 2008). Model of excellence, the basis of quality management, as a model of quality determines some aspects that organization should consider to improve results as well as characteristics of some results that should be considered to obtain excellence through enabling criteria. This model proposes a pattern of relations between enablers and results as well as criteria forming each field (Boul-llusar et al, 2005). Model of excellence include different elements of total quality management in which some bases have been designed to show processes of analysis and change in organization. Outlook of the model is a new action-based method which is based on a systematic principle and structure of thought (Castilla & Rosi, 2008). The model of organizational excellence is useful for promoting quality improvement (Vallejo & Saura, 2006). Also, it is a recognition tool to self-evaluate current health of organization (Mir Fakhrodin et al, 2009). On the other hand, evaluation by helping qualitative systems such as total quality management and the model of

organizational excellence is raised as one of the methods to evaluate performance of organizations and rewards of organizational excellence and quality draw attentions of many organizations and also have helped to clear concepts and key components of quality management (Dale et al, 1997). Using these models exceeds different manufacturing and service sectors and involves public organizations (Johan et al, 2007). Model of excellence (EFQM) is suitable to identify problems of organization but does not any plan and policy for organization (Nazemi, 2008). Also, the other weakness of this model is the problem of making it operating, since terms and concepts used in this model is so general that can be interpreted in different ways and every organization will be enable to create different indicators of evaluation by this headings (Neely et al, 2000).

Kaye & Dayson (1999) believe that this model should be proportionate with the type of organization and is not applicable in any organization but unlike this idea, EFQM founders believe that the model is applicable for all organizations. In past, this model is strange for all service and voluntary organizations because it was rooted in business structure. But now, it has been proved that this model can be a strong managerial tool for such organizations (Donelly, 2000). Of course, implementing the model in a service environment is always difficult (Pitt, 1999). Maybe, it can be said that one of the weaknesses of this model is implementing it in a service environment because it does not consider all related parts in this regard. The other problem to implement this model in service organizations may happen is that the model is complicated and ambiguous for such environments. These are because EFQM is a general model and such model cannot cover professional aspects of an organization (Vallejo & Saura, 2006). However, so far this model has been used in service and health environment with some modifications. Studies show that application of this model is successful in organization of private sector that organization of public sector (Osseo, 2002).

These models are based on values of total quality management and do not solely rely on profit. But it also pays attention to other stimuli and citizens enablers, social responsibilities, human resources, social and personal promotion and excellence and protecting environment (Garvara & Isaksoon, 2001).

This model is based on 9 criteria. Five criteria of the model are related to enablers (leadership criteria, staff criteria, strategy criteria, companies and resources criteria, processes criteria, services and products) which represent components of an organization and how they interact with each other and four criteria are related to results (staff results criteria, costumers results criteria, society results criteria, key results criteria) which include results from organization performance and introduce desired results from implementing enablers (Gerami & Nouralizadeh, 2008).

In the studying service company, an evolutionary way has taken place according to huge policies of service companies which consider evolution and using innovative management patterns and serving people in order. And this company is known as one of the pioneered service companies in including and using innovative management systems and the model of excellence. But in recent years, despite including quality systems such as series of ISO 9000 and some models like organizational excellence and …; what has been obtained is expressing unsatisfactory by staff and directors from including these kinds of systems, in fact, quality systems and models of excellence could not meet staff and service receivers' expectations, adequately. Identifying methods to improve service quality in different areas based on the model of excellence is one the ways through which it can help continuous improvement of service quality in this organization. With respect to what was said, present study tries to investigate the role of some strategies of continuous improvement of excellence mode based on nine-tipple dimensions of the model.

The most important application of the model (EFQM) is doing self-evaluation and identifying improvable fields on an organization (Adel & Tavakoli, 2006). In addition, (EFQM) is an organizational change model. Creating change, regardless of what extent it is profitable, is always difficult. Therefore, it cannot be denied that doing change has always some challenges and faces to some obstacles (Irannejad Parizi & Sasan Gohar, 2003).

Finally, basic indicators of the model (EFQM) constitute the following factors:

- Results: results which have been obtained by organization. (What?)
- Statue of organization and management (How?) (Wester vel, 2003).

Generally, the importance of research can be known in investigating strategies of continuous improvement of innovative management systems with a standard model which is accepted globally. Thus, individual way of successful to come over on current challenges of globalization is organizing organizational plans based on efficiency principles. A plan which focuses is on making efficiency of how to use resources. In this regard, many studies have been conducted on identifying and distributing key factors of organizations success in order to improve their performance which resulted in some national rewards and models of excellence such as Doming and Baldrij Business, and (EFGM). According to what was

explained, the studying service company put the necessity of implementing a broad study in this regard in order to improve serving stakeholders of the company.

Riahi (2009) conducted a study as "investigating factors influencing efficiency of total quality management in 5 governmental ministries located in Tehran". This study aims to investigate the relationship between efficiency factors of total quality management and measuring efficiency to determine their preferences.

Statistical community used in this study, is considered as managers, supervisors and experts of these five governmental ministries in Tehran and also their customers. The results showed that:

1. Attendance of organizations in public sector to human dignity while serving people causes to increase the level of their satisfaction.
2. Trusting customers as an accepted norm in organization and trusting their words cause to increasing their trust on organization services, although organization has to pay the costs of this trust.
3. Accuracy of organization actions, managers' efforts to reduce errors in organization and creating motivating systems to serve customers correctly cause to increase the expected efficiency.
4. Presence of knowledgeable and empowered staff, staff's educations related to services which they serve, and existence of training courses and increasing staff's knowledge and skill cause to increase the expected efficiency.
5. Accountability of organization to people, paying attention to customer satisfactions and commitment to accountability of managers to people demands cause to increase the expected efficiency.

A study was conducted by Mojdehi as "evaluating Office of Standards and Industrial Research of Iran of Sistan-o-Balouchestan Province according to the model of organizational excellence (EFQM) and Iran National Quality Award (INQA) " in order to evaluate Office of Standards and Industrial Research of Iran of Sistan-o-Balouchestan Province by using the model of excellence. By comparing the results from the model (EFQM) and (INQA), it was observed that it should be necessary to pay attention to final product, because process standards are not fully implemented in Iran.

In a study, Dehnavieh et al (2011) titled "the obstacles of using Iran National Quality Award in medical sciences of Iran and giving a strategy" concluded that the most important obstacles included special characteristics of university environment, weakness in performance of top managers, weak participation, weakness in innovation, weakness in communication, weakness in making beds and stabilizing improvement process, weakness in allocating resources and problems coming from environmental factors.

Tari (2005) found in a study titled "using the model of excellence (EFQM) in universities of Spain" with the aim of self-evaluation model of European excellent model in five universities of Spain that increasing management commitment, planning for self-evaluation, forming some teams for training and self-evaluating, determining modifications, implementing modifications and reviewing are some steps that a university can take to reach better performance.

In the model of organizational excellence (EFQM), empirical observations suggest using self-evaluation model to identify improvable areas and evaluate company performance. This study states that paying attention to these items is important for successful of organizations in the way of excellence.

1. Continuous improvement tasks are not only for organization management team, but also staff should be trained and empowered to participate in the improvement process.
2. Staff should be aware of effects and costs coming from giving incomplete information in continuous improvement attempts.
3. Staff should be aware of concepts of quality management and tools to implement quality in organization.
4. Managers should increase abilities and capacities and pay more attention to measuring process and performance of enablers, instead of paying attention only to measuring key results of the performance.

Jung & Vang (2006) conducted a study titled "investigating the relationship between total quality management and continuous improvement of international project management", the results showed that staff's relationships (including empowerment/staff interference, human issues, clear relationships, broad organizational trainings) have had the most effects in reaching continuous improvement of international project management. Leadership factor (including commitment of top managers to quality, existence of goal and strategy, existence of broad culture of organizational quality, existence of goals for quality actions) has been the second factor influencing improvement of international project management.

2-Study questions

1. To what extent continuous improvement strategies of excellence model in field of leadership plays a role, in the service company's staff viewpoint?
2. To what extent continuous improvement strategies of excellence model in field of strategy plays a role, in the service company's staff viewpoint?
3. To what extent continuous improvement strategies of excellence model in field of staff plays a role, in the service company's staff viewpoint?
4. To what extent continuous improvement strategies of excellence model in field of companies and resources plays a role, in the service company's staff viewpoint?
5. To what extent continuous improvement strategies of excellence model in field of processes, services and products plays a role, in the service company's staff viewpoint?
6. To what extent continuous improvement strategies of excellence model in field of customers' results plays a role, in the service company's staff viewpoint?
7. To what extent continuous improvement strategies of excellence model in field of staff's results plays a role, in the service company's staff viewpoint?
8. To what extent continuous improvement strategies of excellence model in field of society results plays a role, in the service company's staff viewpoint?
9. To what extent continuous improvement strategies of excellence model in field of key results plays a role, in the service company's staff viewpoint?
10. Is there any significant difference between responders' opinions with respect to demographic factors (gender, age, level of education, major, work experience, type of employment)?

3-Methodology, community, sampling and methods of analyzing data of the study

Method of the present study is descriptive – survey. Statistical community of this study includes all staff of the company in number of 2584, of which 334 people were selected by using Coockran's formula and stratified random sampling. The tool for gathering data was researcher made questionnaire on investigating strategies of continuous improvement of excellence model includes 32 questions in 9 fields of leadership, strategy, staff, companies and resources, process and services and products, customers' results, staff's results, society result and key results in Likret's 5-degree scale. Formal validity and content validity of the questionnaire were verified by subjects, and experts in the field of excellence model and also consultants, respectively. Perpetuity of the questionnaire was estimated 0.96 by using Croonbach's Alfa coefficient. Inferential statistics including t-variable test and analyzing multi-way variance were used to analyze the data.

4-Analyzing and interpreting findings

Question 1: To what extent continuous improvement strategies of excellence model in field of leadership plays a role, in the service company's staff viewpoint?

Table (1-4): comparison of mean score of leadership with hypothetical average 3

Item	Mean	Standard derivation	Derivation of the mean	t	Degree of freedom	Level of significance
Field of leadership	3.14	0.68	0.040	3.484	287	0.001

According to findings in table (1-4), mean score in leadership of the service company is 3.14. Calculated t is larger than t on the table. So, the role of continuous improvement strategies in the model of excellence in leadership is more than the average level.

Question 2: To what extent continuous improvement strategies of excellence model in field of strategy plays a role, in the service company's staff viewpoint?

Table (2-4): comparison of mean score of strategy with hypothetical average 3

Item	Mean	Standard derivation	Derivation of the mean	t	Degree of freedom	Level of significance
Field of strategy	3.18	0.54	0.32	5.74	287	0.001

According to findings in table (2-4), mean score in strategy of the service company is 3.18. Calculated t is larger than t on the table. So, the role of continuous improvement strategies in the model of excellence in strategy is more than the average level.

Question 3: To what extent continuous improvement strategies of excellence model in field of staff plays a role, in the service company's staff viewpoint?

Table (3-4): comparison of mean score of staff with hypothetical average 3

Item	Mean	Standard derivation	Derivation of the mean	t	Degree of freedom	Level of significance
Field of staff	2.99	0.63	0.037	0.180	287	0.858

According to findings in table (3-4), mean score in staff of the service company is 2.99. Calculated t is larger than t on the table. So, the role of continuous improvement strategies in the model of excellence in staff is more than the average level.

Question 4: To what extent continuous improvement strategies of excellence model in field of companies and resources plays a role, in the service company's staff viewpoint?

Table (4-4): comparison of mean score of companies and resources with hypothetical average 3

Item	Mean	Standard derivation	Derivation of the mean	t	Degree of freedom	Level of significance
Field of companies and resources	3.14	0.69	0.041	3.50	287	0.001

According to findings in table (4-4), mean score in companies and resources of the service company is 3.14. Calculated t is larger than t on the table. So, the role of continuous improvement strategies in the model of excellence in companies and resources is more than the average level.

Question 5: To what extent continuous improvement strategies of excellence model in field of processes, services and products plays a role, in the service company's staff viewpoint?

Table (5-4): comparison of mean score of processes, services and products with hypothetical average 3

Item	Mean	Standard derivation	Derivation of the mean	t	Degree of freedom	Level of significance
Field of processes, services and products	3.18	0.62	0.036	4.901	287	0.001

According to findings in table (5-4), mean score in processes, services and products of the service company is 3.18. Calculated t is larger than t on the table. So, the role of continuous improvement strategies in the model of excellence in processes, services and products is more than the average level.

Question 6: To what extent continuous improvement strategies of excellence model in field of customers' results plays a role, in the service company's staff viewpoint?

Table (6-4): comparison of mean score of customers' results with hypothetical average 3

Item	Mean	Standard derivation	Derivation of the mean	t	Degree of freedom	Level of significance
Field of customers' results	3.21	0.72	0.042	5.063	287	0.001

According to findings in table (6-4), mean score in customers' results of the service company is 3.21. Calculated t is larger than t on the table. So, the role of continuous improvement strategies in the model of excellence in customers' results is more than the average level.

Question 7: To what extent continuous improvement strategies of excellence model in field of staff's results plays a role, in the service company's staff viewpoint?

Table (7-4): comparison of mean score of staff's results with hypothetical average 3

Item	Mean	Standard derivation	Derivation of the mean	t	Degree of freedom	Level of significance
Field of staff's results	3.22	0.68	0.040	3.658	287	0.001

According to findings in table (7-4), mean score in staff's results of the service company is 3.22. Calculated t is larger than t on the table. So, the role of continuous improvement strategies in the model of excellence in staff's results is more than the average level.

Question 8: To what extent continuous improvement strategies of excellence model in field of society's results plays a role, in the service company's staff viewpoint?

Table (8-4): comparison of mean score of society's results with hypothetical average 3

Item	Mean	Standard derivation	Derivation of the mean	t	Degree of freedom	Level of significance
Field of society's results	3.02	0.67	0.039	0.532	287	0.595

According to findings in table (8-4), mean score in society's results of the service company is 3.02. Calculated t is larger than t on the table. So, the role of continuous improvement strategies in the model of excellence in society's results is more than the average level.

Question 9: To what extent continuous improvement strategies of excellence model in field of key results plays a role, in the service company's staff viewpoint?

Table (9-4): comparison of mean score of key results with hypothetical average 3

Item	Mean	Standard derivation	Derivation of the mean	t	Degree of freedom	Level of significance
Field of key results	3.15	0.67	0.039	3.995	287	0.001

According to findings in table (9-4), mean score in key results of the service company is 3.15. Calculated t is larger than t on the table. So, the role of continuous improvement strategies in the model of excellence in key results is more than the average level.

Question 10: Is there any significant difference between responders' opinions with respect to demographic factors (gender, age, level of education, major, work experience, type of employment)?

Table (10-4): multiple-way variance analyzing scores by using the model of excellence in terms of some variables such as gender, age, level of education, major, work experience, type of employment

Source	Sum of squares	Degree of freedom	Mean squares	F	Level of significance	The Eta	Statistical capacity
Gender	0.051	1	0.051	0.158	0.691	0.001	0.068
Age	0.079	3	0.026	0.082	0.970	0.001	0.065
Education	0.436	4	0.109	0.339	0.851	0.007	0.126
Work experience	1.767	4	0.442	1.372	0.245	0.026	0.423

The results in table (10-4) show that the scores of level of using excellence model in service company in terms of variables such as gender, age, education level and work experience are not significant.

5-Discussion and Conclusion

The field of leadership is one of the most important and the most determinant issues in directing organizations. The best asset of a leader is the capacity to help staff in order to direct them. A flexible accountable and finally excellent organization is one which found the capacity to direct in all areas and extended to all levels. In the field of leadership, excellence model determines vision and mission of the organization and facilitates access to it and generates required values for long run success and uses them through suitable actions and behaviors and ensures creating and using management system, by its own. According to what was said, the role of leadership in continuous improvement strategies of excellence model will be explainable. These are strategy, integrated, comprehensive, coordinative

plans which relate organization with its environmental challenges and are based on current and future needs and expectations of stakeholders. This filed was established and known through a framework of key processes. Finally, it determines the process of achieving situations desired by organization. Considering excellence pattern of using policy of organization is a method in which organization achieves its goals through a concentrated clear strategy. In fact, organization's strategy is rally organized. Research's interpretation is that quick changes in technology, shifting the world to a global village, different customers with many needs, increased competition in global markets, ongoing changes in organizations, revolutionize organization's way. Hence, organization's policy and strategy have an important role in this case, therefore the role of strategy in continuous improvement of excellence model will be explainable. Staff is fundamental base of an organization; they are full of inspiration, creativity and motivation in the organization and survive it. Today in this competitive world in which industries should be more efficient, using staff more intelligently will lead them to generate more values for their organization. The model of organizational excellence emphasizes that increasing staff's skills and training is critical in utilizing new technologies and creating necessary changes, the other important remark is that staff's dignity and worth have a critical role in organization's success; hence keeping structural – organizational integration is a major management challenge to allow staff being committed and responsible with adequate motivation and authorities and continue job in a correct framework. In model of excellence, staff criteria is third factor of organization empowerment and has a special importance so that excellent organizations direct, develop and utilize all their staff's potential ability in individual, team and organizational levels. They promote fairness and equality, allow their staff to participate in the affairs and empower them. Such organizations pay attention to their staff, communicate and encourage them so that create motivation and commitment to use required skills and knowledge in direction of organizational benefits. According to what was said, also

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Relationship between human resources development and knowledge management value chain

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Abstract: Present research has analyzed the relationship between human resources development (Employee empowerment, Employee participation and Employee training) and KMVC by descriptive method in correlation type. 1221 employees of one of the Iranian Steal Company with B.S degree and upper took part in the statistical universe of this survey. 232 people were chosen by using systematic sampling method and the sample size formula. To collect data, two researcher-made questionnaires of KM and HRD have been used. Also content validity and face validity of questionnaires in this research were confirmed by experts. The validity of both questionnaires was estimated using Cronbach's alpha coefficient (α) which equals to 0.87 in KM and 0.83 in HRD. The result has shown that there is a relationship between KM and employee participation (r = 82%, α= 0.001), a relationship between KM and employee empowerment (r = 76%, α = 0.001) and a relationship between KM and employee training (r = 63%, α = 0.001).

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Keywords: empowerment, human resources development, knowledge management value chain, participation, training.

I. INTRODUCTION

NOWADAYS, exponential increases in data volumes are increasingly viewed as important and essential sources of information that may eventually be turned into knowledge [1], so that knowledge is increasingly claimed to be a key critical resource and source of competitive advantage in the modern global economy, especially with the rise of the service economy, the growth in the number of 'knowledge workers', and the growing recognition of the importance of intellectual capital and intellectual property rights [2]. Although a firm has access to the knowledge, skills and expertise of employees, it also needs knowledge management mechanisms in place to ensure effective utilization human capital. Knowledge management is an approach to adding or creating value by more actively leveraging the know-how and experience resided in individual minds [3], [4], [5]. Consequently, HRM activities and program must focus on instilling, improving, and evaluating knowledge, skills, and abilities of human assets [6]. Examining the relationship between KM and human resource development activities may lead to increasing organizational efficiency and effectively. The purpose of this paper is to identify the relationship between human resource development activities and KM activities.

II. LITERATURE REVIEW

As the world is becoming more competitive and unstable than ever before, manufacturing-based industries are seeking to gain competitive advantage at all cost and are turning to more innovative sources through HRM practices [7]. Human resource management (HRM) is an inevitable process that accompanies the growth of organizations [8]. The overall purpose of HRM is to ensure that the organization is able to achieve success through people. HRM system can be the source of organizational capabilities that allow firms to learn and capitalize on new opportunities [9]. And to this end Human resource development (HRD) activities are intended to ensure that organizational members have the skills or competences to meet current and future job demands [10]. Strategic human resource development involves introducing, eliminating, modifying, directing and guiding processes in such a way that all individuals and teams are equipped with the skills, knowledge and competences they require to undertake current and future tasks required by the organization [11]. HRD includes: paying attention to change management, to combining learning processes, KM, job development, team making, instructional technology, good job relations, information technology and HRD relation, leadership development and interference strategy [12]. In this survey, three tools such as staff participation, staff empowerment and staff training have been analyzed as the necessary tools to develop human resources in organization.

Training: is a useful investment and a key factor in development and If it is planned and applied well, will have a remarkable economic output. Staff training is a helpful action that can give credit to individual and in organizational level causes

improvement and development to the organization and also in national and transnational level can increase productivity. So we can say one of the basic actions that causes efficiency to organization, is human resource development via training and its improvement continuously. Training and improvement of human force give abilities to the individuals to continue their activities effectively according to organization and environmental changes and increase their productivity and efficiency. So Training and improvement is a continued and planned attempt by management to develop staff competency and organizational operation [13].

Empowerment: is the process of enhancing the capacity of individuals or groups to make choices and to transform those choices into desired actions and outcomes. Central to this process are actions, which both build individual and collective assets, and improve the efficiency and fairness of the organizational and institutional context which govern the use of these assets. It identified empowerment as "the expansion of assets and capabilities of the people to participate in, negotiate with, influence, control, and hold accountable institutions that affect their lives [14].

Participation: there are different definitions about participation that all show the role play, giving ideas and recommendations, issuing solution, work development and improvement. "Employee participation" is a partnership process that aims to stimulate and encourage staff for more commitment and collaboration in organization success [15]. Employee participation embraces employees in the organizational decision making on a collective basis [16].

A number of scholars, such as [17], [18], and [19] have argued on the missing link between HRM practices and organization outcomes. Reference [20] have recognized Knowledge management as the fundamental activity for obtaining, growing and sustaining intellectual capital in organizations and an intervening mechanism between organizational factors and organizational outcomes. Knowledge Management (KM) is an effort to increase useful knowledge within the organization. Ways to do this include encouraging communication, offering opportunities to learn, and promoting the sharing of appropriate knowledge objects or artifacts [21]. Knowledge management is a multidisciplinary approach that takes a comprehensive, systematic view to the information assets of an organization by identifying, capturing, collecting, organizing, indexing, storing, integrating, retrieving, and sharing them. Such assets include (1) explicit knowledge, such as databases, documents, environmental knowledge, policies, procedures, and organizational culture; and (2) the tacit knowledge of the organization's employee, their expertise, and their practical work experience [22].

The goal of KM is to improve tasks and sub-tasks, in most cases the creation or generation; acquisition; identification or capture; validation and evaluation; conversion; organization and linking; formalization or storage; refinement or development; distribution, diffusion, transfer or sharing; presentation or formatting; application and evolution of knowledge, with the help of systematic interventions, instruments or measures [23], [24], [25], [26], [27], [28], [29], [30].

The Art and science of KM is a frame for designing continuous systematic activities to make effective organizational decisions. In this field, KM is a strategic process by the goal of separating organizational from competitors and outstrips from their competitive advantages. To reach to this goal, the organization activities should be organized to create an appropriate model for KM [31]. According to [32], potential sources of competitive advantage are everywhere in the firm. To highlight the idea that competitive advantage grows fundamentally out of the value a firm is able to create for its customers [33] integrate different terminologies used by some authors in describing the KM process and aggregate their work as a simple KM value chain in Fig. 1 the KM value chain is divided into four activities knowledge creation, knowledge storage, knowledge distribution and knowledge application.

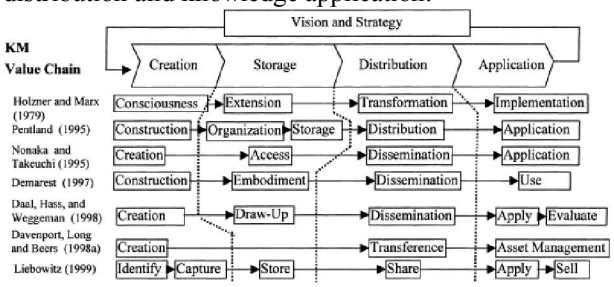

Fig. 1 Knowledge Management value chain

These four activities are defined as a chain to create value for organization via knowledge [33]. Although different researchers have suggested different models to perform knowledge management system in the organization, all of them originate from these four levels. Nowadays the rapid growth of technology has led to an economy where competitive advantage is increasingly based on the successful application of knowledge [34]. The emphasis on HRD is also broadening to a focus on developing themes and creating environments conducive to learning, as well as to the acquisition and creation, sharing and dissemination and application of knowledge within organizations [35].

For instance employee participation is one of key factors in successful KM implementation because the nature of knowledge creation and sharing is unthinkable without employee participation, in addition employee training which can transfer tacit knowledge into explicit knowledge through education, organizations must build employee skills, competencies, and careers, creating "bench strength" [36], and also effective creation and sharing of knowledge will fail if employees do not have a sense of ownership in the overall aim of the organizational KM project so through empowerment, employers can value their employees' expertise and help them communicate their knowledge by creating ways to capture, organize and share knowledge [37].

The resource based view of the firm suggests that organizations will need to be able to combine distinctive, sustainable and superior assets, including sources of knowledge and information, with complementary competencies in leadership and human resource management and development to fully realize the value of their knowledge [38]. HRD in organizations should be structured to promote knowledge creation and mobilization, and how to develop a culture and set of HRM policies and practices that harness knowledge and leverage it to meet strategic objectives [2]. Obviously KM is first and foremost a people issue. The success of KM initiatives depends upon people's motivation, their willingness, and their ability to share knowledge and use the knowledge of others. People in organization, processes and technology will at all times be acting as either enablers of, or barriers to, effective KM practices. Barriers need to be identified and removed. Existing enablers also need to be enhanced and additional ones created. This is often where the greatest KM challenges lie [39]. Consequently, according to the broad goals of HRD in organization, it can help organization to reach its goals along with KM. In the other words, by integrating KM and HRD together, it is possible to follow up the ultimate goals of organization which called the organizational excellence.

III. THEORETICAL FRAME OF RESEARCH

Knowledge management and human resource management are two significant issues in organizations management. In spite of wide literature about both of them, applying them still has been a challenge. When the knowledge capitals of an organization are mentioned, in fact human resources are supposed as the knowledge capitals. So in this situation, human resource systems have to be stated so that support learning environment and have harmony with organizational knowledge management system to respond organization knowledge needs and operate organizational goals. So, present research has tried to survey the relation between HRD (including staff empowerment, staff training and staff participation) and knowledge management value chain in one of the big Irainian industrial companies.

IV. RESEARCH METHODOLOGY

Present research has analyzed the relationship between human resources development (Employee empowerment, Employee participation and Employee training) and KMVC by descriptive method in correlation type. 1221 employees of one of the Iranian Steal Company with B.S degree and upper took part in the statistical universe of this survey. 232 people were chosen by using systematic sampling method and the sample size formula. To collect data, two researcher-made questionnaires of knowledge management and human resources development have been used. Also content validity and face validity of questionnaires in this research were confirmed by experts. The validity of both questionnaires was estimated using Cronbach's alpha coefficient (α) which equals to 0.87 in knowledge management and 0.83 in human resources development.

V. HYPOTHESES

A. There is a relation between employee empowerment and knowledge management value chain in organization:

TABLE I
CORRELATION COEFFICIENT BETWEEN EMPLOYEE EMPOWERMENT AND KNOWLEDGE MANAGEMENT VALUE CHAIN

Predictor variables	Correlation Coefficient	r^2	α
Knowledge Creation	0.614	0.377	0.001
Knowledge Storage	0.658	0.433	0.001
Knowledge Distribution	0.674	0.454	0.001
Knowledge Application	0.785	0.616	0.001
KM (Total Mark)	0.767	0.588	0.001

P<0.01

Findings table I show that the correlation coefficient between employee empowerment and KMVC and its subscales in ($p<0.01$) level is meaningful ($r= 0.767$). It means that there is a meaningful relation between employee empowerment and KMVC and its subscales. According to determination coefficient (r^2) 58.8% variance of employee empowerment and KMVC was common. So the first theory that says "there is a relation between employee empowerment and knowledge management value chain" is confirmed.

The outcome is matched with [40]. They concluded that the role of applied KM in ISACO Company regarding to three indicators such as individual, group or organizational abilities is 68%

TABLE III
CORRELATION COEFFICIENT BETWEEN EMPLOYEE PARTICIPATION AND KNOWLEDGE MANAGEMENT VALUE CHAIN

Predictor variables	Correlation Coefficient	r^2	α
Knowledge Creation	0.727	0.528	0.001
Knowledge Storage	0.656	0.430	0.001
Knowledge Distribution	0.736	0.542	0.001
Knowledge Application	0.802	0.643	0.001
KM (Total Mark)	0.826	0.682	0.001

(3.39 out of 5) from the responders view. So we can conclude that the applied KM in this company has been successful and increased individual, group or organizational abilities. Also this finding is matched with the result of [41] that shows in general level, there is a meaningful relation among transferring implicit knowledge and capability feeling on employee in decision making, taking responsibility of decision making in employee, Their access to related tools for decision making, implementation and finally accepting the responsibility of employee decision result, and its correlation coefficient is 0.6. This survey's results are not matched with [42] results. Because their research shows that there is a trivial relation between employee empowerment and knowledge creation, knowledge storage and knowledge distribution. Empowerment is one of the related factors to KMVC. In modern organizations, organizational knowledge is supposed as a persistent competitive advantage resource and whole KM pays attention to apply present knowledge in organization which results in organizational benefit. In the other hand the importance of knowledge doesn't mean that it is applied all the times in organization activities. So employee empowerment as a strategic and important resource in organizations is caused to persistent development and access to goals, in global approach. If KM process is organized and designed well in an organization, but the employees can't use this new management system, the entire manager's attempts will be useless. Employee empowerment is a factor that facilitates using the KM project and develops the organization. So we can determine the relation between employee empowerment and KMVC.

B. There is a relation between employee training and knowledge management value chain in organization

Findings table II show that the correlation coefficient between employee training and knowledge management value chain and its subscales in ($p<0.01$) level is meaningful ($r=0.634$). It means there is a meaningful relation between employee training and KMVC and its subscales. According to determination coefficient (r^2), 38.9% variance of employee training and KMVC was common. So the second theory of the research is confirmed.

The outcome is matched with the result of [31] that shows there is a meaningful relation in the role of quality management in human resource training and making KMVC in organization. Also it is matched with the outcomes of [5] that shows there is a positive meaningful relation between employee training and knowledge acquisition and also between employee training and knowledge application.

TABLE II
CORRELATION COEFFICIENT BETWEEN EMPLOYEE TRAINING AND KNOWLEDGE MANAGEMENT VALUE CHAIN

Predictor variables	Correlation Coefficient	r^2	α
Knowledge Creation	0.558	0.346	0.001
Knowledge Storage	0.506	0.256	0.001
Knowledge Distribution	0.557	0.310	0.001
Knowledge Application	0.618	0.382	0.001
KM (Total Mark)	0.634	0.389	0.001

Human capital with its knowledge, proficiency and skills is a valuable resource for organizations. Modern organizations which apply and manage the knowledge and proficiency of people mind continuously and effectively, are able to make more value and achieve better competitive advantages. In order to develop KM, they use some useful procedures to expand human resources, such as training which makes them skillful and freed to act. So we can determine the relation between employee training and KMVC.

C. There is a relation between employee participation and knowledge management value chain in organization

Findings table III show that the correlation coefficient between employee participation and knowledge management value chain and its subscales in ($p<0.01$) level is meaningful ($r=0.826$). It means there is a meaningful relation between employee participation and KMVC and its subscales. According to determination coefficient (r^2), 68.2% variance of employee participation and KMVC was common. So the third theory is confirmed.

The outcome is matched with the result of [31] that shows a meaningful relation between the role of quality management in the dimension of human resource participation and producing KMVC in organization. Reference [43] concluded that employee participation doesn't have an important effect to implement KM in the company and this result is not matched with the result of this research.

Knowledge has an abstract meaning and the culture of knowledge sharing and application in organization depends on individual attitudes. Employee participation is one of the most important challenges to implement KM in organization. The culture of participation effects knowledge producing by increasing knowledge exchange in organization and creates suitable situation to transfer knowledge

between individuals and groups, because knowledge transferring needs people who cooperate in exchanging ideas, sharing and creating knowledge. The lack of the culture of participation that supports KM system, limits KM system efficiency.

VI. CONCLUSION

Human resource management is the most important key in organization's success. If policies and procedures related to organization staff are in accordance and have a remarkable share in accessing strategic programs and organization goals, reaching the organizational success is more possible. Culture and general values, organizational situation and managerial behavior which originate form that culture, have a big effect on reaching to desirable excellence. On the other hand, KM causes to establish and improve competitive progresses for commercial organizations. In the other words knowledge improves the ability of competition in an organization and also helps the organizations to survive in present turbulence and changed situation. KM is not just a collection of software and hardware and the organization foundation such as culture and staff have an important role in it. The main duty of HRD is supervision, evaluation and interfering in staff's knowledge visualization, distribution and application. Also all the activities of HRD dimensions are effective in maintaining and making the abilities of organization staff. So if HRD involves human capitals education and if knowledge is the valuable resource for these capitals, in this case HRD and KM are dependent on each other strongly. HRD and KM share general goals and activities when forming work units, teams, multi-duty cooperation and also networks of communications inside and outside organization borders. So by surveying knowledge management cycle and human resource management process together, it is clear that there are a lot of common activities and a two-way relation between them. According to the result of research in this industrial company, we can confirm what was thought in research general. In other words we can say that there is a meaningful relation between HRD (empowerment, training, participation) and knowledge management value chain.

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In Vitro Propagation of Medicinal Plant *Orthosiphun Stamineus* (Misai Kucing) Through Axillary Branching and Callus Culture

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Abstract: *Orthosiphun stamineus* is a herbaceous plant that is popularly known as Misai Kucing. It is widely used in traditional medicine as diuretic agent. This study was divided into two parts that was the *in vitro* production of complete plantlet through axillary branching and callus culture derived from leaf explant. In axillary branching method, sterilization was conducted using 0.02mg/100ml of mercuric chloride followed by rinsing with 20% and 50% of Clorox for 20 minutes and 5 minutes respectively. This sterilization method was able to remove the contaminants from the surface of the axillary stem and almost 70% of the explants were survived. Axillary bud was placed on Murashige and Skoog (MS) basic medium and cultured for 1 month. The *in vitro* shoot was inoculated on MS medium which was supplemented with different concentrations of BAP and NAA. The medium that contained 1.0mg/L of BAP gave the best shoot multiplication (13.25) and shoot length (6.23cm) after 8 weeks in culture. Root formation in term of percentage of root (70%) and the number of root produced (10.50) were the best when shoot inserted into medium contained 6mg/L IBA after 3 weeks in culture. However, MS medium that was supplemented with 2 mg/L IBA enhanced in the root length (3.85 cm). Meanwhile, in callus culture, the leaf explant was placed on MS medium containing with various concentrations of 2,4-D for induction of callus. The optimum level of callus induction and proliferation rate (0.42) were obtained with 4mg/L 2,4-D. The callus cells were tested in medium with Evan's Blue staining and the result showed that the cells were embryogenic. However, the shoot induction from the callus was failed in all tested mediums containing different combinations of BAP and 2,4-D.
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Keywords: In Vitro; Propagation; Axillary branching; Callus

1. Introduction

Orthosiphun stamineus is one of the most valuable medicinal plants which provide rich medicinal compounds. It has been object of interest among researchers since early 20th century (Khamsah et al., 2000). *Orthosiphun stamineus* is distributed from India through Malaysia to tropical Australia, South East Asia, Soviet Union and Cuba and in early 1920 to Europe. It is also called as *Orthosiphun aristalus* Benth with common names of Java tea (Indubala, 2000), kidney tea or bladder tea. It also has local or vernacular names including Misai Kucing or Kumis Kucing.

1.1. Plant Taxonomy and Morphology

Orthosiphun stamineus is a perennial herb which can grow to the height of 150 cm (Ahamed Basheer and Abdul Majid, 2010) with quadrangular, poorly ramified and ascending stem. The leaves are regulated in opposite pairs. They are glabrous, simple, green, and with a lanceolate leaf blade and a serrate margin. The leaf apice is acuminate with an acute leaf base (Ahamed Basheer and Abdul Majid, 2010). The petiole is partially short about 0.5 to 2cm in length with cuneate at base, acute or acuminate at leaf apical. The stem is quadrangle, reddish in color, and erect with profuse branching. Flowers are borne on verticals about 16cm in length and have campanulate shape. They are white to bluish in color with long exerted filaments that make the flowers look cat's whiskers. Bracts are green, minute (1-2mm) and caudiform in shape. In nature, the flowers are hermaphrodite, about 6.2cm in length (including the stamen). There are two calyx lobes which are greenish red in color, having about 6 mm length and partially gamosepalous. One of the calyx margins is toothed and the other one is entire, both covered with white hairs. There are also two corolla lobes that are partially gamopetalous and covered with minute hairs. The corolla is light violet in color with lobes that are much shorter than the corolla tube. The corollas are bilabiate in shape with fringed margin. The labellum is light violet in color, hairy and pinkish on the under surface. There are 4 stamens which are inserted near the base of the corolla tube. There is a single, central, terete style with a clavate stigma. The fruit splits into 4 oblong-ovoid nutlets with 1.5 to 2.0 mm long.

1.1. Growth Condition

Orthosiphun stamineus can grow well in wet soil and be found in both temperate and tropical garden (Hsuan, 1986). In traditional breeding and

propagation, they are generally propagated by stem cutting in about 15-20 cm which has some buds. For plantation, planting in a nursery for a period of 45 days with cutting placed vertically with only one bud visible is preferred. Every 2-3 weeks the upper 4-6 leaves of the shoots are plucked by hand. In modern breeding and propagation, plant tissue culture is widely used as a modern propagation for commercial purposes in many species. Rapid multiplication with the use of tissue culture provides an alternative method for mass propagation of plant to obtain a large number of propagates originating from a small number of elite plants within a short period (Wirjodarmodjo et al., 1988).

1.2. Medicinal uses of *Orthosiphun stamineus*

Higher plants not only are the most important producers of natural products including food, wood, fibers, and oil but also they are the richest sources of medicinal substances (Tabata, 1976). Medicinal plants and herbs contain substances that are known to modern and ancient civilizations for their healing properties. Traditional medicine is well known because of its high nutritional value and its ability to cure illnesses (Muhammad and Mustafa, 1994). Furthermore, it is widespread all over the world (Akerele, 1988). Determination of major phytochemicals from the extract of *Orthosiphun stammineus* leaves confirmed its strong antioxidant potency and total phenolic content (Akowuah et al., 2004). According to Hamann (1988), around 75 to 90% of people in rural areas rely on herbal traditional medicine. It is used as strong diuretic (Englert and Harnischfeger, 1992) against kidney complaints and illnesses, bladder stones problems, rheumatism, abdominal pain, kidney, edema and gout, and urinary tract infection (Ahamed Basheer and Abdul Majid, 2010). It is consumed as tea which cures diabetes. The leaves of this plant are boiled together with *Andrographis paniculata* and consume as tea to bring down diabetes level (Khairina et al., 2000). Moreover, studies have shown that the *Orthosiphun stamineus* leaves exhibit a range of pharmacological properties such as, anti-inflammatory, antioxidant, anti-bacterial, anti-angiogenetic properties (Ahamed Basheer and Abdul Majid, 2010; Abdelwahab et al., 2010).

2. Materials and Methods

2.1. Preparation of medium

Media used in this study was Murashige and Skoog (MS) medium (Murashige and Skoog, 1962). This is prepared by preparing stock solutions for micronutrients, macronutrients, and vitamins. The procedure began by weighing their premixed powder respectively. Each of them was mixed together with additives like sugar, plant growth regulators and distilled water and then poured up to one liter. Then, PH of the medium was adjusted to 5.8 with Sodium Hydroxide (NaOH) and HCl so that 4.0g agar was dissolved into the solution. The solution was later mixed using a magnetic stirrer on a hotplate until it is dissolved and melted. The medium was then poured into each of the test tubes, petri dishes and jars respectively. For petri dishes, after the entire agars were clearly dissolved, the media were poured with 1000 ml bottles and then autoclaved. After that, medium was poured into petri dish before the medium was started to gel. Once medium gelled, petri dishes were closed and the mouth was sealed with parafilm in order to culture. Three types of plant growth regulators were used like BAP, NAA, and IBA. Stock solution of all growth regulators were prepared by weighing 100 mg of each component that was dissolved in a few drops of 1.0M NaOH or 1.0M HCl. This solvent was carefully added drop by drop into a beaker of swirled ultra-pure water, which was then made up to 100 ml volumetric flask. All the stock were stored in cold room and defrosted later for using in the medium.

2.2. Preparation of aseptic condition

Culturing was carried out in a laminar air flow cabinet under aseptic condition. The laminar was exposed to the UV light for 30 minutes to sterilize the surface of working area in the laminar air flow cabinet. The fan blower of the laminar flow was switched on and all the items were swabbed with 70% alcohol. The long-handle forceps and scalpels used were sterilized by dipping them for 10 seconds in 90% alcohol. Then, they were heat sterilized with head bead during the work. The explants were transferred into sterile petri dishes or test tubes using sterile forceps. After that, the plastic cap of test tubes and jars were opened and the mouth was quickly flamed. The explants were then placed on the agar surface. The mouth of vials was then re-flamed before capping and sealed with parafilm before transferring them to the incubation room.

2.3. Source of explants

One year explants were provided by Golden Hope Research Centre Sdn. Bhd, Banting, Selangor and Institute of Biological Sciences Garden, University of Malaya, Kuala Lumpur (Plate 1)

2.4. Sterilization Technique

Two sterilization techniques were used in this study, labeled as A1 and A2.

2.4.1. A1 Technique

The nodal stem segment was washed by detergent for several times and rinsed under running tap water for 30 minutes. It was pretreated by immersion in absolute ethanol for some time and the surface disinfected by 0.02g/100ml mercury chloride ($HgCl_2$) under constant agitation for 5 minutes. After that the stem was rinsed for three times with sterile distilled water and each of them soaked respectively

for at least 5 minutes. The explants then were sterilized with 20% Clorox with addition of three drops of tween 20 for 20 minutes for the first time and sterilized with 5% Clorox for 5 minutes for the second time. Before inoculation, they were rinsed for three times with sterile distilled water to be ready for culturing.

2.4.2. A2 technique

The explants were treated with 0.08g/100 ml mercury chloride ($HgCl_2$) and rinsed for three times with distilled water. Then, they were soaked in 15% Clorox for 15 minutes and rinsed thoroughly three times in sterile distilled water to be ready for culturing.

2.5. Production of *in vitro* explants

The cleaned explants were aseptically cultured into test tube containing basin MS medium. Explants were cut into 15 to 20 mm of nodal stem that were consisted an axillary bud before culturing. After that the mouth of test tube was flamed and closed with plastic cap so that the test tube was sealed with parafilm and kept in 26 to 27^0C under light condition for one month. Then, observation was made weekly to check the contamination.

2.6. Induction of multiple shoots in proliferation culture medium

Segment with three nodes was excised from one month old *in vitro* axillary shoots. The nodes were separated individually to be as explant sources. The explants were cultured in medium supplemented with BAP at 0, 0.5, 1.0, 1.5, 2.0 mg/L and NAA at 0.0, 0.5, 1.0 mg/L levels and were arranged in factorial design. Ten replications were conducted for each treatment. The culture was maintained at temperature 25 $\pm$ 2^0C with 24 hour at 3500 lux photon density. Every week, the responses of the explants and the number of formed shoots were noted.

2.7. Production of *in vitro* complete plantlet

Well-developed shoots from proliferating shoots were separated and rooted on media supplemented with different concentrations of NAA at 0.0, 2.0, 4.0, 6.0 mg/L. Ten replications were conducted and the cultures were maintained at 25 $\pm$ 2^0C for 24 hours at 3500 lux photon density. After that, the number of roots produced was recorded for a period of two weeks.

2.8. Callus culture and sterilization technique

The leaves were washed with detergent and rinsed with sterile distilled water. Then, they were dipped in 90% alcohol for several seconds. Surface sterilized using 0.02/100 ml mercury chloride ($HgCl_2$) for 5 minutes and rinsed with distilled water for three times. After that, they were soaked in 20% Clorox for 10 minutes and rinsed again with sterile distilled water for three times. Following this stage, they were dried on sterile filter paper to be ready for culturing in culture medium.

2.9. Induction of callus

The explants were cut into disk at size 2-3mm and cultured on petri dishes containing 2, 4-D at 0.0, 1.0, 2.0 and 4.0 mg/L. The replication for each concentration was 10. All the cultured explants were placed in dark condition with temperature regulated at 25$\pm$$2^0$C. Diameter of callus was recorded at week two and week eight and the morphology of callus was also recorded. The ability of callus to form somatic embryo in every treatment was confirmed using Evan's blue.

2.10. Induction of shoots

A small portion of friable callus was cultured in medium supplemented with NAA at 0.0, 1.0, 2.0, 3.0, 4.0 5.0 mg/L and 2, 4-D at 0.0, 0.5mg/L and were arranged in factorial design. All the cultured materials were kept at 25$\pm$ 2^0C for 24 hours at 3500 lux photon density. Then, the number and height of shoots produced were recorded.

3. Results

3.1. Production of *in vitro* complete plantlet from axillary bud explants

3.1.1. The effect of using different sterilization techniques on the explants

Figure 1 shows the result of sterilization experiments based on using different concentrations of mercuric chloride ($HgCI_2$), Clorox, and the length of time exposed to the explants. Contamination was visually detected 3 to 4 days after sterilization treatment was given. It was observed that treatment with 0.02mg/100ml of $HgCI_2$ and rinsing with 20% and 5% of Clorox for 20 minutes and 5 minutes were able to remove the surface contaminant on the axillary bud which made almost 70% of the explants to be survived (Plate 2). Meanwhile, in A2 sterilization technique, only 20% of the explants were free from the contaminants and some of the explants were not tend to grow as the tissues of the explants had sign of death after 1 day in the culture media. The contamination could easily note at the edge of the implanted axillary bud which a small white, black or red spot occurred and spread quickly to cover the whole surface of the medium (Plate 3). Fungi and bacteria were believed to be the main causes of the contamination of the explants.

3.1.2. *In vitro* multiplication of shoots

The explants to be used for *in vitro* propagation started to give growth response after 10 days culture in a basic medium. Adventitious buds were in rise at leaf axil and shoot apex. After 2-3 weeks, the vigorous explants were obtained from the survived explants (Plate 4). Besides, it was noticed that some of the explants produced root in the culture medium (Plate 5). The vigorous explants were left for 5 weeks to develop more shoots. Then, the suitable

and healthy segment consisting of three nodes were excised from the 5 week old *in vitro* axillary shoots and the nodes were separated individually to be as explants sources.

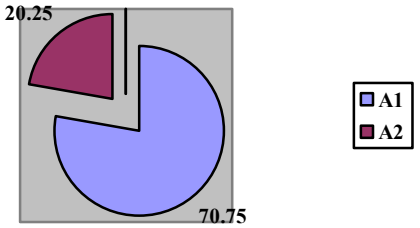

Figure 1. The percentage of explants survived in A1 and A2 sterilization technique

The growth of multiple shoots in various treatments tested in medium containing the combinations of BAP and NAA was recorded as shoots number and shoot length over a period of 4 and 8 weeks (Figures 2 and 3).

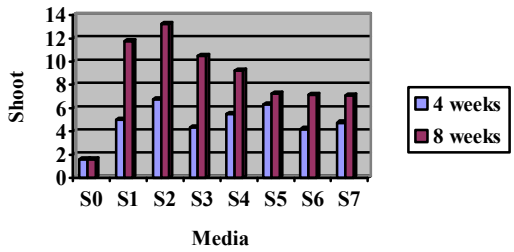

Figure 2. Multiple shoots formation in nodal segments in MS medium supplemented with different concentrations of BAP and NAA

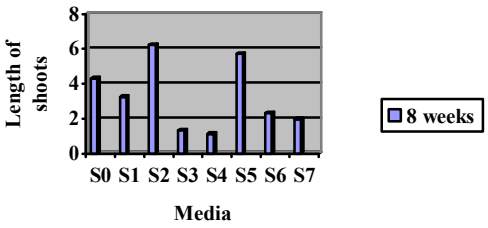

Figure 3. Shoot length in MS medium containing different concentrations of BAP and NAA

The explant cultured in the media produced a cluster of shoots in the all media supplemented with BAP and NAA except in the basic medium. Multiple shoots were initiated from axillary bud explant after 1 week in culture (Plate 6).

The nodal segment of *Orthosiphun stamineus* produced the highest number of multiple shoots per explant when it was cultured in MS + 1.0mg/L BAP alone (Plate 7) where an average 13.75 shoots per explant was obtained after 8 weeks. The number of multiple shoots was increased with the duration of culturing time. The shoots obtained from week 4, resulted an average of 6.75 per shoots showed two-fold greater increase in the next 4 week periods.

On the other hand, the poor growth was seen in MS media containing 1.5mg/L and 2.0mg/L BAP. It was observed that the shoot length was in average 1.34 and 1.13 per shoots (Plate 8). However, the multiple shoots produced in the media are up to almost 10 shoots per explants.

Addition of NAA in the presence of BAP in the MS medium induced callus formation at the base of explants and decreased the formation of multiple shoot number (Plate 9). In the medium without any hormone showed the lowest shoots formation but some of the explants produced complete plantlets with roots obtained after 8 weeks of culturing.

3.1.3. Effect of rooting on explants

The *in vitro* shoots when separated as individual shoot from the multiple shoots produced roots when cultured in basic MS medium or MS medium supplemented with different concentrations of IBA (Figures 4, 5, and 6).

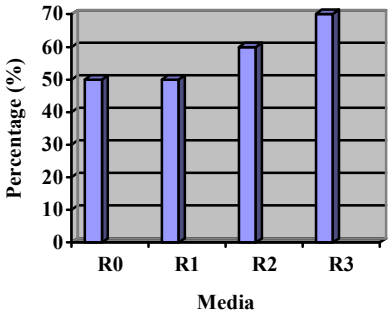

Figure 4. Percentage of shoots producing roots in different concentrations of IBA

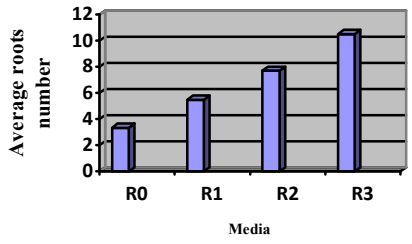

Figure 5. Average number of roots produced per shoot in different concentrations of IBA

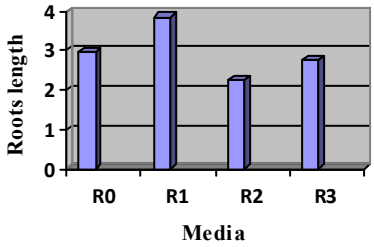

Figure 6. Average length of roots produced in different concentrations of IBA

From the result, the percentage and number of roots formed in each shoot were increased when the concentrations of IBA were enhanced (Plate 10).

The highest percentage of shoots that produced roots was properly seen in shoot grown in MS medium containing 6mg/L of IBA. This medium produced 70% of root and in average 10.50 roots was formed per shoot. When the culture contained 4mg/L of IBA, it was observed in the basic medium that the shoots were also capable to produce 50% of roots. Meanwhile, in terms of production of root length, the longest root was produced in medium containing 2mg/L IBA (Plates 11, 12, and 13).

3.2 Callus culture

3.2.1 Induction and multiplication

The results of the callus induction and multiplication in MS media supplemented with different concentrations of 2, 4-D is summarized in Table 1.

The callus was initiated from the leaf explant after one week in the culture. It was observed that the whole part of the explant was formed into callus after one week later. The callus in medium supplemented with MS medium containing 4.0 mg/L of 2, 4-D was found to promote good callus proliferation. The results showed that the callus in this medium produced the lowest multiplication at week two but the callus diameter was increased to 1.17cm to give the highest diameter incensement (0.42) (Plate 14). Meanwhile, the callus in MS medium containing 2.0 mg/L and 3mg/L of 2,4-D encourage a longer diameter at the first two weeks (0.92 and 0.97) but the proliferation rate decreased with the duration of culturing time which affected the callus incensement (0.29 and 0.23) (Plate 14). Most of the callus textures in all treatments were friable (Plate 15) and the callus colors were light brown and brown (Plate 16). All the callus tested using Evan Blue staining had response to acetocarmine stained material which gave a bright red colors. So, the calluses were recognized as embryogenic cells (Plate 17).

3.2.2. Induction of shoot

The combinations of 2, 4-D and BAP in all used media was failed to produce shoot from the callus after 6 weeks culture. It was observed that the callus color was changed from brown to green after 2-3 weeks in the culture but the color was suddenly changed into dark brown and black and considered to become senescence. Therefore, no result has been recorded.

4. Discussions

Orthosiphun stamineus is currently gained recognition as an important medicinal plant. Generally, the raw used in the production of healthy food from medicinal plants was collected from the wild. Continuous extraction of this material from the forests has led to the depletion of this important raw material. Conventional propagation is faced problems of poor seed viability, low germination, and delayed rooting of vegetative cuttings. There is an urgent need to apply non-conventional propagation methods for conservation and future commercial delivery. Therefore, biotechnology provides a new method for the mass production of elite plants as well as for the *in vitro* production of plant raw materials (Schumacher, 1988). Thus, tissue culture techniques have been reported for conservation and propagation of several endangered medicinal plants (Sharma et al., 1993; Sudha and Seeni., 1996). This study was conducted to produce *in vitro* plantlet through axillary branching and callus culture for rapidly increase the number of propagates of *Orthosiphun stamineus* for cultivation as well as aid in the replacement of natural population.

Table 1. Callus induction and multiplication in MS media supplemented with different concentrations of 2,4-D

Media	*Week 2	*Week 5	Proliferation rate	Callus texture	Callus color	Evan Blue staining
C0	-.	-	-	-	-	-
C1	0.69	1.02	0.34	Friable	Light brown	Red
C2	0.92	1.21	0.29	Friable	Light brown	Red
C3	0.97	1.20	0.23	Friable	Brown	Red
C4	0.75	1.17	0.42	Friable	Brown	Red

* Diameter of callus (cm)

Plate 1. Source of explants obtained from Institute of Biological

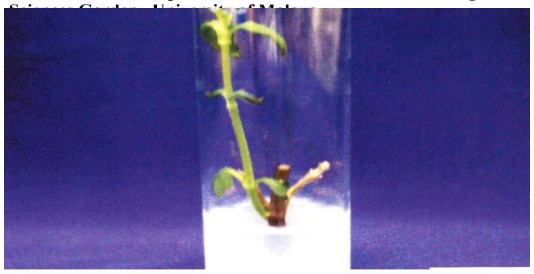

Plate 2. The explant survived after 2 weeks in culture when it was treated with HgCl2 and rinsed with Clorox

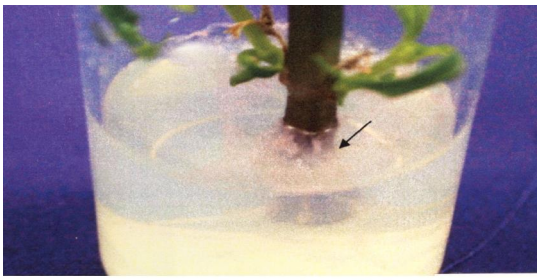

Plate 3. Red spot was spread when contamination occured after 3-4 days sterilization

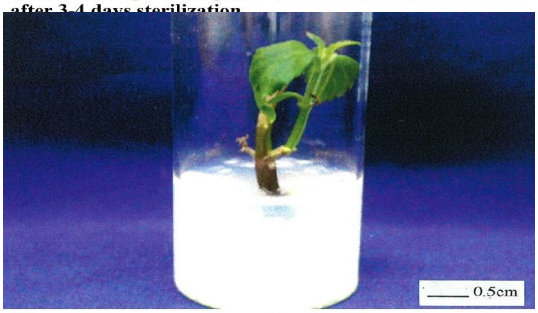

Plate 4. Vigorous explants obtained from the survived explants after 2-3 weeks

Plate 5. The explant produced complete plantlet root in basic medium after 3 weeks

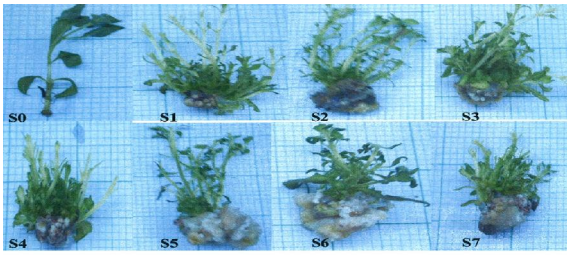

Plate 6. The effect of BAP alone and combination with NAA that produced multiple shoots (S1-S7) compare to the culture in control medium (S0)

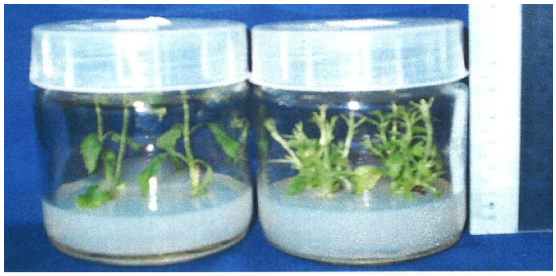

Plate 7. The highest number of multiple shoots and the legth of shoot produced when cultured in MS + 1.0 mg/L BAP alone

Plate 8. The culture with multiple shoots in control medium, MS + 1.0 mg/L BAP and MS + 2.0 mg/L BAP (from left to right

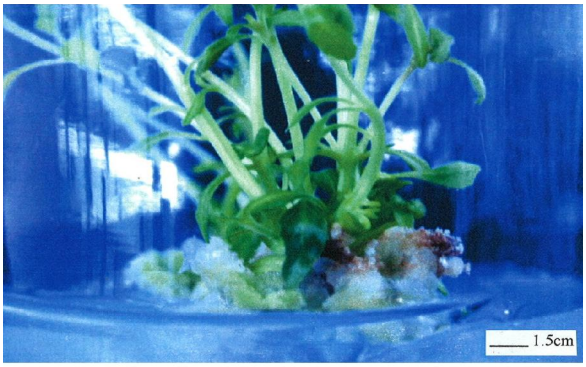

Plate 9. Addition of NAA in the presence of BAP in the MS medium induced callus formation at the base of explants

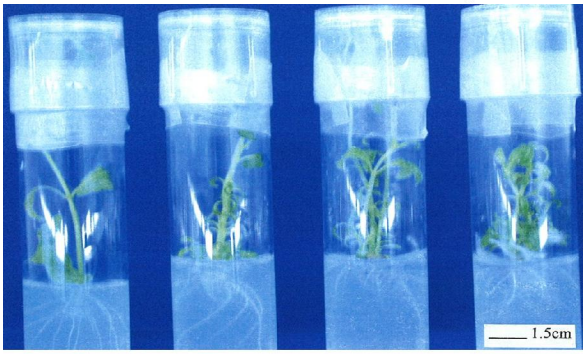

Plate 10. *In vitro* plantlets' root formation in MS medium (control), MS + 2mg/L, MS + 4mg/L IBA and MS + 6mg/L IBA

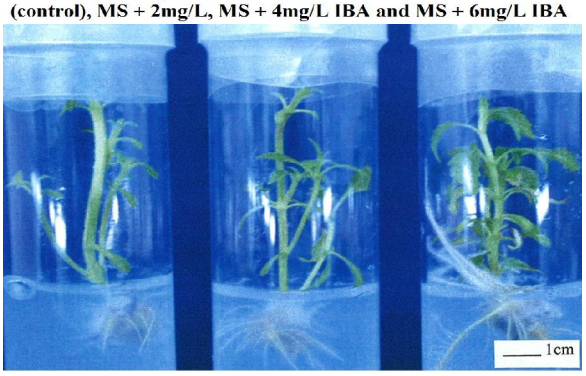

Plate 11. *In vitro* plantlet's root formation in week 1, week 2, and week 3 in MS + 6mg/L IBA (from left to right)

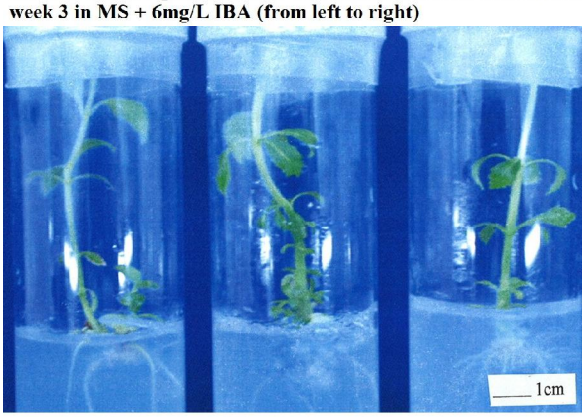

Plate 12. *In vitro* plantlet's root formation in week 1, week 2, and week 3 in MS + 4mg/L IBA (from left to right)

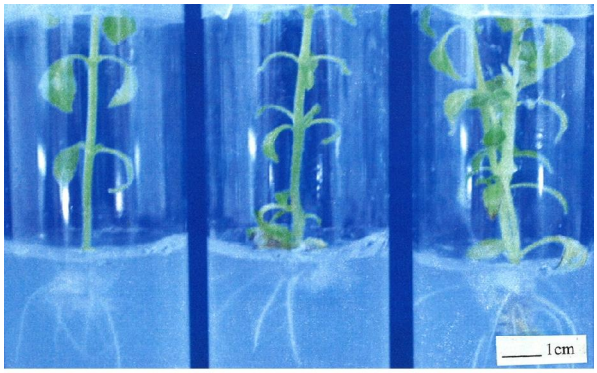

Plate 13. *In vitro* plantlet's root formation in week 1, week 2, and week 3 in MS + 2mg/L IBA (from left to right)

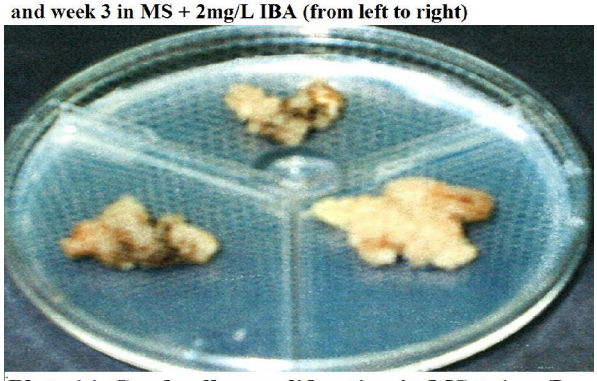

Plate 14. Good callus proliferation in MS + 4mg/L of 2, 4-D after 5 weeks (above) and the lowest proliferation in MS + 3mg/L of 2, 4-D (below)

Plate 15. Friable callus

Plate 16. The brown color callus

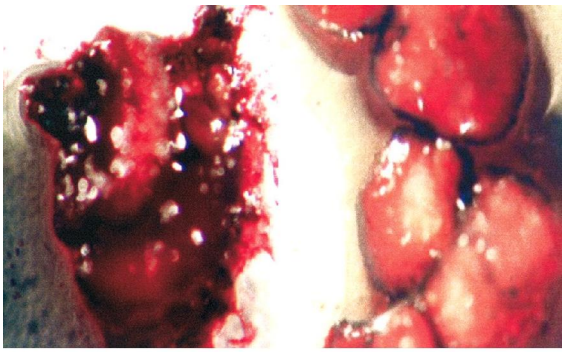

Plate 17. The callus tested using Evan Blue staining which had response to acetocarmine-stained material

4.1. Effect of surface sterilization on explants

In tissue culture, the most important thing to obtain an aseptic culture is the effectiveness of sterilization technique applied to the plant material. The plant material in this study was excised from the axillary stem of *Orthosiphun stamineus* and has been used as a primary explant for the multiplication of shoots and young leaf explants for callus culture. The selected plant materials and the explant should be totally free from any other microbial contaminants. It is because the surfaces of plants carry wide range of microbial contaminants (Bhojwani and Radzan, 1996). Beside the plant material itself which contribute to the contamination of the culture, there are other several possible sources of contaminations such as the medium, the environment of the transfer area, instruments used to handle plant material during inoculation, subculture, the environment of the culture room, and the operator. In this study, it was found that washing with 0.08g/100m1 of mercuric chloride ($HgC1_2$) followed by rinsing with 15% Clorox for at least 10 minutes was failed in eliminating total contaminants in axillary stem of *Orthosiphun stamineus*. On the other hand, 70% of explants became free from the contaminants through using a lower concentration of $HgCI_2$ (0.02mg/1) and rinsing with 20% and 5% of Clorox for 20 and 5 minutes. The contamination on the medium was observed 3-4 days in the culture. The small white, black and red spot of contaminant spread quickly to cover the whole medium. It was believed that bacteria and fungi were the main contaminants. There were various methods to sterilize plants like plant tissue culture, $HgC1_2$ and commercial Clorox which were chosen because they were proven to be effectively decontaminating axillary stem of *Orthosiphun stamieus* (Lee and Chang, 2000). The rinsing processes using 20% and 5% of the commercial Clorox for 20 and 5 minutes have accomplished a sufficient concentration to sterilize the explants with satisfactory result. The commercial Clorox was used containing 99.9% of sodium hypochlorite (NaClO) as the active agent to eliminate the contaminant. The result also showed that application of mercury chloride ($HgC1_2$) at 0.02/100ml was an effective disinfectant; however it contains a danger chemical which poses health risks and disposal problems (Bonga and Aderkas, 1992).

In the case of using higher concentration of $HgC1_2$ (0.08mg/100m1), bleaching of the tissue was caused when the concentration was high enough to kill the microorganism. Dods and Roberts (1982) have stated that the aim in surface sterilization of plant tissue is to remove all microorganisms with a minimum damage to the plant system that need to be cultured. Moreover, they were also indicated that traces of the decontaminant should be removed by several rinsing in sterile distilled water because they can leave toxic residue in the explant and can destroy some components in the medium. Therefore, the concentration of the decontaminant agent and the duration of the treatment should be chosen accurately to minimize the death of the tissue (Bhojwani and Radzan, 1996). Microorganism lodged in crevices in the bark, in leaf axils or at the base of hair may never contact with the decontaminant because air bubbles entrapped in these positions prevent such contact. Furthermore, the tissue surface should be wet firstly by treatment with ethanol. Ethanol partially removes hydrophobic waxes and resins which protect microorganisms from contact with aqueous decontaminants (Bonga and Aderkas, 1992). Pre-cultural treatment can be so essential in that it should be considered as a routine stage of the micro propagation process.

4.2. The influence of media and plant growth regulators on shoot multiplication

Growth and morphogenesis of *in vitro* plant tissue are largely governed by the composition of the media culture (Razdan, 1993). A standard

medium consists of a balanced mixture of macronutrient and micronutrient elements, vitamins, carbon sources, a source of reduced nitrogen supply, organic growth factors, and plant hormones (Narayanaswamy, 1977). The choice of particular medium depends mainly on the species of plant, the tissue or organ to be cultured, and the purpose of the experiments (Dodds and Roberts, 1982). The Murashige and Skoog (1962) medium (MS) was chosen in this study as it is proven to be effective for the growth of a variety of dicotyledonous and monocotyledonous plants (Dixon, 1985). The requirement for the growth regulators varies from the system and the mode of shoot multiplication. Higher concentration of cytokinin to auxin ratio is required for the direct of induction of shoots on explants and higher auxin to cytokinin ratio is necessary for callus initiation and growth (George and Sherrington, 1984). The ability of shoots to multiply rapidly in *in vitro* condition is essential for the establishment of economically feasible micro propagation systems. Usually, once proliferating shoot cultures of particular tree species have been stabilized, they can provide a continuous source of micro shoots. In this study, multiplication of shoots from axillary bud of *Orthosiphun stamineus* have been achieved after 8 weeks in culture in MS medium containing different concentrations of BAP and NAA. It was observed that the shoots buds were differentiated from the sides and developed prolific buds, indicating the easy release of the axillary buds. A BAP was selected for micro propagation of wide ranges of medicinal plants for rapid propagation because of its ability to stimulate shoot proliferation (Lee and Chang, 2000; Sudha and Seeni, 1996; Franca et al., 1995; Sharma et al., 1993). The most effective medium in terms of the number of shoots was formed when it was supplemented with 1.0 mg/l BAP alone and the average shoots produced per explant was 13.25. According to Bhojwani and Razdan (1996), to enhance axillary branching, the shoots can be grown in the medium containing suitable concentration of cytokinin with or without auxin. This is also reported by Lee and Chang (2000) indicating that with the addition of BAP alone in MS medium the highest number of multiple shoots of *Orthosiphun stamineus* can be achieved. George and Sherrington (1984) have state that a high concentration of cytokinin in media can induce shoot formation and at the same time inhibits roots formation. This high cytokinin concentration appeared to stop the apical dominance and allows axillary buds to develop. However, as higher levels of BAP added into the media (l.5 mg/L and 2.0 mg/L), the number of shoots was not increased. Although, the satisfactory number of multiple shoots obtained from these media, the length of the shoots did not support good growth of the shoots. This could be due to high levels of cytokinin that were placed in the media which affected the small shoots and failed to elongate (George and Sherrington, 1984). Moreover, by adding a high level of cytokinin, the number of shoots formed was increased but the growth of individual shoots remained arrested (Bhojwani and Razdan, 1996). The result was actually differed from the study done by Lee and Chang (2000) when the greatest responses of multiple shoots formation were obtained from the medium containing 1.5 mg/L of BAP.

Besides, it is important to consider the physiological stage of explants and the effect of stem bud location on the number of shoots per explant bud (Chand et al., 1999). They also found that the rate of shoot multiplication of medicinal plant *Maytenus ilifolia* was affected by the age of axillary bud excised from the plant source. Meanwhile, the addition of NAA in the presence of BAP in MS medium induced callus formation at the base of the explants and decreased the formation of the multiple shoot number. But NAA was proved to be more efficient than IBA in combination with BAP as reported by Lee and Chang (2000) and in this study the combinations produced the formation of 7 shoots per explant in average. For some species, the combination of auxin and cytokinin seem to be positive for the number and length of shoots. Franca et al. (1995) found that *in vitro* derived shoots of *Stryphnodendron polyphythum* was influenced by the action of cytokinin, whereas height was increased by the presence of both auxin and cytokinin. The result from this study showed that the addition of BAP and NAA into the media did not affect the number and height of shoots. Moreover, the height of shoot may not be significant because in time of sub culturing, a shoot placed on the media were not uniformly in size. According to Bhojwani and Razdan (1996) the exogenous requirements of the hormones depend on the levels of the plant system and it is variable with the tissue, plant type, and the phase of plant growth. Consequently, for the shoot multiplication in this study, the presence of NAA in the medium was not obligatory since a BAP alone was enough for effective shoot multiplication. It is needed to emphasize that the superiority of medium containing 1.0 mg/L of BAP was used as the optimal media for shoot multiplication.

4.3. Production of *in vitro* complete plantlet

Plant roots produced by means of tissue culture is an important step in the clonal multiplication of desirable plants. Propagates have to

have an adequate root system before they can be established satisfactorily (Seabrook, 1980). Rooting can be carried by *in vitro* method as well as *in vivo.* In this study, *in vitro* rooting experiment was carried out using only one type of auxin which was IBA. All *in vitro* shoots when separated as individual shoot, rooted in 4 media experiments including free of auxin medium. Razdan, (1993) expressed that roots are mostly induced in the presence of suitable auxin. Although, a mixture of more than one auxin can be particularly effective for root induction but sometimes root formation is better with only one auxin placed into the media (George and Sherrington, 1984).

In this study, IBA was found to be very effective for root initiation of *Orthosiphun stamineus* especially in MS medium supplemented with 6mg/L of IBA when 70% of roots were formed compare to the medium free of auxin. This result was similar to those obtained by Lee and Chang (2000) but they found that percentage of root formed was almost 80%. On the other hand, the root formation in the medium free of auxin was equaled (50%) to rooting percentage in medium containing 2mg/L of IBA. In this case, the production of root in medium of free auxin was mainly depending on the endogenous auxin. It has been reported in another medicinal plant like *Rauwolfia micrantha* (Sudha and Seeni, 1996) and *Exocoecaria agallocha* L. that a similar concentration of IBA (2.46µM) was required to achieve highest frequency of rooting. In *Hydrastis canadensis,* treatment with IBA at range of 1.0-2.0µM produced the lowest formation of roots as another auxin type NAA was needed for successful result (Bedir et al., 2003).

In this study, it was found that the higher concentration of IBA supplemented on media increased the rooting ability of shoots. For example, the number of shoots was increased from 3.33 in medium containing 2mg/L to 10.50 shoots produced in 6mg/L of IBA. High concentration of IBA added to the media stimulated higher degree of cell division and cell elongation of the shoot primordial but if the excessive auxin was applied, it would cause toxicity of the cell. An increase in the concentration of IBA on medium did not influence the root length. Root production in terms of the number of roots was high i.e 10.5 roots per culture in higher IBA level (6mg/L) used but it had shorter root length than those in the lowest medium containing IBA (2mg/L). In other medicinal plant like *Hydrastis Canadensis,* a longer root length in the lower concentration (1.0µm) of IBA has also been reported (Bedir et al., 2003). According to Pierik (1987), if the high auxin concentration failed to produce roots, callus formation takes place. The callus was observed at the base of the shoot in the media except in the medium of free auxin. There are many factors that influence the rooting ability. Franca et al. (1995) found that the most effective way for rooting in S*tryphnoden polyphythum* was using half strength of macronutrients. Nutrient salt in media affected the rooting due to reduced nitrogen level (Bhojwani and Razdan, 1996). The rooting can also be enhanced by improving the number of shoot multiplication cycles. The other factor is to grow the explants in liquid media since it can provide a good aeration beside development of root hairs. Activated charcoal also has been reported to improve root growth where roots have already been initiated (George and Sherrington, 1984).

4.4. Callus culture

The other method of propagation in this study was through callus formation. Leaf explants were used for initiation of the callus. The type of explants used may vary in morphogenesis which failed to regenerate. The effect of the type of explants on other medicinal plant like *Eurycoma longifolia* (Tongkat Ali) for induction of callus was reported by Chand et al. (1999) when the highest callus production was obtained when petiole was used as explants. The presence of 2, 4-D in all media tested was found to be effective in inducing callus proliferation. 2, 4-D is a phenoxy auxin and has been recognized as strong promoters of callus induction and growth (Bonga and Aderkas, 1992). In this study, the tendency of callus to grow and proliferate was measured in the increments in diameter. The results indicated that callus proliferation was affected by the concentration of 2, 4-D in the media. Maximum callus growth was achieved in MS medium containing 4.0 mg/L 2, 4-D. At the initial stage, the proliferation rate was slow but later as the time passed, the callus started to multiply and proliferate to the greatest result. Since 2, 4-D is a strong auxin, the high concentration provided to the media had created a sufficient condition for callus to continue to develop and therefore keep multiplying as the time passes. The produced callus was friable in all treatments and varied in color. This type of callus was considered as non-embryogenic in which it was unable to produce somatic embryos thorough induction of direct somatic embryogenesis. The appearance of embryogenic callus was white, off white or pale yellow, compact or often nodular and translucent. The compact cell later exhibited synchronized cell division whereas the latter cells were bigger and more vacuolated (Aziz et al., 2000). However, the assessment to distinguish embryogenic culture using Evan's Blue staining offered the other results. The callus cells were treated with acetocarmine and Evan's Blue and it was found that the reactions with embryogenic cell remained in bright

red color with acetocarmin stain. According to Dodd and Roberts (1982), the embryogenic cells are characterized by dense cytoplasmic contents, large starch grains and relatively large nucleus with darkly stained nucleolus. In contrast, with embryogenic callus, nucleus is very small so that the acetocarmine-stained red material is difficult to locate and the whole embryogenic callus were blue when stained with Evan's Blue (Gupta and Holmstorm, 2005). Each embryogenic cell is capable of passing through sequel stages of embryo formation. It starts with repeated cell divisions, cell aggregates progressively and passes through globular, heart and torpedo stages respectively before forming plantlet. In this study, there was no evident of any embryo developed in each stage but the callus was considered in the early stage of somatic embryogenesis. In fact, some cells within callus masses are capable of forming embryogenic cell because they are totipotent which means the cell retains all genetic information required for normal development (Butcher and Ingram, 1976). The callus, however, failed to produce shoot in the media containing combination of BAP and 2,4-D. It was observed that the callus showed green chlorophyll pigmentation that was developed but, then, it turned to black, indicating a fail for shoot initiation. There are many possible reasons for these failures such as combination of BAP and 2, 4-D that did not offer a balance of exogenous auxin and cytokinin or either one of these hormones must be omitted from the medium. Besides, the endogenous hormones may be accumulated and their inhibitory effect on organogenesis was not reversed by exogenous hormones. Dodds and Roberts (1982) found that the level of the hormone provided in the medium must be balanced with the residual amounts of the same in the primary explants, in addition to endogenous hormones that may be synthesized by the newly formed callus. Another possible reason is the cultural condition that involves nutritional and physical factors which may block the onset of the process.

5. Conclusions

The sterilization processed with 0.02mg/l00ml of $HgCl_2$ and rinsed with 20% and 5% of Clorox at 20 minutes and 5 minutes were able to remove the surface contaminant on the axillary stem of *Orthosiphun stamineus,* in which almost 70% of the explants survived. The highest shoots multiplication of *Orthosiphun stamineus* was obtained in MS medium containing l.0mg/L BAP. The optimum medium for rooting of *Orthosiphun stamineus* was in MS medium supplemented with 6mg/L IBA. The effective medium for callus induction and proliferation of *Orthosiphun stamineus* from leaf explants was in 4mg/L 2, 4-D. The combination of 2, 4-D and BAP in all media tested were failed to induce shoot from callus of *Orthosiphun stamineus.* However, further studies should be carried out to identify suitable plant growth regulators which can promote shoot induction from the callus. Different types of the medium or modifications of the medium should be considered and supplemented with other types of cytokinin and auxin. Furthermore, acclimatization of the *in vitro* plantlet should be done to ensure the ability of plantlet in the open field. This will provide very useful information in terms of the establishment of *Orthosiphun stamieus* with the use of this technique.

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Evaluation of Quality of working life in teaching hospitals in Ahwaz Medical University and its relationship with knowledge management from the perspective of senior and junior managers in hospitals

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Abstract: Quality of working life has an effective role in establishing knowledge management, so organizations should pay attention to it because if quality of working life be much better, the establishment of knowledge management will be more successful. This study was aimed to assess the quality of working life and relationship between its components and knowledge management from the perspective of senior and junior managers in hospitals covered by Medical Sciences University of Ahwaz Jundishapur. The present study is a descriptive - analytic and Sectional study that was took place in five Educational Hospitals which were affiliated by Medical Sciences University of Ahwaz in 1390. 56 junior and senior managers formed the study population. Data collection tool were a questionnaire consisting of three sections.Data analysis was performed by SPSSv16 and by using descriptive statistics and Pearson and Spearman correlation test. quality of working life with 22/3 and knowledge management with 3 in the average were in the mediocre level. Among quality of working life indicators, workspace with the average of 3.63 was at the highest status and material privileges with the average of 2.70 were at the lowest position. Participation in decision making, with correlation coefficient of 0.7, and workspace with a correlation coefficient of 09/0 showed statistically the highest and lowest correlation with knowledge management. Among different quality of working life Components, workspace by p-value =0.48, showed no significant relationship with knowledge management.Considering the high participation component in employee decision making was more important, it is recommended that improving programs of working life quality in hospitals affiliated to universities considered in the research, focus on development and improvement of these components. Hospital directors and staff participation in decision making and determining parameters of working life quality program can Increased utilization of managers` knowledge and information, and thus lead to better decisions to be made.

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1.Introduction

In recent years, various organizations and companies have started to join the process of Knowledge and new concepts such as knowledge work, knowledge of work, knowledge management and knowledge organization, announced this trend will intensify. Peter Drucker, using these words heralded a new kind of organization that rather arm strength, the strength of mind is rule. According to this theory, in the future, some communities can expect developments that have more knowledge. So the enjoyment of natural resources cannot be more important that knowledge. Knowledge organizational achieves to capability that is ability to make huge power from low resources (1). Knowledge management, new effort of the century called the Knowledge Age, is for purposeful maintaining, and guidance and improvement of institutions` knowledge capital, and implies that investments in science bring the best and most profit (2).

Because of the health sector relationship with the public health, require the use of efficient techniques in order to improve the quality of services, health care costs down and meet on timeclients' needs which only in the light of using new methods of information management, and allocating appropriate time to knowledge management is possible (3). Knowledge management is organization`s hidden investment management that this process involves the creation,

acquisition, storage, dissemination, sharing and use of knowledge (4).

Purpose of organizational knowledge generation (creation) are capability of a company as a whole, in the production of new knowledge, sharing it in the organization, and embody it in products, services and systems. (5) That this knowledge can be promoted or crystallized at the group level through dialogue, discussion, exchange of ideas, experiences and observations transfer(6). Knowledge storage is an important part of knowledge management; however, when companies are re-organized, valuable institutional memory is often underestimated. But about throwing away the old parts of past experiences should not simply decided (7). When we say a person distribute his knowledge, it means that the person guide another person with his knowledge, insights and ideas to help him to see better his position (9, 8). Organizations should prepare an environment for sharing, transferring and interaction of knowledge among members and teach people to understand their interactions. One of the main steps towards improving the knowledge management is identifying the causes and factors such as job satisfaction, quality of working life programs (10). Optimal use of human resources relies on actions that apply for protection and preservation of employees` Body and Soul. These actions include welfare, health care, job security, job design, emphasis on the role and position of the individual in the organization, providing staff development and growth Background, and as these things, collectively, are considered as quality of working life title. Quality of working life points to job satisfaction, motivation, benefited involvement, commitment and utilizing the people experience in job environment. QWL is one of the important indicators of working life that shows People how much are able to meet personal needs such as the need for independence while working in the organization (11). Since today the quality of working life has been considered as a global concept in the field of human resources management and organizational development and its improvement have been considered as a key to the success of any organization management, QWL as one of the organizational improvement techniques is focused by senior managers (12). Finally, it should be said that, achieving the goals of the organization is dependent on factors such as quality of working life of human recourses. Therefore, in order to achieve the goals of the organization and pleasing its members, managers should recognize employees' quality of working life and be aware of how it affects the organization (10).

Delgoshaiy performed a research to compare the quality of working life in educational and non-educational hospitals of Medical Sciences University of Kashan and its relationship with knowledge management from the perspective of senior and junior hospital managers and did receive that there is no Significant difference between the quality of working life educational and non- educational hospitals of Medical Sciences University of Kashan, and relationship between quality of working life and management in each batch of centers were positive (10). Nissi in the study that was aimed to evaluate effective factors in the successful deployment of knowledge management found that average of Ahwaz telecommunications enterprise Leaders are aware of important factors in knowledge management. However, these factors have been less discussed in terms of the practical deployment (13).

Moharramzade conducted a research entitled " evaluation of relationship between organizational culture and knowledge management establishment in the department of physical education in Western Azerbaijan" and the results showed that knowledge management runs successfully in an organization when already infrastructure cultural context appropriate with the system, since guiding existent knowledge in an organization is a popular action and is a function of organization`s staff culture (14). Fallahi Khoshknab`s research among psychiatric nurses of Medical Sciences University of Tehran, showed that the life quality of 24% of nurses participating in this study was moderate. 67% of them have a good quality of life and 11% reported higher quality of life (15).

Dargahi in his research which titled "evaluation of Quality of working life of hospital nurses in Medical Sciences University of Tehran " came to the conclusion that most nurses are not satisfied with their quality of working life elements or components and the majority of them are not satisfied with work accidents, lack of safety rules and discipline in work, get rid of health conditions in workplace, lack of tests and medical examinations periodically, low salaries, facilities, cash rewards and non-cash benefits, indirect benefits, and also job stress and non clear job prospects (16).

This study aimed at improving hospital management were conducted through the determining the quality of working life component influence on knowledge management based on study population`s comments and it is hoped that the results of this study which are presented in the form of reforming proposals focused by the decisions Centers of hospital covered by Medical Sciences University of Ahwaz.

2. Material and Methods

This study is a descriptive - analytical study that was done in 1390 as a cross-sectional method in five

educational hospitals affiliated to Medical Sciences University of Ahwaz, Imam Khomeini, Abuzar, Razi, Golestan and talaghani. The considered statistical population were 70 people that after distributing the questionnaires, 56 senior managers (including a president, manager, assistant, Metron) and middle managers of hospitals (including educational and clinical supervisor, Director of Administration, Director of Medical Records and director of hospital Services) returned their questionnaires.

This study wasn't conducted due to the limited sample of population and all members of society were evaluated. A data collection tool was a questionnaire consisting of three parts: demographic data, quality of working life and knowledge management. In the first part the demographic information including gender, age, education and experience of managers was studied. The second part of the questionnaire that was related to the quality of working life consisted of 29 questions that was prepared according to the quality of working life components (material benefits such as welfare rights and benefits, such as educational classes and workshops democracy in organization such as organization members` voting right, participation in decision making in order to perform activities, job design such as job's characteristics appropriate with employees, and workplace such as safety in the workplace).

The third section of the questionnaire included 24 questions related to knowledge management component includes creation, transfer and retention of knowledge. To score to questionnaire`s options, five degree Likert scale (1 = very poor to 5 = very much) was used. Justifiability of the questionnaire was approved Based on previous survey. The reliability of the questionnaire was 0.86 and 0.92, similar to previous research (10). To Data analysis, were used descriptive statistics (frequency and percentage frequency graphs, charts and statistical summary) for background information such as age, sex, education and corporate email, and were used inferential statistics (Pearson correlation test, Spearman correlation test) for the relationship between quality of working life variables with knowledge management. Average quality of working life factors were classified as: grades 0-3 as poor, 3-4 as medium and more than 4 as strong and for knowledge management variables: less than 2.5 as poor, between 2.5 to 3.5 as moderate, and more than 3.5 as strong. Data analysis was performed using the SPSSv16 software.

This study is a descriptive - analytical study that was done in 1390 as a cross-sectional method in five educational hospitals affiliated to Medical Sciences University of Ahwaz, Imam Khomeini, Abuzar, Razi, Golestan and talaghani. The considered statistical population were 70 people that after distributing the questionnaires, 56 senior managers (including a president, manager, assistant, Metron) and middle managers of hospitals (including educational and clinical supervisor, Director of Administration, Director of Medical Records and director of hospital Services) returned their questionnaires.

3. Results:

According to the obtained Results, 55.4% (n = 31) of the study participants were female and 42.9% (24 patients) were men. Most participants in the study were in the ages ranging between 30 and 40, 40 to 50 years (respectively 39.3%, 37.5% respectively). Most of them were nurses (about 60.7%) and had bachelor's degree (about 78.6%). Average ages of them were 40.44 years old and standard deviation of age was 6.3 years. The average work experience of them was 16.6 years, and the average managerial experience was 9.9 years. Minimum age of participants was 28 years and the lowest work experience was 5 years. The highest work experience was (about 26.8%) between 15 to 20 year and the lowest was (about 19.6%) less than 10 years.

Table 1: demographic information of participants

variant		number	percentage
gender	male	24	42/9
	female	31	55/4
	No reason	1	1/8
educational major	doctor	2	3/6
	nurse	34	60/7
	Medical evidence	12	21/4
	Other fields	8	14/3
education	diploma	1	1/8
	Associate`s degree	2	3/6
	BS	44	78/6
	MS	4	7/1
	P.H.D	3	5/4
	No reason	2	3/6
age	Less than 30 years old	3	5/4
	Between 30 to 40 years old	22	39/3
	Between 40 to 50 years old	21	37/5
	More than 50 years old	3	5/4
	No reason	7	12/5
Work experience	Less than 10 years	11	19/6
	Between 10 to 15 years	14	25
	Between 15 to 20 years	15	26/8
	More than 20 years	14	25
	No reason	2	3/6
managerial experience	Less than 5 years	15	26/8
	Between 5 to 10 years	8	14/3
	Between 10 to 20 years	18	32/1
	More than 20 years	1	1/8
	No reason	14	25

Among the indicators of quality of working life, workspace was at the highest status with average of 3.63 and material resources with average of 2.7 at

the lowest position. Among studied hospitals, average of the quality of working life components ordered from the highest up to the lowest as follow; workspace, education, participation in decision making, job design, democratic in organization and the material points (Table 2).

Table 2: statistical Summary of component of quality of working life score

variant	avrage	median	SD
Workspace in organization	3/63	3/75	0/48
education	3/48	3/5	0/56
job design	3/16	3/10	0/60
participation in decision making	3/16	3	0/78
democratic in organization	3/13	3/16	0/64
material resources	2/70	2/75	0/62
Total Average	3/21	3/13	0/61

In order to assess the level of knowledge management at three levels: low, moderate, and strong for researched hospitals; scores less than 2.5 was introduced as poor knowledge management, between 2.5 and 3.5 as the average knowledge management and more than 3.5 as the strong knowledge Management. The results showed that most of the people in the studied hospitals had an average knowledge management with a frequency of 62.5% and the strong knowledge management had lowest frequency (about 16 percent). Also among the components of Knowledge Management, Knowledge creation (95/2) was in the highest state and storing knowledge (2.86) was in the lowest state (Table 3).

Table 3: statistical Summary of knowledge management components score, separated studied hospitals

Variant hospital	Knowledge creation		Knowledge transfer		Knowledge storage		knowledge management	
	average	Standard deviation	average	Standard deviation	average	Standard deviation	average	Standard deviation
Abuzar	3/15	0/78	3/18	0/74	2/99	0/77	3/10	0/76
Razi	3/12	0/96	3/22	0/76	2/99	0/76	3/11	0/82
Golestan	3/06	0/71	3/07	0/75	2/95	1/02	3/02	0/82
Imam Khomeini	3/05	0/78	2/96	0/94	2/83	0/85	2/94	0/85
talaghani	2/37	1/02	2/60	0/88	2/54	1/12	2/5	1/00
Total Average	2/95	0/66	3	0/81	2/86	0/9	2/93	0/79

Quality of working life in the studied hospitals was evaluated as an average of 3.22 medium. Highest rate of the quality of working life in the studied hospitals were in Razi and Abuzar with the average of 3.36 and 3.34 and Golestan hospital with the average of 2.97 had the lowest quality of working life (Table 4).

Table 4: Comparison of quality of working life and Rank of studied hospital

hospital	working life quality
Abuzar	3/36
Razi	3/34
Imam Khomeini	3/12
talaghani	3/01
Golestan	2/97
Total Average	3/22

To investigate the relationship between the components of the quality of working life with the knowledge management in studied hospitals, Pearson correlation coefficient was used and their significance was evaluated. The significance level of each component of working life quality with knowledge management from the highest to the least relation were respectively, between participation in decision-making, democratic in organization, job design, education, material privileges, workspace and knowledge management. In the final analysis, the correlation between quality of working life and knowledge management in the studied hospitals was about 0.64, which indicates a positive correlation between these two components in above centers and shows a direct relationship between knowledge management variable and quality of working life. This means that if the rate quality of working life in hospital increase, knowledge management score will increase and vice versa (Table 5).

Table 5: Correlation between working life quality components with knowledge management in studied hospitals

variant	correlation coefficient	P-_value
participation in decision-making	0/7	*0/001
democratic in organization	0/6	*0/001
job design	0/44	*0/001
education	0/37	*0/005
material privileges	0/32	*0/01
workspace	0/09	0/48
working life quality	0/64	*0/001

4. Discussions

Management experts believe that salary and rights are known as a factor of making logical connection between the job responsibilities and salary payments to employees, which is difficult and complicated. Appropriate salary has strong influence on behavior, standards of living, purchasing power and emotional and spiritual health of a community and ultimately increases production efficiency in the organization. Unfair wage also reduces the efficiency, economic crisis, social evils behavior and abnormality in the society. So it should be given adequate attention (17). Due to the results of the present study, it was found that there is significant correlation between the rates of material and knowledge management ($P < 0.05$). This means that the increase in the rates of material benefits, knowledge management will increase in hospital too and vice versa. Meanwhile, material privileges in the studied hospitals were assessed poor (76.8%). Mottaghi and colleagues (10) evaluated that material merit of educational and non educational hospital of Kashan was medium. Fattah Moghaddam and colleagues (15) in their study showed that 51% of nurses had material merit below an average, which the results were contrasts with the first study but were consistent with the second study results.

The aim of educating in the field of knowledge management is training the correct way of doing work and decision making. So considering the fundamental role of education in raising labor productivity, it is possible to have a positive impact on their knowledge management by designing and implementing training courses within and outside the country as scientific workshops for updating managers` information. Findings indicate that the relationship between education and knowledge management is significant and education levels in hospitals were assessed moderate. Mottaghi and colleagues (10) assessed that the education level in Kashan hospitals are strong, which were inconsistent with the results of the present study.

Democracy in organization including the creation of open space, making fairness in payment, giving opportunities to people in the related position, and encouragement: can cause creativity and innovation in the organization. This results in knowledge management improvement in organizations. There is a direct correlation between democracy in organizations and knowledge management in hospitals covered by Medical Sciences University of Ahwaz Jundishapur, and statistically it is significant. The level of democracy was assessed medium.

If some activities are governed participatory ultimately in one hand lead to the working life quality improvement, more human satisfaction and dignity enhancement, personal growth and development and on the other hand acceptance of the changes, and so reduce active and passive resistance and the lack of planed and spontaneous cooperation. In the present study, it has been showed that there is a significant and direct relationship between the correlation variables in decision making and knowledge management, but the level of participation in decision making (51.8%) were weak and it should given more attention. These results are consistent with the result of Garmaseh`s study results (18) in hospital managers of educational hospitals of Isfahan.

Quality of working life are linked with organizational changes that mostly are increasing horizontal (career development) and vertical (job enrichment) flexibility, and certainly contain high levels of engagement and motivation to improve and make attractive the work instead of its conditions. The rate of job design for the studied hospitals was intermediate. Alizadeh (19) indicates that in Social Security Hospital in Tehran this factor is poor that are inconsistent with these results.

Workspace has quality, provided that in it persons are counted as the organization's members, the human mind be faced with thoughts challenge and ideas in the environment, the environment condition cause ability growth and things be done as well in the environment (27). For these conditions are safe working conditions in terms of the physical and also logical work hour's determination. Environment should also reduce the pollution effects that can adversely affect physical and mental condition (24).Most of the hospital managers, evaluated that their workspace is average (66.1%) and only 14.3% of them had a strong workspace. Dargahi and colleagues (16) in a research that examine quality of Medical Sciences University of Tehran hospitals nurses` working life concluded that the vast majority of nurses are dissatisfied with their work environment. In this study, it is found that relationship between the workspace and knowledge management is direct. And also it can be said that the relationship between these two variables is not statistically significant ($P = 0/48$)

What happens for man while working is the way of contacting with them, and also this that their work how might affect their overall life. So giving attention to the quality of working life programs as one of the most interesting ways to motivate employees (25) for managers in a position that directly will affects the quality of others working life, is a necessary and important social responsibility (21). In general, most people in the studied hospitals had moderate quality of working life score with frequency of 67.9%. And only 5.4% of the managers

had a strong working life quality that this number is notable. Mofradnya (20) evaluated that Quality of working life in of Islamic Azad University of Tehran is strong. Also Seifi (22) assessed that the Quality of working life in the Sanandaj academic hospitals is below average, which the results of both research are inconsistent with this study.

Among the key elements in achieving to organizational knowledge management are; knowledge availability, accurate and timeliness knowledge, the right culture, employee participation in knowledge management processes, knowledge leadership, knowledge repositories organizational infrastructure (28). The first step in the evaluation of the provided knowledge is the assessment of possibility of knowledge level. In fact, if organization could not measure their knowledge level and could not evaluate change methods in the knowledge level, so the knowledge management cycle will remain at the bottom, Because no feedback will be conducted to make modifications in different components of knowledge management including knowledge creation and development if necessary(7). Knowledge management in studied hospitals mostly was with a frequency of 62.5% and the 16.1% of surveyed executives noted that knowledge management was strong. While Zanjani moghadam evaluated that (26) knowledge management was in his research. Findings from the study showed that the highest correlation between the quality of working life and knowledge managements component was participation in decisions of the Senior and middle managers. While Asgari (23) in the Ministry of Labor and Social Affairs noted that the most important thing to promote knowledge management is work structure. In the present study, between the working life quality variables and knowledge management there is a direct relationship, it means that to enhance the quality of working life score, a score of knowledge management in hospital will increase, and vice versa.

Since among the six components of QWL in this study, participation in employees` decision-making were more important component, it is recommended that programs of improving working life quality in universities` dependent hospitals, to focus on these components development and improvement. Hospital directors and staff participation in decision making and determination of indices of working life quality programs can increase knowledge utilization and managers` information, and thus lead to make better decisions. Working life quality is effective in maintaining the knowledge management, and organizations in order to effectively discipline knowledge and disseminate it into the entire organization, should at first, consider the quality of working life, because if Quality of working life be much better, the knowledge management establishment will be more successful. The obtained results of this study can identify the key needs of employees in the workplace and evaluating strategic plan to enhance the quality of their working life, be used by senior managers and decision makers in the health field.

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Gap between the pattern of food consumption with interests in boy student's

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Abstract: Many of habits and food's profferings are forming in the age of adolsece and youth. One of the most important obstruction's factors of degenerative disease in elderly, is following of balanced and accurate diets. The aim of this study was determining of Gap between the pattern of food consumptioon with interests in boy student's in Mazandaran University of medical sciences in 2012. This cross sectional- descriptive study was done on 350 boy student in Mazandaran University of medical sciences. Researcher questionnaire was used to collect data. In oreder to comparision of food consumtions with students interest in the main meal and the middle meals and also all of food's goods used of t-test and to comparision of gap number in main meal food and middle meal food was used of repeated measurerrs and in ordet to comparision of the gap between consumption and interest of students who is living in dormistory and out of dormitory was used of one-sided variance test and Post Hoc test likeLSD test. Result showed that there is difference between consumption and interests in five main meals (two middle meal and three main meals). Difference mean in three main meal and two middle meal is obtained (-14.3) and (-11.9), respectively. Difference between gap between food consumption and interests in three main meal was significant (p=0.0001).If education presents with food needs and attention to correct food's pattern, habit and food resources, it could be hopefull about development of health education in university students.
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Keywords: Gap; food patterns;interests; boy student

1. Introduction

Nutrition has an important and key role in creation, maintance and health promotion, because the nutrition is necessary for all of vital activity of body (WHO,2001). Malnutrition is one of the roots of poverty, economical retardation and political dependence (Contento,Randell,Basch,2003). Some of the reasons of death and disability among adults, such as vessel- cardiac diseases, cancer, diabetes, brain and lung disease is related to life style and diets in United State and about 65% of adults who were above 20 years old are overweight and fat (Khalili et al. ,2011). In each community, provision of nutrition health make saves the costs of medical services, productivity in investment of educational section and finally, the best education for future generation that is warrantee for political and economic's independence. Respecting to quality and quantity about situation of children's nutrition has placed above the health outline (. Pipes ,Trahms1996). It's seemed that one of the important ways to challenge to problems which associated with malnutrition in children and adolescence is education (Yoon et al, 2000).

Behavioral properties such as food patterns are rare in teens and this is the creative causes of many of mortalities in elderly age, because many of health and unhealthy behaviors which is based in this age will be shown them as consistent behaviors in future (Gupta, Kochar, 2009). Since, young human sources is mentioned as national wealth for sustainable development in each community, planning is uncover able for improving nutrition and health situation in this group. By identifying adolescences problems, presentation of proper procedure is possible (Gracey et al,1996). With respect to the importance of food receiving according to interests and main meal and middle meal to provide nutrition needs and boy student's health, this study was done with goal to assign the gap between pattern of food consumption with boy student's interests. The results could be a good guide to managers in planning, change of identity, and an enrich program for food educational planning.

This cross sectional- descriptive study from practical type has been done on 350 boy students in Mazandaran University of medical sciences in 2011. The method of sampling was multi step process. Students determined base on faculty, educational degree and educational course and students who were finished at least one semester. Then in each category the number of required test were selected randomly. In this study researcher questionnaire to was used to collect nutrition's data in 3 month. This questionnaire was included two questionnaires that called the main

food meals and the middle food meals with whole food groups of 60 goods. For completion this questionnaire, it is wanted from students that anything which have consumed at 3 recent months, at first, they ticked in checklist and whatever was significant consumption entered in main questionnaire. In second stage, it is wanted from students expect of any things that they have used, each goods that they would like to consumption in main and middle meals determine in checklist and those food were more interested inserted in main questionnaireIn the present study we investigate herb species richness (spermatophyte) in terms of taxonomical diversity and species composition in relation to oak and pine forests in Central Himalayan forests.

2. Material and Methods

This cross sectional- descriptive study from practical type has been done on 350 boy students in Mazandaran University of medical sciences in 2011. The method of sampling was multi step process. Students determined base on faculty, educational degree and educational course and students who were finished at least one semester. Then in each category the number of required test were selected randomly. In this study researcher questionnaire to was used to collect nutrition's data in 3 month. This questionnaire was included two questionnaires that called the main food meals and the middle food meals with whole food groups of 60 goods. For completion this questionnaire, it is wanted from students that anything which have consumed at 3 recent months, at first, they ticked in checklist and whatever was significant consumption entered in main questionnaire. In second stage, it is wanted from students expect of any things that they have used, each goods that they would like to consumption in main and middle meals determine in checklist and those food were more interested inserted in main questionnaire. For analyzing data was used of statistical program (Spss 13). In oreder to comparision of food consumtions with students interest in the main meal and the middle meals and also all of food's goods used of t-test and to comparision of gap number in main meal food and middle meal was used of repeated measurerrs and in ordet to comparision of the gap between consumption and interest of students who is living in dormistory and out of dormitory was used of one-sided variance test and Post Hoc test likeLSD test.

3. Results

Findings showed that there is difference between consumption and interest in five main meals (three main meals and two middle meals). Differenc mean in three main food meal and two middle food meal was obtained (-14.3) and (-11.9), respectively. Differenc between gap of food consuption and interests in three main food meal was significant (p=0.0001). By assigning all of goos, there was negative gap in some cases in the both of main and middle food meals. The most gap in main food meals was related to dried fruits and grains (pistachio) 46.6 and the least gap is related to meat groups (hen) -0.6. Also, the most gap in the middle food meals was related to dried fruits and sweet noodles and crushed ice (Faloodeh) -34 and the least gap was related to grains group (split pea) -3.1. frequency distribution of samples in 13 course and six faculty peresented in table 1 from experienced and expert's level. (Table 1: Frequency distribution of samples).

According to findings, if consumption was more than interestes, then this gap considered positive between consumption and interestes. But rice versa, if consumption is less than interests, this gap has cosidered negetive. Table two has shown the percentage of consumption and intereste and also negative and posetive gap rate. (Table 2: Comparison of gap between consumption and interest of the main meal) (Table3: comparison of Gap between consumption and interest in the middle meal).

Table 1: Frequency distribution of samples

Faculty	Major	Sample value	level
Health	Public health	30	undergraduate
	Environmental health	30	undergraduate
	Occupational health	30	undergraduate
paramedical	Laboratory sciences	30	undergraduate
	Anesthesiology	30	undergraduate
	Operating room	30	undergraduate
	Radiology	30	undergraduate
	Medical emergency	30	PhD
	Information technology	10	undergraduate
Medicine	Medicine	30	PhD
Pharmacy	Pharmacy	30	PhD
Nursing and midwifery	Nursing	30	undergraduate
Dentistry	Dentistry	10	PhD

4. Discussions

Today the social convenient in different counteries is evaluated based on various variables such as their nutritional situation and those communities is considered advanced counteries which its people have appropirate health and nutrition. Adolsence ages is the best time for consolidation of correct habit in order to prevent of health and nutritional problems in the next periods of life. Nutritional science is essnetial for developing of nutrition and health (Alam et al, 2010).

Table 2: Comparison of gap between consumption and interest of the main meal

Food group	Food items	Consumption (percentage)	Interest (percentage)	Gap (percentage)
Bread	Bread	97.1	88.9	+8.2
	Rice	93.4	85.1	+8.3
	Pasta	75.7	82	-13.7
grains	Bean	59.4	69.7	-10.3
	Pea	46.9	68.9	-22
	Lentil	60.6	75.7	-15.1
	Spilt pea	50.6	64.3	13.7
Vegetables	Potato	82.9	90	-7.1
	Onion	54.9	66.3	-11.4
	Tomato	82.3	88.4	-6.1
	Cucumber	70.6	78.9	-8.3
	Leave vegetables	64.6	79.4	-14.8
Fruits	orange	59.4	74	-14.6
	Kiwi	45.7	74.9	-29.2
	Banana	59.1	84	-24.9
	Pear	38.3	79.1	-40.8
	Peach	42	82	-40
	Nectarine	38.9	65.7	-26.8
	Cherry	36.6	69.4	-32.8
	sour cherry	32.9	63.4	-30.5
	Apples	71.1	89.1	-18
	Other fruits	37.4	68.6	-31.2
Meat	Red meat	74.6	82.3	-7.7
	Hen	84.3	84.9	-0/6
	Fish	80.3	86.9	-6.6
	Egg	90.6	87.1	+3.5
Dairy products	Milk	84.3	90.9	-14.6
	Yogurts	86.6	86.3	+0/3
	Creamy cheese	68	86.3	-26.3
oils	Vegetable oil (solid)	55.1	42.3	+12.8
	Animal butter	58	47.1	+10.9
	Vegetable butter	48	66.6	-18.6
	Margarine (vegetable oil (liquid)	66	64.6	+1.4
Candy and sweets	Sugar	71.7	71.1	+0/6
	Dates	74.3	87.7	-13.4
	Honey	67.7	90.9	-23.2
Carbohydrate salts and sausage	Pickled cucumber	57.1	59.4	-2.3
	Pickled cabbage	38.6	46	-7.4
	Other pickle	57.7	62.9	-5.2
Grains and dried fruits	Walnut	68.3	82	13.7
	Almond	37.1	76.9	76.9
	Pistachio	33.1	81.7	81.7
	Sunflower seed	38.3	74.6	74.6
	Squash seed	33.1	61.1	61.1
	other	23.7	35.1	35.1
Candy products (confectionary)	Candies	42.9	72.3	72.3
	Ice cream	38.6	52	52
	Faloodeh	28.3	49.4	49.4
	Industry cake	43.7	66.3	66.3
	Biscuit	42	50.3	50.3
Drinks	Doogh (yogurt and water)	73.7	80	80
	beverage	53.1	59.1	59.1
	Barely water	48.3	63.1	63.1
	Industry juice	40	56	56
	Natural juice	44.9	60.6	60.6
	Tea	71.4	87.7	87.7
	other	34.3	35.1	35.1
Other foods		29.1	45.1	45.1
Total		56.7	71.02	71.02

Table 3: comparison of Gap between consumption and interest in the middle meal

Food group	Food items	Consumption (percentage)	Interest (percentage)	Gap (percentage)
Bread	Bread	84	71.7	+12.3
	Rice	-	-	-
	Pasta	-	-	-
grains	Bean	30.9	33.4	-3.5
	Pea	20.3	29.4	-9.1
	Lentil	29.1	36.9	-7.8
	Spilt pea	22.3	25.4	-3.1
Vegetables	Potato	52.9	51.4	+1.5
	Onion	34.7	32.6	+2.1
	Tomato	57.1	63.1	-6
	Cucumber	51.4	73.4	-22
	Leave vegetables	41.7	55.1	-14.4
Fruits	orange	57.4	89.4	-32
	Kiwi	48.9	69.7	-20.8
	Banana	67.3	96.3	-29
	Pear	44	75.7	-31.7
	Peach	44.9	65.7	-20.8
	Nectarine	43.1	61.1	-18
	Cherry	40.3	63.4	-23.1
	sour cherry	40.6	60.6	-20
	Apples	72.6	90.9	-18.3
	Other fruits	37.7	47.1	-9.4
Meat	Red meat	32.9	54.3	-21.4
	Hen	38.3	53.1	-14.8
	Fish	-	-	-
	Egg	71.1	64.6	+6.5
Dairy products	Milk	73.7	86	-7.7
	Yogurts	61.1	55.4	+5.7
	Creamy cheese	50	66	-16
oils	Vegetable oil (solid)	-	-	-
	Animal butter	31.1	20	+11.1
	Vegetable butter	30.3	22	+8.3
	Margarine (vegetable oil (liquid)	31.1	28.9	+2.2
Candy and sweets	Sugar	48.3	57.1	-8.8
	Dates	58.1	70.6	-12.5
	Honey	47.7	77.1	-29.4
Carbohydrate salts and sausage	Pickled cucumber	26.6	19.7	+6.9
	Pickled cabbage	20.3	12	+8.3
	Other pickle	28.9	34	-6.9
Grains and dried fruits	Walnut	56	65.4	-9.4
	Almond	43.7	71.7	-28
	Pistachio	47.4	76.6	-29.2
	Sunflower seed	46	67.1	-21.1
	Squash seed	34.9	68.3	-34
	other	24	53.4	-29.4
Candy products (confectionary)	Candies	45.1	45.1	-19.8
	Ice cream	51.7	51.7	-16.6
	Faloodeh	34.3	34.3	-34
	Industry cake	47.7	47.7	-12.9
	Biscuit	46.3	46.3	-18.3
Drinks	Doogh (yogurt and water)	50.6	50.6	-20.3
	beverage	34.6	34.6	-7.7
	Barely water	48.9	48.9	-7.1
	Industry juice	38	38	-25.1
	Natural juice	42.9	42.9	-10.2
	Tea	74	74	+3.1
	other	25.7	25.7	-
Other foods		26.3	26.3	-3.3
Total		42.6	42.6	-11.9

According to result of this study, the main meal is very important because of the most energy is obtained of them and also defined in the food program of all community. In this study, there is significant difference from the comparision of gap between consumption and interest in the main meal (p=0.0001) and this gap is related to fruits group. But the most of energy's providing in main meal in both interests and consumption field, is related to bread, grains,meat, hen and dairy products. The least consumption is allocated to dried fruits and seeds and the least interests is related to salt and spices groups in the main meal. Students has stated parts of energy and food menu about interesting and consumption to the middle meal as well as light, energetic and nutrition foods. In this study, there was significant difference between consumption and interests in the middle meal (p=0.0001)., as the most gap in middle meal was related to dried fruits and seeds (squash seed) and bakery products group (faloodeh) (-34), and the least gap was related to grains group (split pea) that was -3.1. The most consumption was related to bread and grains in middle meal and the least interest was related to fats and oils groups. In Decarli study and et al, in Switerland, the part of breakfast, lunch and dinner in the procurement of daily energy were 31%, 19% and 29%, respectively. And the part of middle meal was 23% (Decarli et al, 2000).

In middle meal, afternoon snack and lunch in main meal have the most part of consumption. Apple and banana has the most consumption in fruits grups. In the study of consumption pattern in Iran, apple is introduced as the most consumption in families (Kianfar et al,2006).other study state that middle meal has a lot of effect on improvement, concentration powerness, learning and even fatness (Benton et al. 2007). In Esferjani study and et al, in east area of Tehran, the most consumption in middle meal of adolsence were cake, sandwich, fruits, chips, artificial juice and beverage (Esferjani et al, 2006). In current study, ten food of that have the most consomption of main food in student were: bread, rice, egg, yougurt, milk, hen, potato, tomato, fish and date but interested food in main meal were milk, honey, tomato, bread, potato, apple, rice, egg, yogurt and fish. But in middle meal the most consumption food were bread, tea, milk, apple, egg, banana, yogurt, date, citrus fruits and tomato, but the most interested food in middle meal was related to banana, apple, citrus, milk, honey, pistachio, cucuber, bread, almond seed, yogurt and water.

In Santich study and et al, on American teenagers, the most consumption in middle meal was related to rock candy and cabonated drink (Santich, 1995). In Dadkhosh's study, the most procurement of daily energy in main meal was related to lunch (28%) and the middle meal was allocated to afternoon snack (22%). The middle meal was forms the 40% daily energy in students. The middle meal had the key role in the procurement of daily energy even in some parts is more important than main meal like breakfast (Dadkhah et al,2009). Findings in the most students confirm each other, especially in consumption main and middle meals. In the end, the following possibly reasons for describing the gap between intersted and consumption showed that may be it didn't seem in other simillar studies in various population groups and those are:

1. Dormitory,s life in most students
2. having closely nutrition clusters
3. some of nutrient foods are not available easily
4. simillar level of economic situation in students

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The Paradigm of Mobile Government Establishment, Basic Necessity in the Third Millenary

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Abstract: In the new age that attention of all companies and institutions is following to representation of desirable and quick services to all of citizens, we can mention M-government as one of these services. Electronic government and M-government are not separate from each other ,rather, M-government is subset of electronic government that is consist of tool for public operation from governmental services without time and local limitation. M-government makes the new methods for representation of governmental services to citizens that are more comfortable and cheaper than customary methods that cause presentation of desirable services of government to citizens. In relation to M-government, information and communication technology limit to mobile or wireless technology like, cellular cell phones, laptops and personal digital cooperators which connect to wireless regional networks. M-government can help to assemblage of public information and governmental services in everywhere and every time, whereas provide chances to improve the interior operations in governmental organizations. M-government has some premiums like, potential of disruption of frontier, help to creation one digital neural and solid system for government that extremely, cause the increase of efficiency and performance of government personnel, improvement of representation of governmental information and services, increase of channels for public transactions, decrease of cost, increase of participation through to decrease time and increase the communication efforts .This essay proceed the checking concept of mobile government and we try to specify citizen preparation in M-government usages and presenter services of M-government for citizens.
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Key words: mobile government, developmental countries, mobile democracy, mobile government services, global village.

Introduction

In the later decade of twentieth century, governments of all of world establish the proceeding to create extensive amplitude from electronic services by information technologies especially, applications based on internet. These improvements occurred in the countries with slight development at slow rate and when the governments increase the usages from information and communication technology, public requests increase fore more active services. M-government enable citizens to have participation in economical, social and information society activities and will have providence in cost and time for government, people and commerce part. Furthermore, users of information governmental services will be enjoy services with perennial, efficient, seamless and better quality. Citizens need information and sometimes, this information is biotic for them. Information representation to public makes the fortification democracy in suitable moment in countries. Mobile and wireless technologies developments improve the some applications and services of electronic government that are provide by mobile machines and evolve the methods for new applications and services. Mobile technologies prove that mobile is an important way for presentation of immediate information to citizens. The purpose of electronic government is usages from all the forms of innovation, information and communication technology for better accomplishment of government basic rules. Now development of mobile technology with personal digital assistant (PDA) and wireless networks make a new medium for governmental services delivery to citizens with more activities. These developments which new medium has made for governmental services delivery to citizens, is named **Mobile Government**. (Fat hi, 1384)

The countries that do not begin yet or they are in the primary stages of implementation processes electronic government strategy, probably they have more advantages because of problems that government faces with them. In developmental countries, M-government usages can be a key method to citizens and support for information exchanges, especially in faraway villages. It can be an important advantage of M-government in faraway villages

because of insufficient transmission substructures and acceptance of cell phones. (Ibrahim Kushchu, M. Halid Kuscu, 2004). Recently, the accomplished developments in mobile wireless technologies zone have made new ways for more comfortable and cheaper services to citizens. Complex of these modern procedures is named M-Government. Some experts define M-Government to another form: M-Government is a strategy that is consist of making implementation of all kind of mobile and wireless technologies, instrument and services intercommunicate with it and gradation of services representation between components and general institutions include of citizens, commerce part and governmental managements. (Noori, 1386)

Deliberation of global village history and concept

"Global village" is one of the considered discussions in modern society that has absorbed itself to social science and other sciences experts. Global village is derived from one of the Mack Loohan (1911 – 1980) books, Canadian sociologist and thinker, in the name of " fight and peace in global village "that is edited in the 1968, in New York and had a new social theory.

Global village of media (such as, press, radio, television and …) has changed many lifestyles. Now our vast world becomes a big village that acts and thoughts of individuals and populations are not hidden from each other's opinions and the exchanges of thoughts, information, sciences, techniques, arts and advertisements occur surprisingly at high rate and speed. Nowadays, mankind does not need to gather messages and thoughts; also they can read and know messages of thinkers and scientists at their homes. Forming and developing such a space, intimate appearance and development of individualism.

Nevertheless, scientific and technical developments such as, growth of information and communication technology, majority of people have problems in usages of these technologies and they do not utilize basic communication tools and modern knowledge yet.

According to this, we encounter to "global pillage" term against of "global village" term (that discussed about that and history of its pattern), in political science books. This term has been used by a group of contemporary west critics to show that the "global village" which is considered by mack loohan, despite its relative realization, (following to destruct cultures, religions and ethnos) is transferred to global pillage (to pillage reaches part of the world by another part). Looking quickly to formal statistics and reports of international institutions about improvement indexes of two sides of the world, show this pillage explicitly. Global village is a step toward the growth and generalization of ICT culture, promotion and utilization of communication technology and facilitation of its uses, persuasion of industrialists, suppliers, merchants and administrators to present at internet and the other goals.

Mack loohan believes that the modern world is an electronic world. He also says: Electronic environment of modern age will make the old spaces of images which have accustomed to them, unpleasant and worthless.

In the electronic world, events, objects and creatures immerge to each other, have relations with each other, divorcements are wasted, differences are forgotten, resemblances are paraded and in the circumstances, new environment is engendered. We can name it "global village". Fame of " mack loohan" in communication arena, was because of his predictions about future universe that global village was one of the most important of them; the term that was accepted from the all of world.

He predicted that gregarious mediums depredate local distances and all of people are informed from all events which occur in corner of the world such a village. This prediction that occur by element which involve all of people simultaneous in problems such as: '11th September or events of attack to Iraq 'and make a global common sense.

Herbert Marshan Mack Loohan is a Canadian theoretician in literature and communications extents that is known well because of his theories about gregarious mediums. He began to study in university of Torento in 1964 and become famous for his fuller sentence "medium is message".

Her well-known phrase "Global village" shows this thought which collective mediums diminish world to a village by their pervasive cultural effects.

Mack Loohan invited many opinions to competition by his fuller sentence "medium is message". His purpose is that when a message is transferred from any forms of communications, is mainly impressed by the medium which send it.

He believed that mutation of information engenders by modern mediums and said "when humans need information, they can get them quickly from four angles of world. Daily, the vast world becomes small and humans whether want or not, live in such a global village or global tribe. Electronic facilities will obtain this situation.

He believed that each little news of event spread quickly in the world, the village where behaviors of each group are not hidden from the other. (Kazem Zadeh, 1387)

Global village is a fact, whether we want or not, and satellites deny geographic frontiers. At the end, we can conclude that Global village is a grown process which changes all of the systems (especially family system); the changes that we have to continue them

carefully to destroy the plague of this process and attract the advantages.

Description and deliberation of M-Government concept

M-Government can be defined as one of the subsets of E- Government (electronic Government). In this definition, M-Government can be defined as a tool for public efficiency of citizens from governmental services without local and temporal limitation that its purpose is gradation of services representation between all of the social institutions and factures such as, citizens, commerce part and governmental managements. In recent years, mobile and wireless technologies have rapid developments and new methods are engendered for representation of governmental services to citizens which are more comfortable and cheaper than common methods. Complex of these modern methods which makes more desirable representation of governmental services to citizens, is named M-Government. (Omid Malayeri, 1387)

In a comprehensive definition of M-Government, we can express it: 1) M-Government is a strategy that can be for amelioration of different groups profits which work with electronic government consist of, citizens, companies and governmental units (Ibrahim Kushchu, M.Halid Kuscu, 2004). 2) Implementation of governmental services by a mobile platform to secure its users (both people and employees) with usages of information services at every time and every where (Ibrahim Kushchu, M.Halid Kuscu, 2004). Extension of activities in wireless devices enables countries which want to spend their enormous capitals for implementation of electronic government, to be active trough obtain new and actual information to employees of M-government and more extensive limitation from switches to people for transacting with government. Implementation of M-Government makes an advantage to transfer data and mechanism of exchange between governmental units.

M-Government and E-Government are not separate from each other. Concept of E-Government includes usages of all modern technologies for servicing citizens, efforts for decreasing direct references of people to different social managements and institutions and replacing virtual connections to personal references.

With due attention to this definition and M-Government description which was submitted previously, simply M-government can be defined significantly as a subset of E-Government and by its discussion, concept of E-Government enjoys more extension because of instruments which are itinerate in M-Government such as, cell phones, PDAs and laptops.

Although mobile instruments such as cell phones have unique ability to give services presentation, they have some limitations. One of the most important of limitations is impossibility of massive files transmissions on second generation of cell phones (include the most subscribers of cell phones). Now (2006) just 3 percent of describers use third generation of cell phones.

In another phase, SMS is the most usual services which are presented on second generation, only can insert 160 characters in each message. However, these limitations have bounded variety and span of services presentations in M-Government, attractive services are presentable by usages of these existent abilities (Farshid Ghyasi, Ibrahim Kushchu, 2004). Although E-Government and M-Government have common funds, they have some differences: 1) private information: computer can be subscriber between several users, but mobile device have been contoured for one user.2) switching on: contrary to personal computers, most of the mobile devices always are switched at a light mood.3) ability of transport: mobile devices are always assistance with users and one of the applications of them is presentation of information to users momentarily (Betty Yu, Ibrahim kushchu, 2004).

We hope that the concept of M-Government and offered services, with due attention to speed of penetration factor growth of mobile networks technologies in this zone, develop until that represent the concept of E-Government! (Noori, 1386)

Purpose of M-Government utilization

M-Government is like the cash machines**.** Utilizable tools are quick and comfortable in both of occasions. But this is terminal delivery channel to citizens. There is one complicated and expensive substructure. M-Government can be applied as four goals and main functions in governmental part which are abbreviated below:

Mobile communications: improvement of communications between government and citizens (G2C; C2G)

Representation of information to people is a significant activity and strengthening basis of citizens. Without related information, citizens can not represent intelligent opinions and they can not have wise functions in basis of their around issues. Information is necessary to increase transparency and promote accountability.

Mobile tools fix important channel at the disposal of governments so that they can become in communication with citizens **(G2C)**. For example, Singapore's people have election rights to receive warnings in the mold of message for different spectrum of electronic services likes, extension of Roads symptoms, medical experiments for internal

labors, announcement of passport extension, seasonal reminder for parking and parliamentary announcements. The Maltese can register to receive message announcements for court meetings and their delays, extension of driving license, result of tests and direct credit pays from the social security organization. In England, London polices put sending messages in their warning services and they send warnings to the owners of jobs in London for security threats such as bombings. Despite monthly receiving expenses for sending messages, pagers and existence of free electronic post, the numbers of persons who register receiving warnings and pagers, are more than persons who want to use system of electronic post warnings.

Also we can use G2C communications to send necessary and Urgent news. At the height of Sars crisis, Government of Hong Kong sent a text message to six millions cell phones to end pubic wave of fear.

Message services also are a communication channel between citizens and government (C2G). In Philippines, half of the ministries have message services that let to citizens to request information or opine about governmental services and officials or complain from them. In China, cell phone owners can send message to 2987 representatives of people national congress (Hariri, Noori, 1386).

Mobile services: Mobile transactions and pays

Messages and other mobile tools provide a communication channel between citizens and government and make a possible interaction between them.

Karnataka state government has computerized records of lands in India. A problem is that servers which save records are at regional headquarters and villages that do not have telephone lines, can not have access to the records.

Singapore government has concluded that use messages in program of its population increase. Its social development unit Works as an agency to put appointments for educated single persons. This unit sends 40 free messages to its members so that they can contact with eligible professionals. Board of Singapore national library has offered the message service that let their users to check their account situation and fiduciary books and receive reminder messages before the date of fiduciary books. They can also do some works such as, extension of deadline books or fines pays by cell phones (Hariri, Noori, 1386).

Mobile democracy

Mobile polling and usages of messages and mobile tools for participation citizens in political decisions is one of the usages of M-government which have more potential to increase democratic participation. Now there is not any salient Experience in basis of mobile democracy in the developing countries. We have gotten evidence of this article from England experiences. The most of England experiences in basis of electronic polling such as polling by cell phone cause to discovery easy ways to involve citizens in political decisions.

Some concerns must be attended to vote through cell phones that the most important of them are security and secrecy. In traditional polling method, existence of person in polling place is enough. In mobile polling system, the assurance must be existed that the message sender is recorded as a voter and no person can vote more than one time or in stead of the others by this system. In local selections of Liverpool and Sheffield in May 2002, Payne numbers are given to voters that they use them to vote.

Another problem is easing system as far as possible for users. If Payne becomes very long, the possibility may be exist that most of the people forget their Payne. Then there is a problem of phone keyboard to enter the name of parties and candidates. At the end, polling machine must let voters to repeat data and options. In addition to this, capacity of system should be enough so that can work at the peak of work because busy phone lines are as boring as long rows.

Even thought, they are technical problems which overcome with them are more possible than with voters to use cell phones and messages. The recent studies in Scotland and Wales show that 40-50 percent of investigated people have interest in electronic styles include of mobile polling. Even thought, another study that is done recently in England shows that with due attention to this general inclination in electronic and mobile polling, most of the citizens have no inclination to use text message. According to this study, older repliers feel that this style is not proper for them because they do not know how to send text message. The interesting issue was that younger repliers and somebody who use text message feel this style is an easy option, but a little number of them wants to use that. "Why the repliers who believe that mobile polling is quick and easy, do not use it?". They answered this question: "The presented issue is that text message is casual so it can not be used for polling and, sending text message is a fancy communicate tool which is not proper for polling. Apparently the views that are present about technology are the main factor to determine citizens desires to use electronic polling styles.

These findings may have more extensive tacit meaning for all of the M-Government usages and have adaptability with the presented problems in basis of sending unfathered messages to mobile government systems in Philippines. They also show that mobile polling systems should be known as a spiracle

between several polling spiracles such as: internet, post and telephone (Hariri, Noori, 1386).

Mobile management: improvement of domestic operations in governmental part

M-Government provides chances to improve domestic operations in governmental organizations. In these cases, a little example have presented in these usages in developing economics.

Another mobile technology potential is that it can provide inscrutable environment for government staffs so they become permanently in communication with electronic instruments. Information and services can be represented in basis of communication between government and staffs in every time (G2E); Data may be presented in internet of organization network or under control portable instrument (Hariri, Noori, 1386).

M-Government characters and features

Using mobile technologies and usages separate M-Government from other developments in governmental part that use modern technologies such as E-Government.

Based on several researches that are done about M-Government usages and using them, several insulator factors can be defined such a, better attention and personalization in purpose of users and data delivery, being of more proper accessibility, availability and being of more extensive users perfections (Fathi, 1384).

More proper Accessibility and availability

_ M-Government increase usages of internal linear governmental services. People can enjoy external governmental services in everywhere but not every time.

_ Mobile devices are always on. This is different with personal computers because most of the mobile devices always switch on.

_ Mobile devices can be contoured as portable devices that are assistance always with users and for providing quick information to users.

Better attention and personalization in purpose of users and data delivery

_A computer can be shared between several users but mobile devices are contoured for one user. It means that personal information can arrive to special users of especial device in every time.

_M-Government increase usages of internal linear governmental services by people with more personal devices.

Being bigger and more extensive user society:

_M-Government represents services to most of the people through mobile devices.

_M-Government represents services to the many of audiences include some people who do not have any experience about computers and internet, but are active users of mobile communication (Ibrahim Kushchu, Chester Borucki, 2004).

The examples of M-Government usages in developing countries

We should not see M-Government as a new phenomenon. For example, wireless technology always has been a main part of law enforcement. Nowadays, police officers use wireless laptops connected to internet as well as use old two sided radios. When they face to suspicious cars, they can find directly some information through database about car owner, theft of it, existent of it in crime scene, under prosecution of its owner and etc. Hygiene and safety controllers can record their reports in the inspection at real time by a pocket computer or handy terminal. In this way, they do not need paper forms or entering collected data after returning to their offices.

On the other hand, citizens can economize energy and time by having accessibility of governmental network and internet through cell phones and the other mobile instruments. For example, in Malaysia, citizens can deliver information of polling by using of short messages.

M-Government is not only for efficiency, but also give possibility of civil activities to citizens. In Philippines, citizens can help to implementation of laws fighting with the pollution by reporting buses and the other smoke generator universal vehicles through short messages. Short message can be used to make people interfere in fighting of crime and drugs. (Hariri, Noori, 1386).

Norway: Norway tax offices have offered tax pays by using short messages. The tax payers who do not want to fill tax forms and return them in post offices, can send a text message with code words, their social security number and a Payne code.

This new service is beneficial for about 1.5 million Norwegian taxpayer that return tax forms through posts in normal way.

Finland: In Finland, SMS tickets can be used to Helsinky public transportation systems. These tickets can be ordered by sending text message so their bills are published in cell phones bills. The ticket also has been delivered to the transportation company (Hariri, Noori, 1386).

Turkey: Cell phones have penetrated in Turkey. 2303 millions from 6906 millions of turkey population (34%) have cell phone in compared with 403 millions internet users (6%). Numerous mobile applications exist in Turkey that most of them are focus on SMS technology; for example, in Seasye province, SMS technology is used by citizens for tax paying and voting.

Czech Republic: Cell phones have penetrated in 90% of 10 millions population of Czech Republic. Most of the M-Government applications have been

started and experimented especially for representing information to citizens in natural events and criticizes. For example, in several years ago, agency of natural events (storms, earthquakes and etc) use megaphones to inform people that have high costs for governments. In recent years, SMS have been replaced instead of this system and have clinched that this mobile system is more proper and more efficient (Fathi, 1384).

Philippines: The influence of cell phones is 23.8% in Philippines. Most of the users use SMS technology in this country. For example, the service that its purpose is increase of efficiency and delivery speed, is utilized by Civil Service Commission (CSC). People use this system as a weapon to wrest on governmental agency for moving toward this purpose. Before this, services are represented by using the other electronic tools like electronic post and telephones. But in addition to the limitations and high costs of these tools, usages of these services were not easy: With the arrival of SMS in the year 2001, CSC simplified sending complains because usage of SMS is cheaper and faster (Farshid Ghyasi, Ibrahim Kushchu, 2004).

Though the usage of mobile payment in electronic government still limited, we hope that its use becomes increase in interactions with the governments in parallel with development of mobile payment systems from a simple payments for content and digital service to complicate payments between cell phone, bank and operator.

M-Government service for citizens

With regard to the growing use of cell phone technology between most of the citizens, the most important and applicable method in using of M-Government services are using of message services of cell phone to represent services that we introduce several services (Omid Malayeri, 1387).

Function of short text message or SMS in government now is one of the main examples of M-Government that represents message information service through cell phone whether in form of short text message or multimedia message (MMS). SMS is the old technology that has different applications in the all countries of world. Although MMS technically needs cell phone networks based on the 2.5 generation and is presented in less country, can have a more extensive role to represent M-Government services with more abilities compared to SMS such as sending image, text, audio and picture files.

Nowadays, SMS is immensely used to represent all kind of services especially government services to people (G2C). For example, in India, citizens after passport requesting, can send number given by the issuance of passports and be informed from their passport status or in Oman, examination scores obtained more than 200 thousands students of high school is sent through SMS (Noori, 1386).

Possibility to done personal affairs like paying bills and extension of passports:

In developed countries, one of the M-Government services is allocating cultures to users for their personal affaires by government such as, extension of passport, paying all kind of bills and etc. In this method, personal information of each person is recorded completely and with high security, and a security code is sent by using of cell phone and short message to them. In times of using this service, possibility of how deciding and doing costs locomotion is done by using of special security code and user confirmation (Omid Malayeri, 1387).

Preparation of transportation ticket by using of short message service:

In this way, user sends a short message to center of ticket issuance and the Center sends a serial number to a person after deducting ticket sum from electronic account or credit cart of user. User can use vehicles after showing the sent serial number to operator. For example: in German, 40% of passenger ticket reservation is preselled by cell phone. In this way, customer sends a SMS to center of ticket issuance and the Center exports a serial number as a ticket for a person**.** The cost of ticket is calked on monthly bill of customer. When a customer wants to get on common vehicles, can show the serial number (=ticket) and use vehicles after acknowledging of ticket by operator (Noori, 1386).

Park Mobile (using of short message service for paying car park):

The cars that use this service should have special labels on its glasses. Because That ,special controllers can check the cars by sending this labels special bar code to central control system and be informed from validation of users. For using this service, customer sends a short message include of serial number of his car account and parking location code to center, at the beginning of parking and at the during of time completion and leaving the parking time, subscriber send a message of usage time ending to center and central control system adds the relevant costs to cell phone bill based on parking time and location of parking. (Omid Malayeri, 1387).

M-Teacher service:

The purpose of this service in the developed countries is making of more and better communication between home and school. For using this service, parents fill a special form that the status of education students is sent regularly to parents by short message service of cell phone. In this service, the cases such as, student grades, education log, absences, educational progress and degradation of student, announcement of camp celebration, ceremony programs, requesting of personal reference and etc are sent to parents by SMS

(Omid Malayeri, 1387). Also in suitable cases, they want the emails of parents by sending a SMS to observe logs and the other references or refer to school.

Mobile commerce (M-Commerce):

The meaning of M-Commerce is buying and selling goods by wireless tools like, cell phone or personal data assistant (PDA). With regard to the growth increasing of information technology, using of this kind of commerce is strongly usual and has allocated many percent of the daily trade volume of in the world. The way of using short message service in this M-Commerce is that, at the beginning, user goes to services that can be a bank or a credit institute, and make a credit in form of bank account o credit cart. The stores and shopping centers that want to represent services through cell phones, goes to the same services and make an account. The services send a code to user and relevant shopping center that this code is their cultures during buying, customer send a short message that include control cultures, shopping center cultures and relevant cost to center of services and regard to person credit, it check the accuracy of buying and add the cost to the account of shopping center owner. Then the center send a message based on success or failure of doing operations, to shopping center owner and buying is done by approval bought health.

Many countries have funded in M-Commerce part like German, Italia, Japan, South Korea, Singapore, United state, Philippines and China. In German, 25% of cell phone describers purchase by cell phones (Omid Malayeri, 1387). This method is proper for stores that do not have cart reader devices or for people who do not have credit carts.

Polling:

Using of electronic polling systems and its promotion in society is one of the discussion that is not attended enough even in the developed countries. Only small examples of short message service using are seen in polling in different countries that they have been used in different polling programs of television (Omid Malayeri, 1387). One of the electronic methods of polling is mobile polling. Even thought, another study that is done recently in England shows that with due attention to this general inclination in electronic and mobile polling, most of the citizens have no inclination to use text message. According to this study, older repliers feel that this style is not proper for them because they do not know how to send text message. The interesting issue was that younger repliers and somebody who use text message feel this style is an easy option, but a little number of them wants to use that. "Why the repliers who believe that mobile polling is quick and easy, do not use it?". They answered this question: "The presented issue is that text message is casual so it can not be used for polling and, sending text message is a fancy communicate tool which is not proper for polling. Apparently the views that are present about technology are the main factor to determine citizens desires to use electronic polling styles. According to statistics, this country has increased level of people participation to 62% by using different methods of wireless. These findings may have more extensive tacit meaning for all of the M-Government usages and have adaptability with the presented problems in basis of sending unfathered messages to mobile government systems in Philippines. They also show that mobile polling systems should be known as a spiracle between several polling spiracles such as: internet, post and telephone (Noori, 1386).

Status finding Service by cell phone: we can represent services based on status or LBS with installation Software on network by operator. The first LBS with status finding of emergency calls is operated (in Europe with the telephone number 112 and in America with 911). Network finds the exact location of contacter and sets at the disposal of relevant relief's forces (Police, Emergency, fire-fighting). The main advantage of this service is quick helping in emergency cases. Federal Communication committee of America (FCC) obliged all of the operators that operate this service until October 2001. LBS services can have the other applications such as friend finder service. In this service, local situation of person's friend (of course, with satisfaction that previously has been announced to operator) is set at the disposal of person. The advantage of this service is for parents who want to be informed from situation of their children.

SMS and presentable services with it in M-Government:

In this part of article, we will have a review to several services of M-Government that are represented in numerous countries. One of the services features that, we referred to them in the continuance, is being creative. "Creative" sometimes is imagined wrong with "modern". The purpose of innovative products presenting is the processes or new mechanisms which are entered by using of its special advantages in economic cycle (Noori, 1386).

Challenges of M-Government services usages in societies

In addition to M-Government advantages, it has some faults that we deliberate some of them:

Cost: M-Government typically is a still extra channel for electronic government that will cause the additional costs. This situation will have been continued as long as M-Government can become another successor of channels to represent services. Still, with regard to the number of people who will be

probably without mobile tools in expectable future, M-Government will make serious problems for relevant systems with citizens. So, these systems probably cause the cost increasing instead of being successor of costs. At least, some governments have used innovative cost finder strategy like, the arrangements of sharing in costs.

The break of mobile digital: existence of digital break is the most important problem in using of M-Government services in different societies. As it is clear, in one society, all of citizens do not have accessibility to cell phone and most of them use their traditional approaches and do not use modern systems based on M-Government because of its modern and complicated features. So, if government represents service only with this usage of service, some people will not have possibility to use that service. Thus, the most important point to represent M-Government services in each society is creation of proper elimination and correct and normative education of citizens to use this service in society (Omid Malayeri, 1387).

Public mentality: most of people know mobile tool especially cellular telephones, as an entertainment tool. But politics is serious that has difficult choices. A sign that appear from the infrastructure of this tension, is Using of M-Government systems for buffoonery such as factitious messages.

Trust/security: if M-Government wants to include mobile payment system or the other governmental service, should have a good security and trust ability. Still, a break exists in trust ability that should depredate.

Too much of the data: mobile instrument increase world pressures that users are always connect in there. This permanent connection increases the number of messages in circulation and can make a communication storm that sometimes is valuable and sometimes is worthless. (Hariri, Noori, 1386).

Conclusion:

With regard to the represented in this article, we should pay attention that one of the most important challenges of M-Government services development is citizen readiness level. The meaning of citizen readiness is possibility of access and using of information and communication technologies like, internet, cell phones, computers and etc. Although this readiness is high in the developed countries, the status is different in the developing countries. Low readiness citizens can have different reasons including low level of literacy and education, weakness in English language knowing, weak telecommunications infrastructures, low economic incomes of people and government, lack of coherent planning for ICT part and etc. Also to reach to bilateral development, checking the citizen demands are necessary because the success of a society is impossible without considering the people needs of society, so, we have to attend to these points, not only in theory but also in practice:

_Represented services by M-Government cause the better and quick representation services with less limitation to citizens. The basis of more and better using of this service can be engendered by suitable elimination. Using of wireless technologies is one of the best solutions to represent services to citizens with regard to the low-cost and speed of this technology and growing of its growth in societies. Representation of this service is good by relying on service such as SMS for beginning, but we should attend to this point that citizen expects from the government and providing better services become daily increased. So, we should gait toward the use of newer technology with less limitation and faster speed to represent services well.

_Mobile networks technology is daily in developing and the number of their subscribers is on the rise. As with continuation of this process, active companies of this zone and people profit, governments can use these developments as a way to present better and faster services. In addition to this, people predict and need from governments to present suitable services daily become increased. Up to now, the advanced countries use their wire and wireless communication substructure for this purpose and now follow this process on wireless technologies with more emphasis. For the developing countries that have less attention to this field, wireless technologies make a good chance for development of communication and main part compensation of lags because of needing the cost, less time and more extensive usages ability in compared with wire technologies. It seems that the M-Government concept introduction be a good beginning with services such as SMS. But certainly with the widespread and satiate market, progress toward more advanced technologies like MMS is necessary.

_Evolutions in internet services and technologies have redounded developments in E-Government efforts to provide services for citizens and companies. Development in services and relevant technologies with E-Government is happening with noticeable speed in all of the word. One of the revolutionary developments is using of wireless technologies in governmental activities. The number of people who have accessibility to internet by cell phone Become increased quickly and accessibility of cell phone will be a daily usual part of life and government must register its activities with user requests for easier and better interaction.

_Global village is a step toward growth and implementation of ICT culture, promotion and using

of communication technology, facilitating of its usage and also encouraging artisans, producers, merchants and managements to present in internet and the other purposes, certain that Mack Loohan have assimilated the present time to global village.

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Application of Mendel Accountant in Population Genetic Studies

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Abstract: During the last years gene interaction networks are increasingly being used for the assessment and interpretation of biological measurements. Knowledge of the interaction partners of an unknown protein allows scientists to understand the complex relationships between genetic products, helps to reveal unknown biological functions and pathways, and get a more detailed picture of an organism's complexity. Being able to measure all protein interactions under all relevant conditions is virtually impossible. Hence, computational methods integrating different datasets for predicting gene interactions are needed. Mendel's Accountant is an advanced numerical simulation program for modeling genetic change over time and was developed collaboratively by Sanford, Baumgardner, Brewer, Gibson and ReMine.Mendel's Accountant (hereafter referred to as "Mendel") is a user-friendly biologically realistic simulation program for investigating the processes of mutation and selection in sexually reproducing diploid populations. Using a standard personal computer, the MENDEL program can be used to generate and track millions of mutations within a single population.MENDEL provides biologists with a new tool for research and teaching, and allows for the modeling of complex biological scenarios that would have previously been impossible.
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1. Introduction

Population geneticists have used mathematical modeling for over 75 years to understand better how mutation and selection affect population dynamics. Recent advances in numerical simulation and the wide availability of low cost computational resources now make possible an alternative way to understand how populations change over time. Numerical simulation offers the ability to treat complex biological situations where an analytical solution would be cumbersome, if not impossible. Numerical simulation allows the study of the complex interactions of many biological factors simultaneously. This is generally not practical using traditional methods. The numerical approach provides great flexibility and allows a researcher or student to explore parameter space quite rapidly, without detailed knowledge of the mathematical techniques that underlie the classical theoretical approach.

At its most basic level, the task of modeling mutation and selection in a population over many generations can be viewed as a bookkeeping problem in which random events play a major role. Mutations are continuously entering and leaving any population.

When a new mutation arises, it may or may not be transmitted to an individual's progeny, depending on whether or not the chromosome segment carrying the mutation segregates into the gamete from which the progeny is derived. Generally speaking, mutations that occur near one another on the same chromosome are likely to be inherited together. Therefore, tracking mutation location in the genome is important if one desires to account for mutational linkage. In addition, in higher organisms during meiosis there are about two crossovers per chromosome pair (Santiago & Cabellero, 2000). This random phenomenon of crossover also must be part of the simulation in order to treat linkage in a realistic manner.

Random mutations tend to differ greatly from one another in their effects on genotypic fitness. The fitness effect of a given mutation can be positive or negative, can range from lethal to beneficial, and can vary from fully dominant to fully recessive.

How the effects of multiple mutations (at different loci within the same individual) combine with one another (additively or multiplicatively) also influences the overall genetic fitness of an individual. The effectiveness of selection (that is, its power to alter individual mutation frequencies) is limited by the surplus population available, which in turn depends on the population's average fertility level. Selection efficiency is further limited by factors such as random fluctuations in environmental conditions. Generally speaking, reproduction in nature has a significant random component and is only partially correlated with the fitness of the genotype. All these variables influence actual genetic change over time

and must be modeled accurately if a simulation is to be biologically relevant.

2. Approach

Although there are many programs for genetic data analysis, comparatively little effort has been devoted to software development for detailed simulation of the processes of mutation and selection (Balloux, 2001). Numerical strategies for population genetics modeling have been under discussion for several decades (Crosby, 1973; Fraser & Burnell, 1970), yet it is only recently that computing resources have become widely available to allow large realistic forward-time simulations. The forward-time approach offers the distinct advantage of being able to treat random mutations and natural selection under complex mating/recombination scenarios.

Mendel represents an advance in forward-time simulations by incorporating several improvements over previous simulation tools:

(1) Mendel adds the ability to model mutations as having a continuous, natural distribution of mutation effects.

(2) Mendel allows a user-specified ratio of dominant to recessive mutations.

(3) Mendel uses an infinite sites model, where segregating mutations are distinct and their number is unlimited (or limited only slightly by computer capacities).

(4) Mendel incorporates the concept of heritability and accounts for environmental variance.

(5) Mendel uses realistic chromosome structure with realistic stochastic crossover and recombination, and a high number of linkage blocks (up to order 105).Users can specify the number of chromosome pairs.

(6) Mendel is tuned for speed-efficiency and memory usage to handle large populations and high mutation rates.

(7) Mendel allows control of genetic parameters via a graphical user interface (Figure 1), thereby allowing non-programmers to construct sophisticated simulations.

(8) Mendel provides several forms of graphical output, allowing the user to see the results as the simulation proceeds (Figure 2 shows one of the plots).

In addition, Mendel provides a variety of options for mating, bottleneck events, and population substructure. It is computationally efficient, allowing many problems of interest to be run on ordinary personal computers. In addition, because Mendel is parallelized with MPI (Message Passing Interface), it can exploit multiple processors to run:

(a) Multiple interacting heterogeneous tribes (b) multiple replications of a single case, or (c) a very large population comprised of sub-populations but with sufficient migration to maintain a high degree of genetic homogeneity.

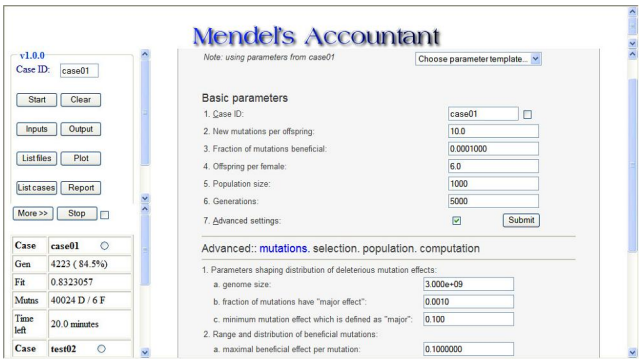

Figure 1. Web user interface of Mendel ' s Accountant showing a portion of the input window

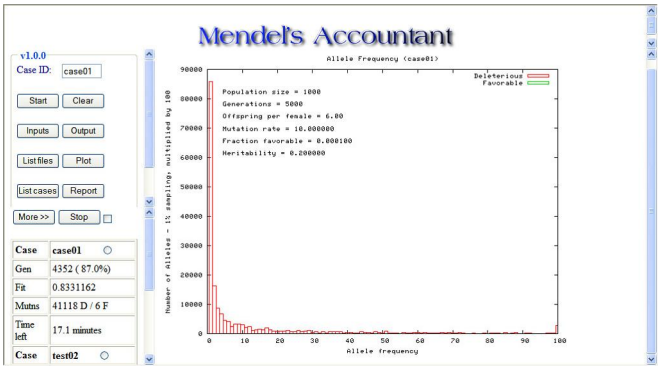

Figure 2. Web user interface of Mendel ' s Accountant showing one of the several output plots the program generates. This plot displays the distribution of deleterious mutations with respect to fitness effect. Red bars represent mutation distribution in the absence of selection. Blue and green bars represent actual accumulated recessive and dominant mutations, respectively, in the presence of selection. The two bars representing mutation classes with effects nearest zero extend beyond the vertical scale of the plot.

3. Analysis

Mendel's input parameters include: number of offspring per female, mutation rate, fraction of mutations which are beneficial, fraction of mutations that are recessive, high-impact mutation threshold, fraction of mutations with effect greater than threshold (two parameters that specify the distribution of mutation effects), number of linkage blocks, number of chromosomes, genome size, mutation effect combining method, heritability of genotypic fitness, type of selection, number of generations, and population size. Mendel's output report is provided at regular generation intervals and includes summary statistics on number and types of

mutations, mean population fitness, fitness standard deviation, and related information. In addition, data for each generation is stored in various files and is also plotted in output figures.

In the example we present below, we employ the following input parameters: number of offspring per female = 6 (4 surplus offspring selected away), mutation rate = 10 per offspring, fraction of mutations which are beneficial = 0.01, fraction of mutations that are recessive = 0.8, high-impact mutation threshold = 0.1, fraction of mutations with effect greater than threshold = 0.001, number of linkage blocks =1000, number of chromosomes = 23, genome size = 3 billion, mutation effect combination method = multiplicative, heritability of genotypic fitness = 0.2, type of selection = probability, number of generations = 5,000, and population size = 1000. Although the current human population size is more than six billion, we have found that population sizes above 1,000 result in only marginal increases in selection efficiency. It is reasonable to expect that, beyond a certain level, larger population size will not result in more efficient selection, because of increased environmental variance.

Some of the output from this example is displayed in the following figures. Fig. 3a shows the mean mutation count per individual plotted with respect to time. A noteworthy aspect of this figure is a nearly exact linear accumulation of mutations, a feature we observe consistently across a broad region of parameter space. The slope of this line is governed primarily by the mutation rate. Selection intensity modifies the slope of this line only to a limited degree. This is because of the preponderance of unselectable "nearly-neutral" deleterious mutations (as further described below).

Fig. 3b shows an initial non-linear genotypic fitness decline, which soon becomes essentially a linear decline. We observe this pattern across most of the parameter space we have explored. Mendel defines an individual's genotypic fitness as 1.0 plus the combined positive and negative effects of all the individual's mutations. In this case mutation effects are being combined multiplicatively. We have found that the slope of this curve (fitness change over time) is determined primarily by three things – the mutation rate, the average mutational effect, and the selection intensity.

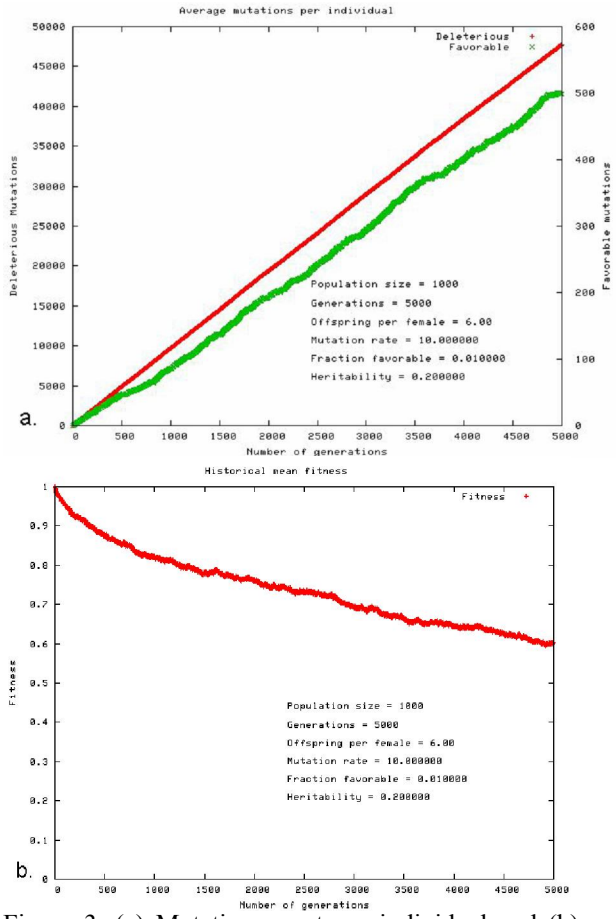

Figure 3 .(a) Mutation count per individual and (b) mean population fitness, plotted for 5,000 generations. (a) shows that deleterious mutations accumulate in close to a strict linear fashion (reaching 47,730–scale on left). Beneficial mutations also accumulate in a linear manner, but their lower number results in sampling error fluctuations (reaching 498– scale on right). (b) shows a progressive decline in population fitness. oak forest (at HB, 30) as compared to pine forest (at HB, 23). Species richness was higher (7.4) at HB and lower at HT (5.0) in oak forest. Similar pattern was found in pine forest, i.e., maximum species richness was at HB (10.5) and minimum at HT (4.7).

Fig. 4 shows the distribution of mutation effects of accumulating deleterious mutations. Mendel employs a distribution of mutation effects (prior to selection), which reflects what is found in nature – a continuous distribution essentially exponential in character. Input parameters such as genome size and the fraction of high-impact mutations define the exact shape of the mutation-effect distribution curve. Because of the shape of the mutation-effect curve, lethal mutations will always be very rare, and a large fraction of deleterious mutations will have near-zero impact.

When strong selection is applied, regardless of the other input parameters, high impact mutations are consistently eliminated quite effectively – especially the dominant ones. However, across a wide range of parameter space the bins nearest to zero fill at essentially the same rate, regardless of whether or not selection is being applied. Experimentally, these "nearly-neutral" mutations are consistently found to be un-selectable – in accordance with mathematical theory (Kimura, 1979; 1983). Mutations with intermediate levels of impact accumulate at intermediate rates. The transition zone between selectable and un-selectable mutations is very wide, especially for recessive mutations. The actual point at which mutations become un-selectable depends on numerous input parameters, but is readily apparent in Mendel's output for any given scenario.

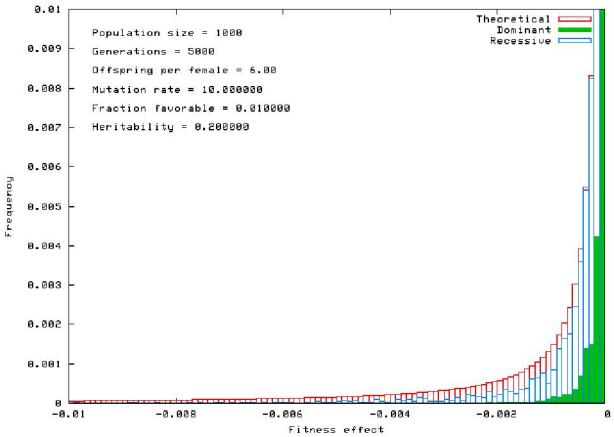

Figure 4. Distributions of accumulating mutations are shown above. Red bins represent the expected mutation accumulation when no selection is applied. Blue bins represent actual accumulation of recessive mutations. Green bins represent actual accumulation of dominant mutations. The magnitude of each mutation's effect is shown on the x-axis, which is a linear scale. The bin nearest zero represents mutations which change fitness by a factor between .0001 and .00001. Mutations with a magnitude of less than .00001 were not tracked or plotted.

4. Conclusions

The program Mendel's Accountant provides a biologically realistic platform for analyzing the problem of mutation accumulation. This program demonstrates that the problem of deleterious mutation accumulation is very serious under a wide range of scenarios and across a vast portion of parameter space. The relentless accumulation of deleterious mutations is primarily due to the existence of un-selectable "nearly- neutral" mutations, but the genetic load problem is greatly amplified when mutation rates are high. Intensified natural selection only marginally slows the accumulation of deleterious mutations. Preliminary Mendel experiments indicate that the most effective means of slowing mutation accumulation and reducing a population's genetic load is by reduction of the mutation rate. This study clearly indicates that more research is needed. Mendel's Accountant is freely available to users and can be downloaded at either http://mendelsaccountant.info or http://sourceforge.net/ projects/ mendelsaccount.

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Histological and ultrastructural studies on the effect of Costus Plant and Amphotericin B on male lung rats infected by *Aspergillus niger*

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Abstract: This work amid to study the use of costus plant in the treatment of pulmonary infection caused by *Aspergillus niger* instead of the chemical drug (Amphotericin B), The experiments were conducted using 90 white male rats that were divided into the following groups : Group one consisted of the control 30 rats which orally administrated with distilled water. Group two comprised 10 rats treated with the fungus suspension. (0.4mg/ kg b.wt.) Group three comprised 40 rats treated with costus plant extract and divided into four subgroups. Group four comprised 20 rats treated with Amphotericin B (0.2mg/ kg) which was divided into two subgroups, then sacrificed and dissected. Then biopsies were taken from the lunges of the various groups of rats and placed in various fixatives in order to conduct the histological and ultrastructural studies. It could be observed from examination of the histological and ultrastructural sections of the lungs of the rats infected with the fungus, the appearance of numerous lymph inflammations especially around the bronchioles. In addition, some reduced with degenerative walls, In case of rats treated with costus extract, the lung tissues appeared normal. Also the rats infected with fungus then treated with costus, the interstitial tissues nearly restored its normal shape and appeared free from cytoplasmic degeneration. On the other sides the histological and ultrastructural sections of rats treated with Amphtericin B after fungus infection showed deformed lung tissues.

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Keywords: *Aspergillus niger (Aspergillosis)* - Costus Plant - Amphotericin B – Histological and Ultrastructural studies - lung - male rats

1. Introduction

I-Aspergillus niger:

Alexopoulos and Mims,(1979) The Family : Eurotinceae, is considered from the important families because it comprise famous genera with of the major factions great economic importance (Alexopoulos and Mims,1979). This Family is called also Aspergillaceae because the conidial fungus were obvious which preferred in studies than the complete phases. *Aspergillus niger* one of the most famous species of the genus *Aspergillus*.

Species belonging to this genus are from the most non living medium prevalent in nature, where fungus spores present in the soil,air and grow on any one of the most causes of pollution in the laboratories. Aflatoxil B was discovered in 1962.

1-Aflatoxil B1:

The most of species are harmful and cause diseases for Humans and animals which known as *Aspergillosis*. Furthermore, Hoshino *et al.* (1984) indicated that with fungal infection *A.niger* cause Allergic bronchopulnonary *Aspergillosis*.

According to Bennett (1980), the role played by *Aspergillus* in lung diseases is not clear, where as the Fungal hyphae bronchitis, in addition, the effects and the sensitivity to anti fungals that can. cause serious damage to bronchial constriction, and that the fungal invasion of lung tissue confined entirely in patients suffered from immune deficiency.

The infection with *Asperigillus* is characterized by the presence of inflammations,of the skin, external ear, sinus, eye socket, eye, lungs, pleura, chest cavity, bones, brain and meninges, heart valves, rarely throat, vagina and uterus Mashni (1998).

Abdel Hameed (2000) concluded that the spore extracts of some species of *Asperigillus* have carcinogenic effect on the young ducks. Johnson *et al*., (1998) indicated that the development evolution of SARS arises from abnormal immune system unable to resist infections, causing an inability to blood oxygenation, leading to lethargy and shortness of breath associated with excessive mucus secretions in the airways leads to coughing breathing and difficulties and an increase in the number of white blood cells, neutrophils and monocytes.

The diagnosis for this disease indicated inflammation of the air bags and narrow bronchi and pulmonary Odema and an increase in the number of pulmonary acidic blood cells (Davidson, 2006)

II-Costus:

Costus means in Arabic Oud, so it is possible to say marine costus or marine Oud, because Arabs brings it from the sea.

There are two types of Costus, the first is marine or white or sweet, and the second type is an Indian or

black or bitter, and the Indian is more hotter than the marine type.

The Oud is taken from Costus germling whith 1.5 mm length which has leaves, stem and root, and present in India.

The root peels are the active therapeutic used parts which are white or black (Alamuel, 2006).

Scientific classification of plants Costus:

Kingdom : plant
Division : Spermatophyta
Sub.D: Angiospermae
Class : Monocotyledoneae
Order : Scitamineae
Family :Zingiberaceae

Lawrence (1969) has pointed that the family Zingiberaceae divided into two sub families Zingiberadeae and Costusedeae.

The second one comprised four species from which the genus Costus.

The benefits and medical uses of Costus plant :

Al-Tirmidhi narrated from Zayd ibn Arqam that the prophet peace be upon him said: (Treat pleurisy installment by marine Costus and oil).

In the novel of the Albokhari from Umm Qays bent Muhsin said: I heard the prophet peace be upon him say: (you must use the Indian Oud which has seven cures: Istatt its virginity and the evidence of pleurisy).

Also, prophet peace be upon him said(which has seven cures) Bukhaari said: Rawi said: "I heard Al-zahrni say: he showed us two not five Hajar said: signed as well as the shortcut to talk about two of the seven is either shortened by the narrator or talk about two for its existence only. And he (the evidence of pleurisy) means Isagah in a naughty mouth, a warning as to the method of watering when the remedy for the patient to sit Aeetmkn believe eating raises his hand when he had severe pain arch is watered rights in a naughty mouth.

Baghdadi said the conciliator (and collected peace be upon him straight between the cupping and the secret to a nice, if painted by a scalpel cupping with no lag in the skin effect Almcharit This is an oddity of medicine, these effects if it grew in the skin may fancy that they saw the flash and vitilligo or dislike where these effects with the cupping aware of what he believes it is.

The Costus may make it the prophet be upon him represent what many take any medicine by the benefits of semi-paralysis and move the benefit Beh, a snake venom antidote, and Achtmamh dispelled by the cold, fat and useless back ache.

Vool and Maleeva (1952) stated that there are many kinds of Costus, they emphasized the medical benefits of three types: *Costs arabicus* which is common in South-East Asia and cultivated in India and Indonesia and is used therapeutically in the chest diseases cough and asthma.

Costu afrri which is common in tropical Africa, the powder of dried stems used for cough treatment whereas, the dye prepared from roots used in a pharmaceutical formulation for sleeping sickness, leaves are boiled and applied topically to treat Alrthyp, and the boiled roots used as local cure to heal skin ulcers. *Costus spicatus* is wide spread in Colombia and tropical America, especially in Peru, Guyana and used medically to treat chronic bronchitis and enteric fever and typhus.

Dutt ***et al.*** (1960) and Sastry & Dutta (1961) confirmed the effectiveness of Costus plant in the treatment of chronic bronchitis inflammation and asthma.

Also, Cruz (1965) found that the injection with derial parts of Costus was effective in the treatment cold and sore throats, dysentery and diarrhea.

The results obtained by Whistler ***et al.*** (1976) indicated the polysaccharides present in Costus and the use of its roots in traditional medicine in Brazil improve the phagocytosis and provide vascular protection in the reticulo-endothelial system of the blood vessel.

Otrero *et al.*(2000) proved that 13 of the 74 extracts of Costus,used by the premium traditional healers to snake bites in the north-western Colombia were effective against the lethal effect of the poison Bothropsatrox.

Pandey *et al.*(2007) isolated many biologically active compounds from Saussurea Costus which were costunolide, dehydrocostus lactone and cynaropicrin. So it is appropriate to develop its use as medicine.Vijayalakshmi &Sarada (2008) stated that the polyphenols content of extracts of Costus than in leaves was higher in the roots and stem cortex. Thus, they proved the effectiveness of these parts as alternative for chemical antioxidants.

III-Amphotericin-B:

Amphotericin-B was discovered in (1956) and isolated from the spore of *Streptomyces nodosus* and the radial anaerobic fungus isolated from the Orinoco river in Venezuela.

Amphotericin-B belongs –to the family of a Polyene macrolide and effective against the fungal mucosal inflammation and Aspergillosis, and has limited activity against the protozoa and does not have antibacterial action. The drug release deoxy cholale in the blood whereas, the remnants of Amphotericin-B in the plasma constitute more than 90% in the plasma that sour rounds protein mainly beta lipoprotein. The effectiveness of anti-fungal A mphotericin – referred to the part interact with the sterol and ergosterols in the plasmal membrane of the fungus such interaction with the. cell membrane leads to the formation of

holes or channels which leads to an increase in the permeability of the membrane and allow the passage of small molecules, furthermore, oxidative damage was observed in the cells of the fungus. (Goodman and Gilmans, 2001)

Demarie *et la.(*1994) observed the accumulation of Amphotericin-B in the liver and spleen, they also noted that liver toxicity is not considered the principle of the drug.

Johnson *et la.,.*(1998) indicated that the pain associated with injection as in the back, abdomen or sometimes the chest usually occur in patients who take the first few does.

Bekersky *et al.*(1999) mentioned that doses above 10 mg/kg that rarely given to patients, causing no deaths, where the doses of 8 mg/kg were highly toxic in dogs.

The therapeutic dose of Amphotericin-B ranged from 0.5 to 0.6 mg/kg b.wt. with daily slow intravenous injection from the most fundamental effects of Amphotericin-B, fever, chills, and sometimes an increase in respiratory rate, reduction of blood pressure or hypersensitivity, also the metabolic requirements may be disturbed. And Amphotericin-B decrease the production of hemoglobin, causing anemia of red blood cells and which supressed the following treatment slowly and sometimes a lake of platelets and white blood cells was observed also furthermore, tissue damage sustained to the renal tubules and disturbance in renal function were observed by Goodman and Gilmans, (2001).

Vogelsinger *et al.* (2006) indicated that the levels of Amphotericin-B were high in the liver and spleen, followed by kidney, heart muscle and brain, while concentrations of lipo- Amphotericin-B were high in the lung.

2. Materials and Methods

1-Costus: (Fig1)

Costus extract was prepared by heating 100 ml of water, then 10 grams of powdered roots of Costus, were added boiled for 3 minutes, covered and stored in a refrigerator at a temperature of less than 25 (Adzu *et al.,*2001).

The animals were treated by this extract orally using stomach tube.

2-Amphotericine-B:

The Molecular formula of Amphotericine-B is $C_{47}H_{75}NO_{17}$, and its Molecular weight is 924. 084.Ten ml of sterile water were added to the vial content for injection.

Thus, the concentration will be 0.1 mg/ ml and kept at temperture2-8^{o}C.

The drug was given to the animal by Alveli intravenous injection(Goodman and Gilmans,2001)

3-Aspergillus niger:

A. niger was obtained from the National Research center Microbial and Natural Products- Arab Republic of Egypt. Cultivation and preparation of spore suspension were carried out in microbiology laboratory-faculty of Education-Jeddah.

A – Preparation of spore suspension of *A.nger*:

The spore suspension of *A.niger* was prepared from two days old culture grown on solid Sabourad dextrose medium by adding 5ml of phosphate buffer solution according to the method adopted by Hadecek, and Greger, (2000).

The animals were treated by diluted spore suspension(1x10-3) by distillation in the nose.

B-Determination of dry weight of *A. niger:*

The conical flasks containing different concentrations of costus extract (5, 10, 15 ml/ 100ml of culture medium) in addition to control flask were inoculated with 5 mm diameter discs from 6-days old cultures of *A.niger*, incubated at 25-2^{o}C then filtered after three days.

C- Antagonistic tests:

To test the antifungal of activity Costus against the pathogenic fungus, a disk of *A.niger* (5mm diameter) was inoculated in the center of sterilized petridish containing sterilized solid sabourad dextrose medium. Thereafter, 0.25 ml of water extract of costus were added in a hole (5mm diameter) was obtained in the solid medium. Control samples were obtained without the addition of Costus extract to the fungus. The dishes were incubated at 27^{o}C. Six replicates were done for each treatment (Sastry and Dutta,1961)……

4-Experimental Animals:

In this study, 90 male Albion rats *Rattus norvegicus,* of 21 days old (the age of post-weaning) and weights ranging from (50-60 grams), were obtained from the King Fahd Center for Medical Research of the University Of King Abdul Aziz.

All transactions injection and autopsy, taking of samples, dying of histological sections were carried out in laboratories for Faculty of Education for girls in Jeddah.

Experimental animals were divided into the following groups:

First Group:

This group comprised 30 control rats, they were given distilled water through mouth during the experimental period.

Second Group:

This group included 10 rats treated with the spore suspension of *Aspergillus niger* and injected with a dose (0.4mg/ kg b.wt.) by nasal distillation, six doses every other day for two weeks and then two weeks after the last dose.

Third Group:

This group comprised the animals treated with extract Costus and the 40 mice are divided into 4 subgroups as follows:

A- Comprised 10 rats treated only with Costus extract by oral administration with a dose of (0.2mg /kg), daily for three weeks and then examined.

B- Included 10 rats treated only with. Costus extract and given at dose of (0.4mg/ kg) by mouth, daily for three weeks, and then explained.

C- This subgroup comprised 10 rats treated with *A.niger* spore, suspension(0.4mg/ kg) by distillation in the nose six doses every other day for two weeks and then treated after Two weeks at a dose of Costus extract (0.2mg/ kg) by though daily oral injection for 10 days and then examined.

D- and included 10 rats treated with *A.niger* a dose of (0.4mg/ kg) through Distillation intranasal six doses every other day for two weeks and then treated after two weeks with Extract Costus at a dose (0.4mg/ kg) by though daily oral injection for 10 days and then examined.

Fourth Group:

This group comprised 10 rats infected with *A.niger* (0.4mg/ kg) by nasal distillation, six doses every other day for two weeks. Then they treated with Amphotericin-B(0.2mg/ kg) by daily intravenous injection for 10 days and then explained according to Vanetten *et al.* (2000).

Note that he has been appointed effective doses, and also different concentrations of all transactions after Make several initial test results for each of the materials used in this research.

Histological and ultrastrustural studies:

At the end of the experimental period, rats were killed by chloroform, lung samples were taken, cut and placed in various fixatives in order to conduct the histological and ultra structural studies.

Dehydration, clearing, paraffin embedding and cutting of samples (3micron diameter) were carried out. The sample painted by haematoxylin and Eosin stain and blue Toluedin for histological studies. (Lillie, 1965).

Ultrastructure studies by using the transmission electronic microscope (Robenson *et al.,* 1987).

3. Results and Discussion

I-microbial studeis:

As appeared from fig. (2), the extract of Costus plant showed highly effective ant agonistic activity against the pathogenic fungus *A.niger*. As shown, inhibition zone surrounded the holes containing the Costus extract and prevented the fungal growth compared to the control sample at which the fungal growth occupied the hole petridish.

Also, it could be observed the sharp decrease in the biomass of the fungal mycelia as a result of Costus extract treatment Thus, the inhibition of fungal growth reached 97.2% at conc. of 15% of Costus extract compared to the control sample (Fig.3).

II: Histological and ultrastructure studies:

First Group (Control samples) :

Histological studies:

The respiratory system consists of the lungs and air ways, which in turn are divided into respiratory passages. In the embryo in a manner similar to the emergence of glands of the dermis, and Bronchi the lungs protected inside the respiratory girdle. Lung consists mainly of and Bronchlotes and alveoli in addition to the blood vessels nerve fibers and a few connective tissue. The pulmonary components the external side to are alveoli as follows: primary Bronchi, Secondary Bronchi, Tertiary Bronchi, Bronchioles, Terminal bronchioles, Respiratory bronchioles, Alveolar Ducts, Alveolar Sacs and Alveoli.

Bronchioles:

It is composed of inner layer in the form of clear by which have ciliated columnar epithelial cells with few goblet cells and smooth muscle layer surrounding the original Lamina propria and surrounded from the outside by Adventitia. There are no glands or cartilage, near the Bronchioles near of the bronchlole there is a branch of pulmonary artery, and surmounted by Alveoli (Fig. 4).

Terminal bronchioles:

Terminal bronchioles are Lined with cuboidal ciliated epithelium without goblet cell and replaced by Alkosip Clara cells (tall columnar cells with apical secretory granules), thick layer of smooth muscle thin plate and surrounded from the outside by adventitia terminal Bronchioles have no cartilage or glands and accompanied by branch of the pulmonary (Figs. 5 & 6).

Respiratory bronchioles:

Respiratory Bronchioles are lined by cubidal ciliated epithelium lining the epithelial cells and the number of few of non-ciliated cells called Clara cells which replace goblet cells they are surrounded by smooth muscle layer which surrounded by the elastic fibrous connective tissue and each respiratory Bronchiole divided into several alveolar ducts which turn end by alveolar sac which open into several alveoli.

Alveoli & Interalveolar septa:

Each Alveoli include pocket open on one side of the route of alveolar septum composed of three components: epithelial surface, connective tissue and blood vessels. Epithelial layer which constitute the continued lining vesicle include two types of cells (Fig. 7): The first type includes most of the liposome surface area that are heavily covered with cells called squamous cells lining the vesicles P1 air pneumonia (cells lining follicular) (Pneumocytes) type II

epithelial cells known as P2 cells lining respiratory alveoli (Pneumocytes) which occupy a small proportion of about 5% of the liposome surface. Capillaries form most of alveolar septum and that branch and intertwine to form basket-like arrangement on each alveoli. Based on the cells lining the convex side the membrane while the basal cells lining the vascular poetry and the concave side next to the red blood cells within the capillaries. The barrier between alveoli composed of capillaries surrounded by vesicular network formed of elastic collagen fibers with of squamous epithelium of neighboring on both sides of the capillary network, also contains barriers acinar vesicular pores, which allows some movement of air between neighboring vesicles. Thickening of collagen fibers and elastic fibers around alveolar opening and constitute the support of the lung tissue (Garner and Hiatt, 2006).

Ultrastructural studies :

Bronchioles:

Terminal bronchioles are lined by cubic ciliated epithelial cells or Non-ciliated cells. The ciliated cells move the secretions and prevent the arrival of particles into the throat whereas the non-ciliated cells called Clara cells are the main characteristic of the terminal bronchioles secretory function.

Clara cells have a head like a dome for filling the region apical granules secretory dense irregular shape of the article kleikoz Ominoclaekanat that may maintain the lining of the bronchi. Clara cells contain mainly large mitochondrial and the base contain a nucleus and a rough endoplasmic reticulum with patches of glycogen, and the tops of the network are smooth and a Golgi apparatus which is not developed (Fig. 8) (Johnson,1991)

The Clara cells have three important functions:

1. Produce components of Surfactant which kleikozominokleikinat.
2. Serve as producing cells. They are capable of dividing (Figures 17,18) and replacing affected cells.
3. Contain a device capable of enzymatic detoxification of harmful substances.

Alveoli:

Vesicles are the unit of the basic structure and function of the lung. A vesicular wall called the Interalveolar Septum consists of five major types of cells:

- (Endothelial cells) 30%.
- (Type I Pneumocytes) 80%.
- (Type II Pneumocytes) 16%.
- (Interstitial cells) include (Fibroblasts) (Mast cell) 36%.
- (Alveolar macrophages) 10%. (Young *et al.,* 2000).

Endothelial cells:

Endothelial cells of the blood vessels capillaries are very thin and can be easily suspected with cells of type I squamous P1, which is based on cells of the first type found on the convex side of the membrane base while the cells lining the vascular noodles on the concave side and next to any red blood cells within capillaries,

the endothelial cells of vessels are related and non-perforated, gather the nuclei and other organelles to help areas in the cell to be very thin in order to increase the efficiency of gas exchange. A very notable appearance in the flat parts of cytoplasm is the presence of many Pinocytic vesicles as in (Fig 9).

Type I Pneumocytes P1:

Most of the surface area is covered with a large Squamous alveolar cell, called cell lung Type I Pneumocytes (P1) Alveolar lining cells.) Alveolar lining cells are highly squamous splayed and essentially intertwined in a lined vacuum vesicular, these cells have a strong nucleus distinct from the nuclei of cells lining the capillaries, cytoplasm has organelles such as Golgi apparatus and endoplasmic reticulum and mitochondria that accumulate around the nucleus and thereby reduce the thickness of the blood-air barrier, leaving large areas of cytoplasm free of organelles.

Cytoplasm contains in the thin parts Pinocytic vesicles, which play a role in the transformation of turnover of surfactant and the removal of pollutants in small plywood from the outer surface, ribosomes are organized in bundles within the cytoplasm, even in most areas and vulnerable cells contain pneumoniae P1 contacts applied Occluding junctions working to prevent the leakage of tissue fluid into the vacuum vesicular. The main role of these cells is to provide a thin barrier which douches gases easily

Type II Pneumocytes P2:

Among squamous cell lung P1, there are spherical cells called (Type II Pneumocytes P2), or Surfactant cells. There are connections associated between the cells of P1 and P2.Usually P2 is at the sites which combines the alveoli composing angles that have almost cubic shaped cells. These cells have a large spherical Nucleus large,Foamy cytoplasm vesicular shape due to the presence lomellar bodies that contain concentric or parallel plates. Studies in Chemistry tissues showed these Lomellar bodies contain phosphorous laminate fat from Type Dipalmatoyl Lecithin and Kleikozominoclaekanat and proteins.

These objects are considered as granular glands (Surfactant) which is working to reduce the strain on the surface tension of the cells in the alveoli and prevent closure of lung during exhalation and reduces the amount of energy required to emphysema during inspiration. Also, cytoplasm contains cells of type II,

the mitochondria and a rough endoplasmic reticulum, free ribosomes, a well-formed Golgi apparatus and and a number of vesicular objects and Cytossmes. P2 is surrounded by basal membrane and a small proportion of its surface exposed to vesicular vacuum and showing microvilli related to secrete a material called Surfactent as in (Fig.10)

Interstitial cells:

The barrier in the alveoli contains cells of a connective tissue as Fibroblasts (Fig, 11) and Mast cells (Fig, 12). The interstitial fibroblasts compose collagen fibers and elastic fibers which are intensified in the barrier of the vesicular to strengthen the fabric of visceral lung.

Alveolar macrophages:

The lung Contains big phagocyte cells which are launched in the liposome blanks and barriers. They are active cells and have a surface of irregular shape because of the false underfoot movement. They are Aptlaip and secretory cells, where it protects and cleans the surface of epithelial vesicles from the microbial damage and particles of organic and inorganic dust by ingesting the exotic materials.

Macrophages contain many secondary lysosomes and fatty drops. Also the number of reflect the size of the phagocytosis process of those cells. In the fullness of macrophages with dust, they transmit either to the top of respiratory tree, (Fig.13).

Second Group:

Histological studies :

Rats treated with the suspension of *Aspergillus niger* (0.4 mg/kg).

Examination of the hisological sections of rats infected with the fungal suspension indicated a significant loss of the normal lung structure due to the degeneration and distortion as a result of infection dominated by the appearance of aggregations of inflammatory cells mainly lymphocytes around the bronchioles (Fig.8).

Also, some bronchioles loosed its normal shape due to distortion, dilatation, lysis of the internal lining layer and fibrosis (Fig.9).

The epithelial tissue of the distorted respiratory bronchioles showed irregular structure with congested blood vessels with accumulation of red blood cells further, significant increase in the thickness of alveolar walls and interalveolar septum with narrow lumen of some of them (Fig. 16).

Respiratory bronchioles:

Respiratory bronchioles are lined by cuboidal ciliated epithelium and few non ciliated cells called Clara cells instead of goblet cells. They are surrounded by smooth muscular cells which surrounded by elastic fibrous connective tissue. Each respiratory bronchioles divided into several alveolar ducts which in turn ended by alveolar sac which open in several alveoli.

The examination of the semi-thin section revealed that the vesicles were distorted with thick wall with narrow lumens which is known as collapse phenomenon (Fig.17). Also, the number of pulmonary cells of type P_2 increased in the alveolar wall. As a result of the sever injury, the rats lung were subjected to closure of the alveoli and decrease in the number of terminal bronchioles with their distortion which led to the disappearance of Clara cells. Bennett (1979) indicated that *Aspergillus* grow in the human tissue through the respiratory passages such as trachea or pneumonic cavity which is known as fungal pulmonary diseases or Aspergillosis.

Trachea and lung can be infected by fungal spores which affect pleura, air can enter to it through the rupture of alveoli (Al gamas and Dia Eldin, 1983).

Asperigllosis was known to affect the respiratory system causing fungal pneumonia and funal bronchitis, also the inhaled fungal spores cause hypersensitivity (Abduel Hamid, 2000).

Luther *et al.* (2007) mentioned that the pulmonary macrophage cells constitute an important part of early immune defense mechanism against the *Aspergillus* infection, accordingly, engulfing of fungal spores is essential to git rid of the infection.

The clotting of blood in the vessels is considered the responsible factor for the death resulting from *Aspergillosis* (Lai *et al.,* 2007).

Ultrastrustural studies :

A group of rats infected with fungus *Aspergillus niger* dose (0.4 mg / kg). When examining the sectors of the ultra-structure of the lungs of rats

A.niger infected dose (0.4 mg / kg) and found many areas covered with large amounts of bleeding. A study of alveoli increased histopathological changes in comparison with those of prior infected rats. These changes were in the small number of cells P1 surrounded by bleeding and the occurrence of cytoplasm decomposition, while the number of cells P2 largely increase which led to a lack in air spaces in the tissue, which reduces the area of gas exchange in the tissue.

Those changes weren't only in the number, they exceeded the internal structure of P2 cells, it was observed a significant increase of laminate objects compared to former infected rats and the small number of mitochondria, where an analysis occurred to its customs and interior membranes as well as to the nucleus division and a large increase in the size in some of the P2 cells or an atrophy of the nucleus and the distorted in other cells, and reduction of the Golgi apparatus.

As a result of infection, the macrophages largely increased in the vesicular which confirms the infection. Also lysosomes frequently appeared to communicate to the process of phagocytosis of these

defensive cells (Figures18,19,20). Studies carried out by (Parke, 1994) have shown that when the animals' lung tissue is expose to jet fuel particles, it melts in a lining epithelial liquid of the bronchioles and primarily aimed at macrophages,Clara cells and P2 cells, where all these cells partially absorb it.

(Ochs *et al.,* 1999) reported that pneumonia servictnt is one of the active ingredients in a defense mechanism against pneumococcal infections. Both the (Hawgood, 1997; Gunther *et al.,* 1999) pointed that the importance of pulmonary surfactant change comes through its prevention of the collapse by reducing the surface tension of the vesicles.

Hays *et al* (2003) studied the impact of Propulsion jet fuel -8 on the respiratory efficiency of male rats' lung weighing (200-250 gm). He studied the fine structure of vesicular cells type II P2, when rats exposed 24 hours for doses (0,5 0.1 0.0, 1,5 mg / g) many of the micro-structural changes appeared. Mostly an increase in the number of laminate objects, while high dose (1,5 mg/g) was decomposed and P2 cells lost their normal shape.

Alokail & Alarifi, 2004 tested the use of the Arab incense smoke on rats' lungs. He found that it causes changes in the histology of the intra- barriers and the blood tissue in treated animals. The study showed the presence of infections which appeared in lymphocytes and plasma cells in the connective tissue around the vascular and inherent class to some bronchi, while the liposome barriers that surround bronchioles increased in thickness and filled with lymphocytes, acidic and neutral cells. the use of incense smoke caused an increase in the pneumonia damage which is represented in the increase numbers of macrophages liposome that spread in the lbranchema as well as the increase in size, so-called Hyperplasia phenomenon.

Alarifi *et al.,* 2004 and others studied the minute structural changes in the fabric of rats' lung exposed to the smoke of the Arab incense. One of the main results of the study was minute synthetic changes for most organelles, and appeared to be evident the lung tissue the Hyperplasia phenomenon for the vesicular cells and increase in presence of neutral cells in the infected alveoli with a crash and changes in interstitial cells and necrosis in the liposome, as seen for the deposition of collagen fibers in the liposome walls. The study conducted by the electronic microscope confirmed that the use of incense smoke made minute synthetic changes in the alveoli that led to a lack of respiratory efficiency

Both (Harrison, 2004 and Davidson, 2006) mentioned that the disease of the lung indicates an inflammation in the lungs due to fungal infection. It is accompanied by pneumonia and pain in the chest concentrated in the shoulder or top abdominal wall, difficulties in breathing and dry cough at the beginning of the disease, most often accompanied by green or yellow or rust-colored expectoration. It might be smelly with symptoms of fever, loss of appetite, the diagnostic procedure observed violation, such as chronic pulmonary air sacs, small bronchi, odema and an increase in the number of acid and pulmonary blood cells. Luther *et al.,* 2007, mentioned that pulmonary macrophages are an important part of the early immune defense against *Aspergillus* infection and thus the process of fungal spores ingestion is a necessary condition to eradicate it.

Third Group:a.b

Histological studies :

Rats treated with Costus extract (0.2 and 0.4 mg/kg).

The histological examination of lung section of rats treated with Costus extract (0.2 and 0.4 mg/kg) showed that the lung tissue appeared with its traditional normal structure and most of its components appeared in the normal position.

As appeared in (Fig. 21) air bronchioles present in regular lung tissue and consists of internal epithelial layer which in turn consists of columnar ciliated cells with goblet cells in between, with extended invaginations inside the cavity and surrounded by muscular layer in addition to the blood vessel.

It could be observed that the terminal bronchi structure is similar to that of the air bronchi (Figs. 22,23). However, the internal epithelium consists of ciliated Cuboidal cells and characterized by the presence of Clara cell which replaced the globlet cell. The cavity of the terminal bronchi connected to that of the respiratory bronchi which in turn branched to several vesicular channels ended with alveoli and alveoli air sacs. As appeared from (Fig.24) the wall of alveoli if p1 and p2 cells. With complete absence of callapse phenomenon.

C- Rats treated with *Aspergillus* suspension (0.4 mg/ kg) then treated with costus extract (0.2 mg/ kg).

The microscopic examination of the histological lung structure of rats infected with fungal suspension (0.4 mg /kg) then treated with Costus extract (0.2 mg/ kg) revealed the restoration of the interstitial tissue of lungs nearly its normal structure and appeared devoid of degeneration (Fig. 25).

The histological sections of lungs showed the air bronchioles characterized by normal and regular structure, the internal epithelium consists of clear invaginations around nearly regular cavity. Also, the alveolar sacs showed regular structure at which the alveolar consists of normal walls with clear distinguished cells.

D- Rats treated with *Aspergillus* suspension (0.4 mg/ kg) then treated with costus extract (0.4mg/ kg):

Examination of lungs of rats infected by *Aspergillus* suspension (0.4mg /kg) then treated with costus extract showed the positive effect of costus plant in the restoration of the lung tissue to its traditional and normal structure and the disappearance of the negative effects and pathological changes caused by fungal infection.

Fig. 26 showed well formed and regular air bronchioles in the treated rats compared to those of infected once.

The air bronchi characterized by internal epithelial layer consisted of ciliated columner cells with globlet cells arranged in folds inside well developed lumen. The blood vessels with regular walls were non-congested and located adjacent to air bronchioles. Furthermore, the air sacs appeared with regular walls characterized by clear P1 and P2 cells with complete absence of collapse phenomenon (Fig 27), The high prevalence of diseases, the side effects of some drugs in addition to its high cost have led to an increase in demand for use of natural product in pharmaceutical industry. Metwally (2005) indicated that costus plant prevent sputum, addresses common cold, costs and pleurisy pain, tetanus and provides protection against toxins and its adverse effects.

Costus arabicus was effective against chronic bronchitis and asthmas (Dutta *et al.,* 1960,& Sastry and Dutta, 1961).

Tsarong *et al.* (1994) indicated that the popular traditional uses of costus were to address lung inflammation, coughs, colds, ulcers and rheumatism. Recently, Habsah *et al.* (2000) confirmed the antioxidants and antimicrobial activities of costus.

Although many published evidences support the effectiveness of costus plant and safe use, little information were known about the active ingredients in the plant, its bioavailability. So, studies on the physiological pathways and pharmacological importance were needed to provide good entries for new pharmaceutical uses of the plant (Pandey *et al.,* 2007).

Costus is considered from the famous medicinal plants described traditionally specially in India, China and Korea.

The effectiveness of Costus aginst cancers, infections, liver hyperactivities was confirmed by Pandy *et al.* (2007).

Parekh and Chanda (2008) indicated that the Methanolic extract of Costus and Saussurea Lappa, belonging to the same family composite showed higher antifungal activity compared to the chemical antifungal Amphotericin B and fluconazle. Furthermore, methanolic extract of costus was very effective against three species of *Aspergillus* and the effect of the extract depend on the fungal species. Thus, low concentrations inhibit *A. Flavus* where, higher ones inhibit *A.niger*. In addition, extract of costus plant showed antioxidant activity due to the presence of polyphenols which inhibit the oxidative stress of free radicles (VijayalaKshmi and Sarada, 2008). This study was conducted to lest the effect of costus plant on the histological structure of lungs of rats infected with *Aspergillus*. The results revealed the effective effected of costus as natural and safe antifungal. Further medical and pharmacological studies were needed.

Ultrastructural studies :

(A - b) A group of rats treated with a dose of Costus extract (0.2 mg / kg -0.4 mg / kg). Examination with the electronic microscope for a sector of rats' lungs treated with Costus extracts dose (0.2 mg / kg - 0.4 mg / kg) showed that most of the components of the textile were natural developed position. The vesicular emerged thickness similar to what it was in the control samples, Also regularity of endothelial cells of the poetic shell, P1, P2 cells were well-formed where a cascade ideally capillaries in P1 cells in the vesicular wall. P2 cells seeming installing an internal regular and the nucleus of a clear and a number of objects laminate natural mitochondrial and endoplasmic reticulum clear (Fig., 28), as well as proven microscopic examination presence of mast cells are similar to the control samples (Fig., 29) and macrophages was characterized by a number of natural lysosomes (Fig., 30).

(C - d) A group of rats infected with *A.niger* dose of [0.4 mg / kg] treated with a dose of extract treatment[0.2 mg / kg - 0.4 mg / kg]: The exact examination for the infected rats' lung with *A.niger* dose (0.4 mg / kg) treated with dose (0.2 mg / kg - 0.4 mg / kg) confirmed the return of the natural appearance for thickness of the walls liposome and low number of P2 cells the optimum recovery to be installed, usually in terms of shape of the nucleus and the return of the mitochondria and customs of semi-natural appearance, also macrophages that have few lysosmes appeared as evidence of the decline in the process of phagocytosis resulting from the damage of injury (Figures, 31,32,33).

Due to the ineffectiveness of drugs which lost its original value in the treatment and their destructive effects on other intact, in addition to its high cost, we want to shed light on the wealth of the great field of prophetic medicine, which is honest, and the safest and most effective medicine to exist because it is originally from God The Almighty, where he says: (that is only a revelation revealed) Al-Star: Aya (4). Prohet Mohammad peace be upon him in the novel of Al-Bukhari from Umm Qais the daughter of Mahsen, she said: I heard the Prophet peace be upon him say: (use this Indian lute in which the seven heal: snort of its faces and generate tags).

Neuwinger, 1996, reported that species Aframomum (Zingiberacea) contain Quercetin and Kaempferol and both have the ability to prevent the growth of fungus, yeast and viruses also contain Syringic acid, which is an important and topical anesthetic for anti-Parkinson.

Also (Schmidt, 1999) found that the plant is rich in sesquiterpene lactones with the inhibitory activity and its ability to develop new drugs, especially used for the treatment of acute and chronic inflammation such as chronic arthritis. It is also the main component of the premium composite (Brahmyadi Ghanavati)) and the user as an officer for high blood pressure Rath *et al.,* 1999

Habsah *et al.,* 2000 reported that the methanolic and methanolic bilateral chlorine plant extracts species Zingibeeraceae including installment showed an antifungal activity of microbes and anti-oxidants where the methanolic bilateral chlorine extracts is stronger than methanolic extracts and interpreted so as to lower component terminals located in methanolic bilateral chlorine extracts (not polar) contributed towards increased activity for methanolic extracts (polar).

Both (Anjaria *et al.,* 2002;& Sriram *et al.,* 2004) say that the roots of abstracts of the premium is used as a treatment for asthma, bronchitis and a swollen abdomen and leprosy. Jeong *et al.,* 2002;& Cho *et al.,* 2004 mentioned that cynaropicrin and costunolide that contain premium is one of the factors used against cancer

A study conducted by (Tane *et al.,* 2006), he demonstrated that extracts and compounds (Aframomum,Zingiberacea), including the installment of vital activity of an anti-fungal toxins and cellular and anti-bacteria, parasites and viruses and anti-cholesterol increase. Pandey *et al.,*(2007) explained the uses of traditional Costus and proven its therapeutic effectiveness against cancer, ulcers, infections, liver protection activities. Magassouba *et al.,* 2007 Pointed out that active chemical compounds distinguish plants' extracts which are used in traditional medicine such as Costus that is responsible for the observed effect against bacteria. In the study conducted by (Vijayalakshmi & Sarada, 2008) on types of of Costus extracts, they verified that they contain Polyphenol and possession of an anti-oxidant activity shown in frequent presence of hydroxyl radicals, which works to quell the activity of free radicals.

The fourth group (G4): Rats infected by *Aspergillus* suspension 0.4 mg/ kg then treated with Amphotericin B.

Histological studies :

The lungs of rats infected with *Aspergillus niger* (0.4 mg/ kg) and treated with Amphotericin B generally showed deformed tissue as a result of infection. Microscopic examination showed irregular deformed air bronchioles with increased wall thickness of alveoli which filled with infiltration and bleeding (Figs. 34,35). Some of the bronchioles were continued with each others, while others loosed its normal shape inside deformed pulmonary tissue. Inflammatory invasion was detected on the epithelial layer of the bronchioles, with distorted blood vessel filled with bleeding (Fig.36).

Examination of the semi thin sections of terminal bronchioles did not show any detected improvement after amphotericin treatment where clara cells secretions filled the bronchiole lumen which lost its normal shape. Furthermore, the alveoli walls were highly thickened leading to atelectasis in some areas of the lung tissue (Figs.37,38)

Amphotericin -B showed dangerous side effects on brain and kidney. Toline and Raij (1988) treatment of rats with amphotericin –B caused renal toxicity lead to increase in the resistance of renal capillaries. Also Chavanet *et al.* (1992) comfirmed the occurrence of renal toxicity with lmg amphotericin /kg. Carlson and Condon (1994) indicated that 80% of patients treated with amphoterici B suffered from hyper nitrogenaemia. Wingard *et al.* (1999) observed that renal toxicity coused by amphotericin B- lead to disturbancein glomerular infiltration. Also, tissue damage of renal tubules and disturbance in lenal functions were detected with small doses of amphotericin (Walsh *et al.*, 1999).

Olson *et al.* (2006) tested the effectiveness of amphotericin B on rats infected with aspergillosis and other immune dificients non-infected.The observed high levels of blood urinary nitrogen in the non-infected rats, and degeneration in the renal tubules in the infected rats after drung treatment. Furthermore, amphotericin B caused encephalopathe and its daily repeated intravenous injection (0.5mg/kg) resulted in the appearance of amphotericin in plasma (1-105mg/ml) and in different body and few concentrations penetrated to cerebrospinal fluid (Blamaceda *et al.* 1994). Amphotericin was found to decrease hemoglobin production (anemia), platelels and white blood cells (Goodman and Gilman`s, 2001).

Nrajvar *et al.* (2004) indicated that amphotericin B up to 5mg/kg not sufficient for the treatment of rats from sever aspergillosis.

Amphotericin B with its different lipoid structures character zed by different, patterns of accumulation, thus it is accumulated in the lung tissue and after 24hours of treatment. whereas, liposomal amphotericin B showed more accumulation in the epithelial lining fluid (Groll *et al.,* 2006). In this

concern,Vogelsinger *et al.* (2006) indicated that the levels of accumulated amphoterici B were in the lungs of patients treated with colloidal amphotericin B than those treated with liposomal one.

Ultrastrustural studies :

A group of rats infected with a dose of *A.niger* (0.4 mg / kg) [and treated with Amphotericin - B: examination pointed out thorough the installation of histological lung, rats *A.niger* dose (0.4 mg / kg) and treated with Amphotericin - B continued negative effects of infection in spite of treatment with the drug, as there was no significant improvement after treatment with the drug Amphotericin - B cells pneumoniae P1 and P2, which appeared cells P1 atrophic and of abnormal nuclei, while cells P2 cells emerged frequently as there has been no improvement to their interior structures of the difference form the nucleus and increase number of objects laminate and the degradation of mitochondria, and capillaries appeared deformed and atrophic,also endothelial cells with the appearance of Sitoblazmi decomposition, The macrophages cells appeared large shapes, numerous lysosomes (Fig., 39,40,41)

Borgeat & Samuelsson, 1979; Turk *et al.* 1982;& Fels *et al.* 1982) mentioned that the neutral, acidic and macrophages cells in human lung epithelial cells, as well as Epithelial cells which are considered as materials for air passages are key sources for the production of Lipoxygenase enzyme. (Tolins and Raij, 1988) Found in the treatment of mice with Balomvutricin B an increase in renal vascular resistance as the result of renal toxicity. (Chavanet *et al.,* 1992) also stressed the emergence of renal toxicity when using Amphotericin - B for patients at 1 mg / kg.

According to both (Liu *et al.* 1995, 1996, 1997) when treated with the drug (DEC) of cases of asthma disturbance in physical activities as well as in the metabolic processes of Servictnt pneumonia. Nomura *et al.* 2001 found that drug (DEC) analyzes Lipoxygenase enzyme and thus inhibits macrophages liposome-producing enzyme by preventing the launch of its chemical activity.

The results observed from microscopic examination in a study carried out by Florencio *et al.* 2005 to determine the effect of drug diethy carbamazine (DEC) binary ethyl Cirbemaizin on the cells of the rats' lungs after treatment for 12 days and compared to Control Cells. That the P2 liposome cells of active nuclei by chromatin dense nuclei and clear vesicles secretory large number on the other hand, synthetic change wasn't noticed in P1 liposome cells, As macrophages have a number of ways to harmonize the cellular activity appeared with the nuclei of real chromatin and central nuclei(endossomes) in the stages of different growth (early and late) spread in the cytoplasm and these results support that the drug (DEC) plays a role in stimulating important cellular pathways which is likely to be related to therapeutic improvement in as a result for (asthma symptoms). Groll *et al.,* 2006, confirmed that Amphotericin - B and its different components are growing in the tissues of the lung and respiratory pulmonary macrophages bags.


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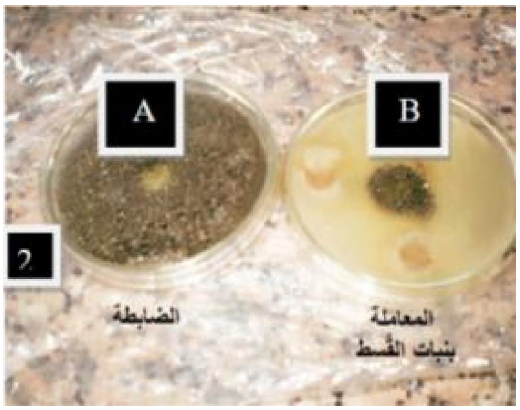
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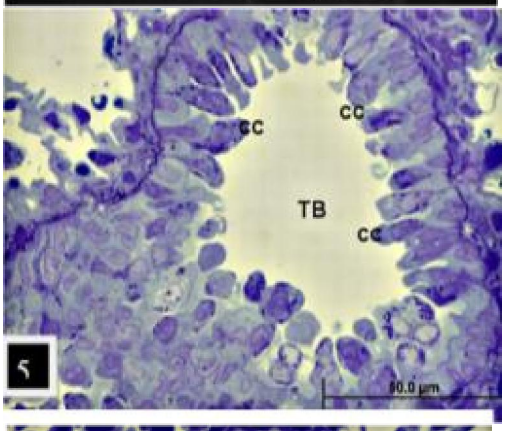
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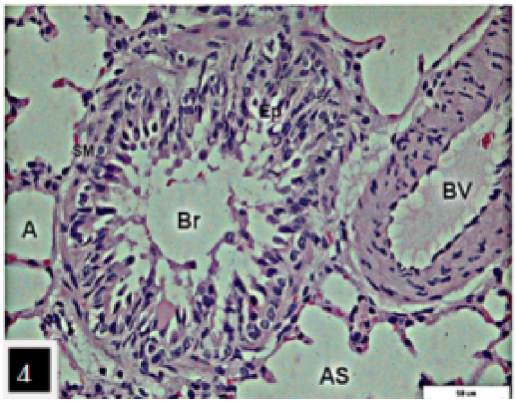
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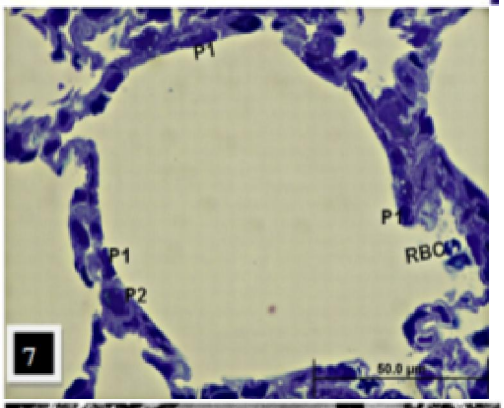
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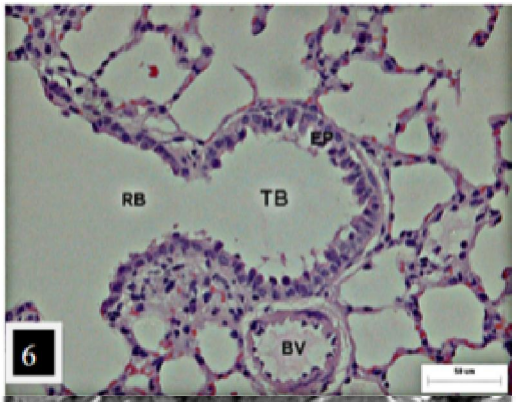
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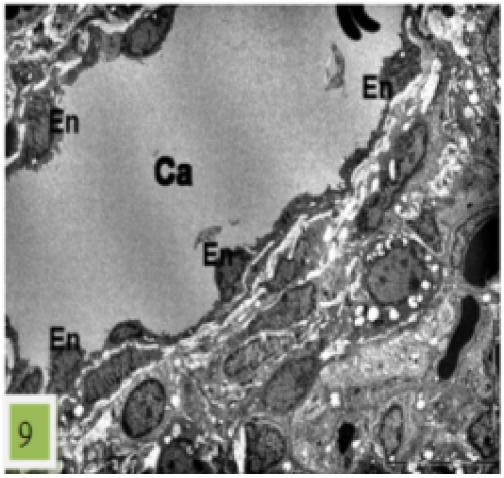
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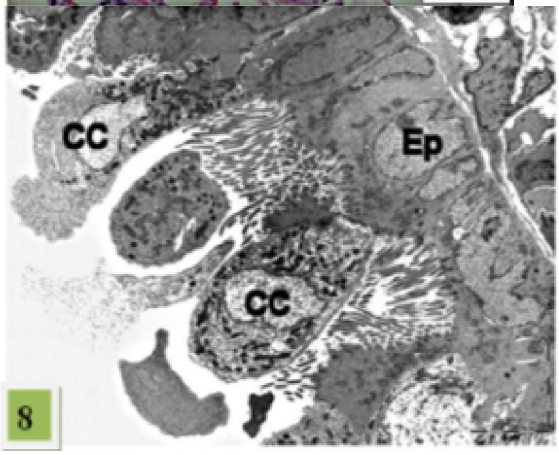
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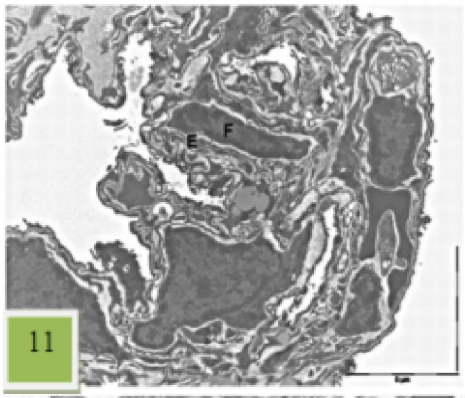
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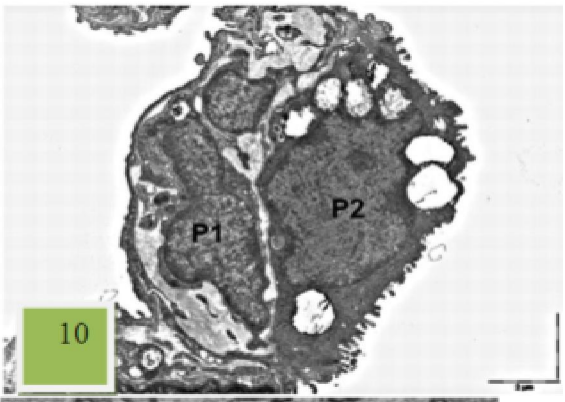
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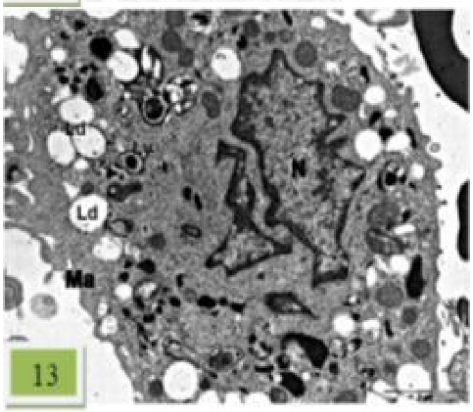
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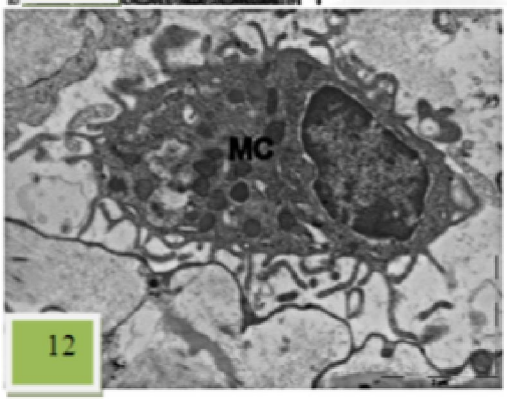
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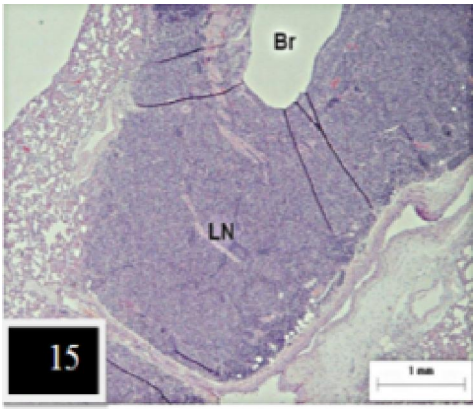
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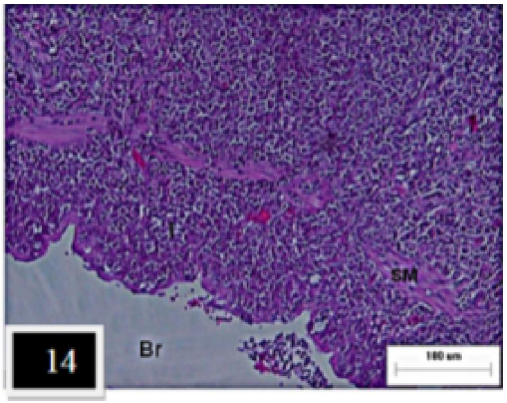
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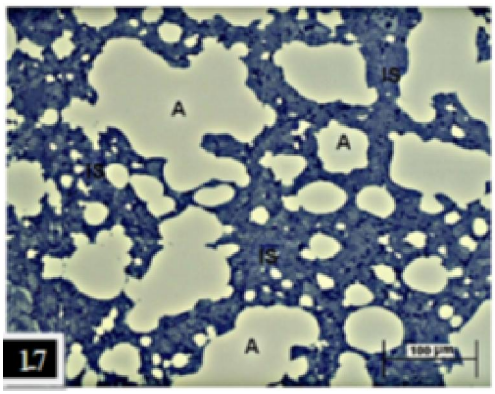
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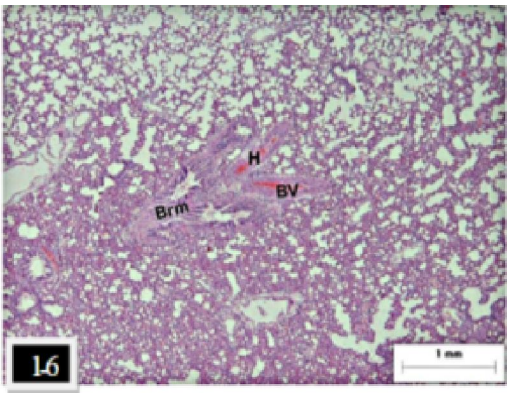
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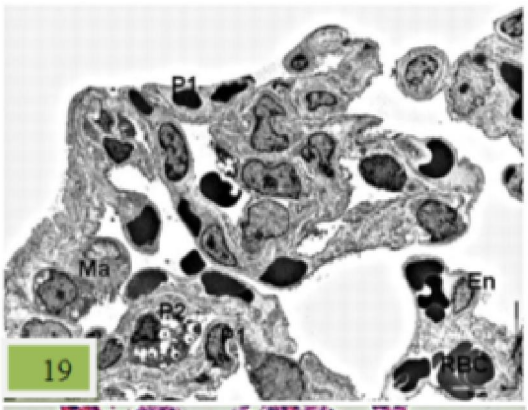
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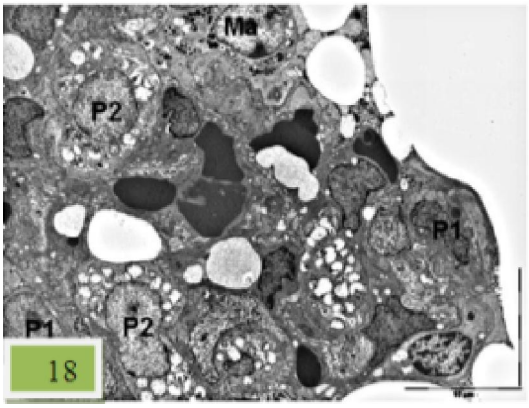
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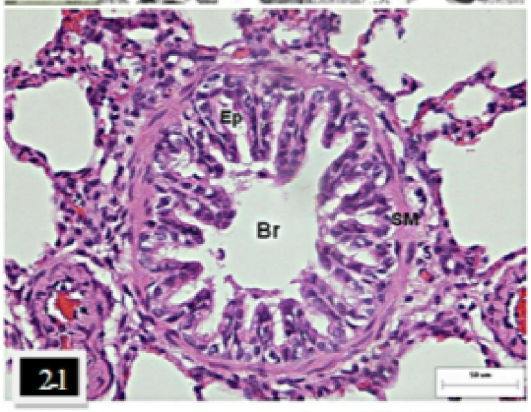
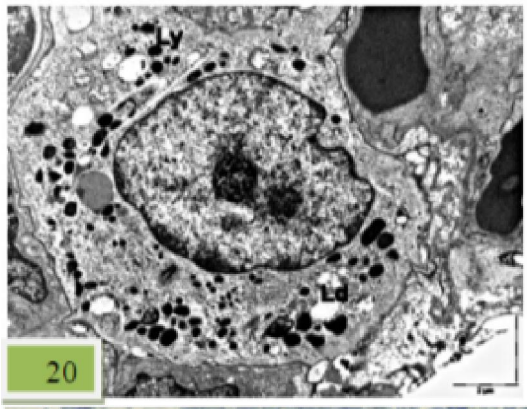
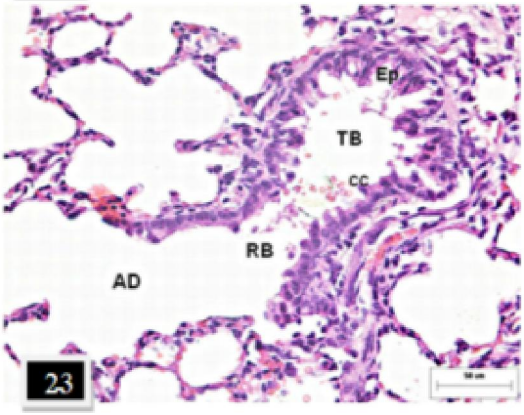
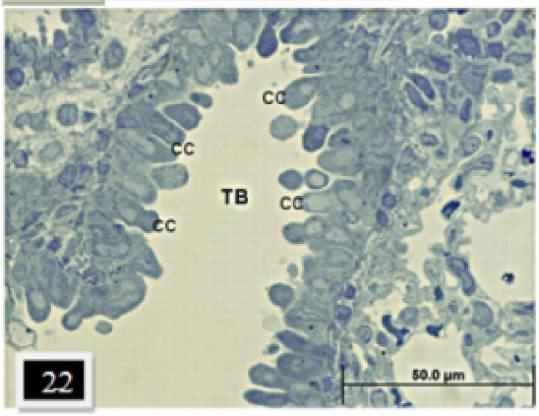
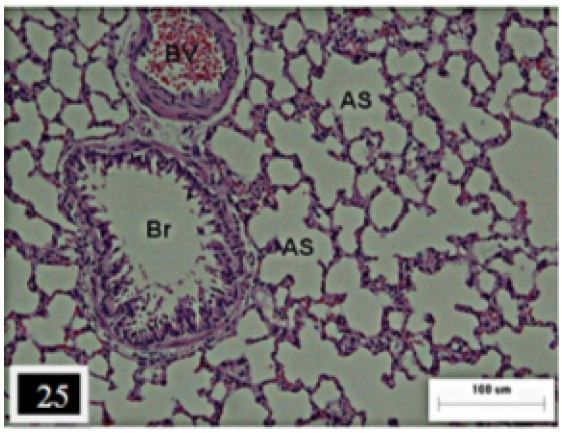
P2
18

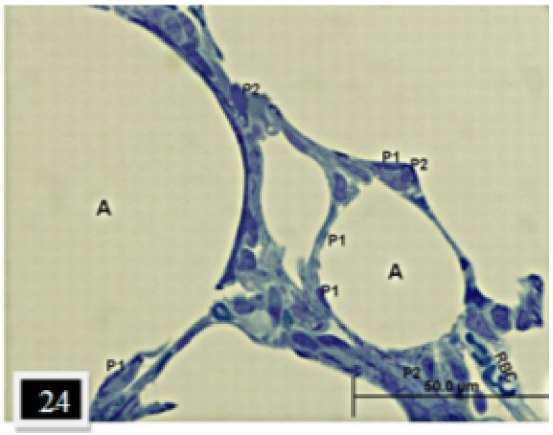

Ep
Br
SM
21

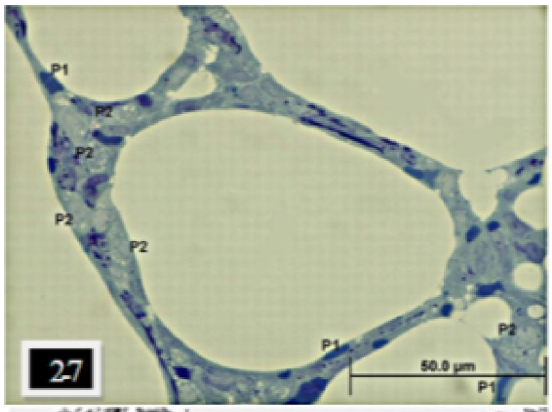

Ly
Ld
20

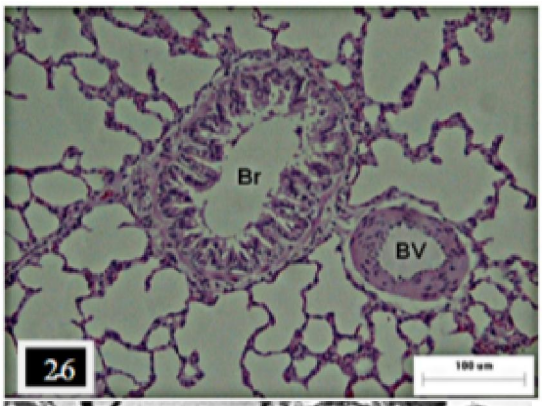

Ep
TB
CC
RB
AD
23

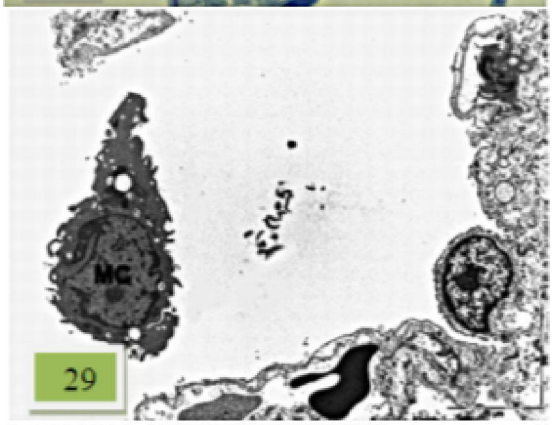

CC
CC
TB
CC
CC
50.0 µm
22

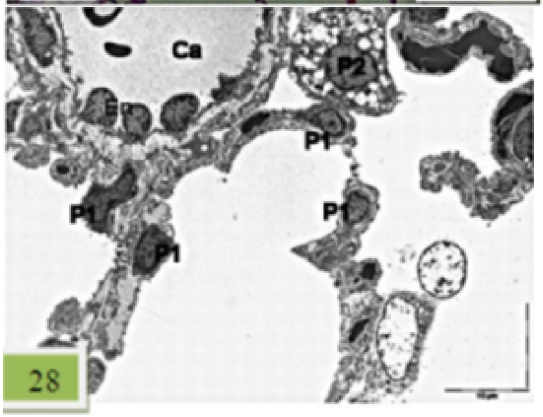

BV
AS
Br
AS
25

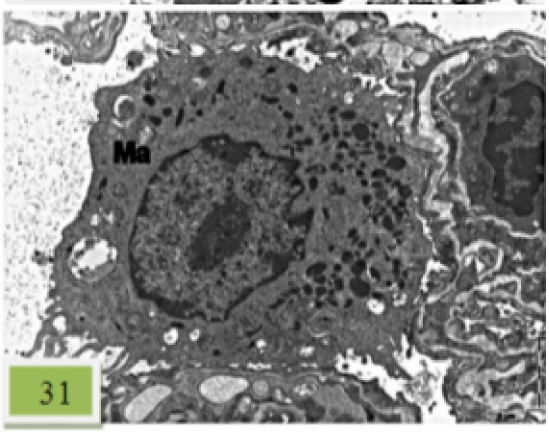

P2
P1
P2
A
P1
A
P1
P1
P2
RBC
50.0 µm
24

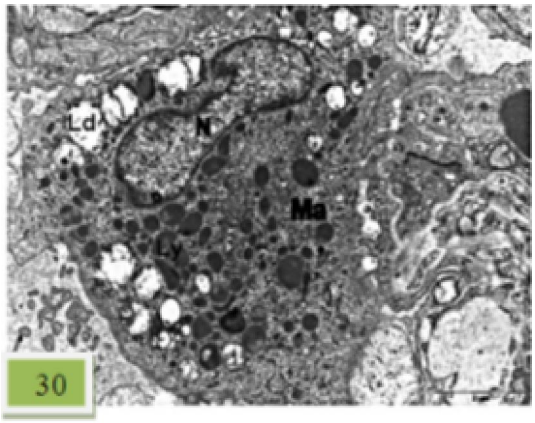

P1
P2
P2
P2
P2
P1
P2
50.0 µm
P1
27

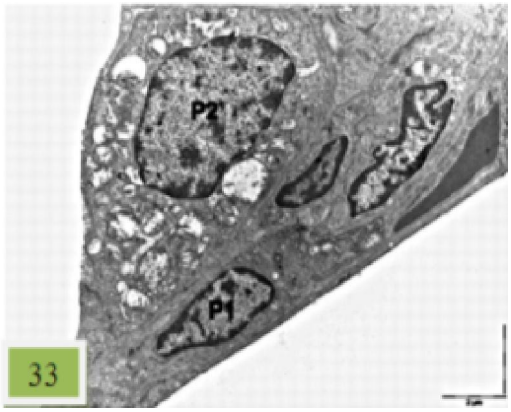

Br
BV
26

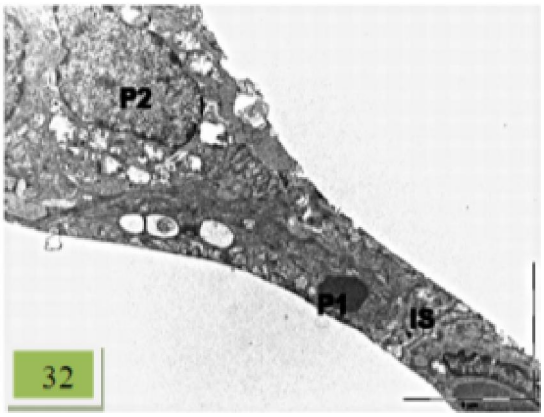

MC
29

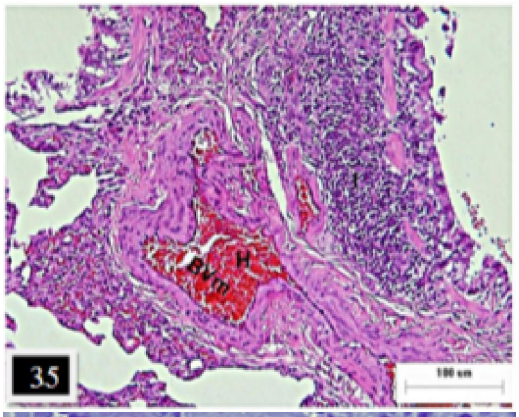

Ca
P2
P1
P1
P1
P1
28

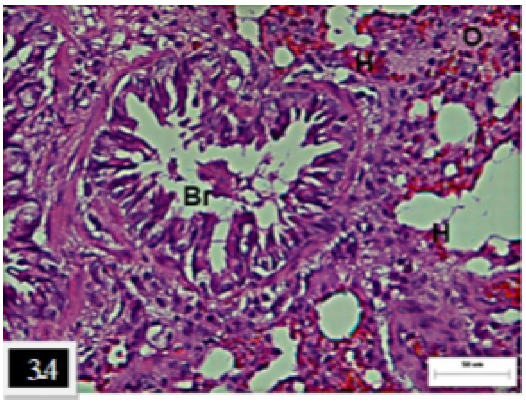

Ma
31

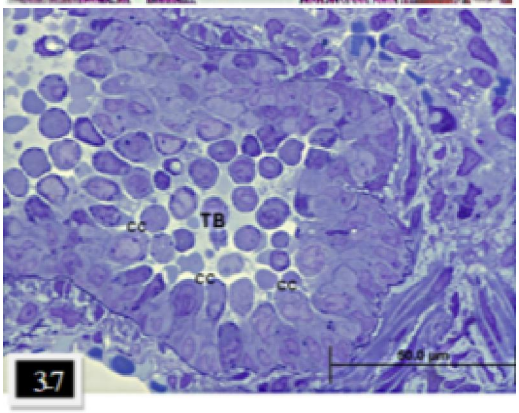

Ld
N
Ma
Ly
30

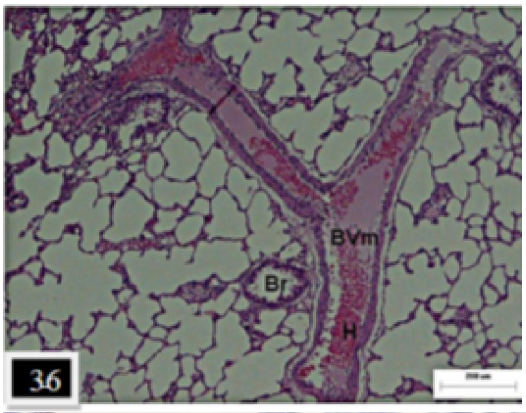

P2
P1
33

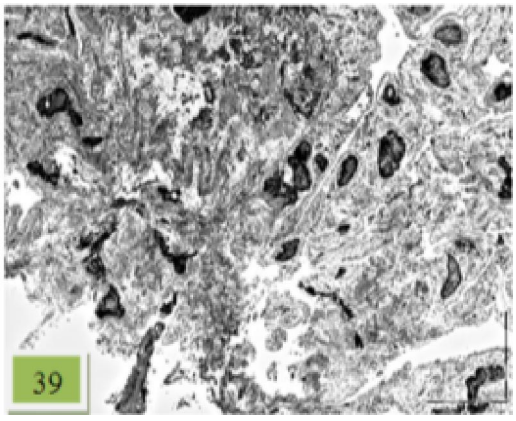

P2
P1
IS
32

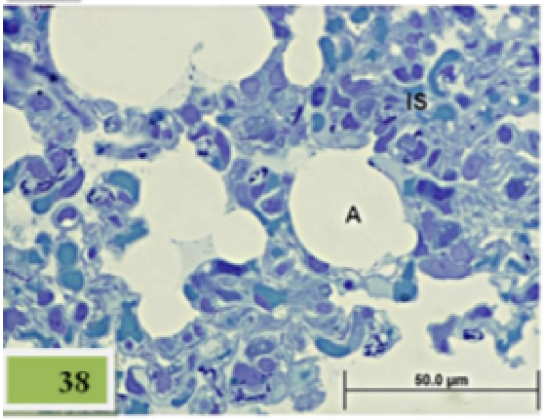

BVm
H
35

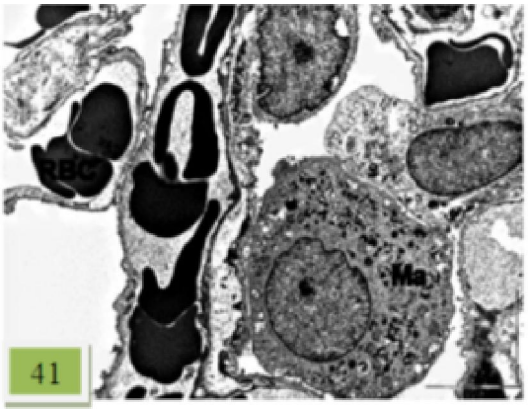

O
H
Br
H
34


CC
TB
CC
CC
37


BVm
Br
H
36


39


IS
A
38


RBC
Ma
41

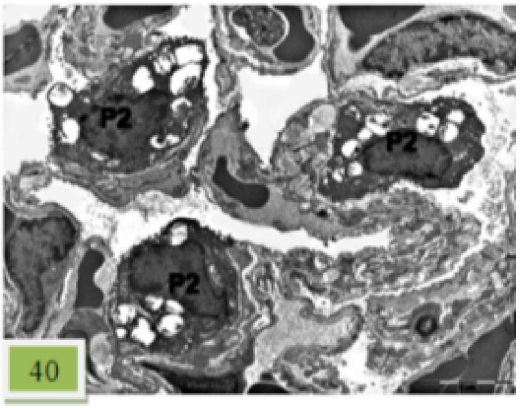

P2
P2
P2
40

Fig (1): Roots of Costus Plant.
Fig (2): (A), Control group, the fungal growth occupied the hole Petridish. (B), Inhibition zone surrounded the holes containing the costus extract and prevented the fungal growth.
Fig (3): The sharp decrease in the biomass of the fungal mycelia a a result of costus extraet treatment (A), Control and (b) costus treatment.
Fig (4): Section of rat lung of control group showed bronchiole, (S M) Muscle layer, (EP) epithelial layer, (B v) Blood vessel, (A S) Alveolar sac and (A) Alveoli. (H&E) stain.
Fig (5) : semithin section in rat lung of control group showed, (E P) epithelial layer, (T B) terminal bronchiole and (C C) clara cells. Toluedin blue stain,
Fig (6): Section, in rat lung of control group showed, (TB) terminal bronchiole, (EP) epithelial layer, (RB) part of respiratory bronchiole and (BV) Blood vessel. (H&E) stain.
Fig (7): semithin section in rat lung of control group showed (P 1) type one, (P 2) type two of lung cells. (RBcs) red blood cell. Toluedin blue stain.
Fig (8): Electron section in rat lung of control group showed ciliated epithelial cells (EP) in terminal bronchioles and non-ciliated cells, clara cells (CC).x 2600.
Fig (9):Electron section in rat lung of control group showed the cell (En) lining the vascular noodles on the concave side of capillaries (Ca). X 1950.
Fig (10): Electron section in rat lung of control group showed lung cells p□ and p□.x 1450.
Fig (11): Electron section in rat lung of control group showed the cells of a connective tissue a Fibroblasts(F). x 4600
Fig (12): Electron section in rat lung of control group showed Mast cells (MC). X7900.
Fig(13):Electron section in rat lung of control group showed phagocyte cells (Ma) with its nucleus(N),lysosomes(Ly) and fatty drops (Ld).X 7900.
Fig (14): Section in rat lung of infected group with *A.niger* (0,4 mg/kg) showed epithelial layer (Ep) was degenerated in bronchiole (Br) which around with inflammatory cells(I). H & E stain.
Fig (15): section of rat lung of treated group with the suspension of *Aspergillus niger* (0, 4 mg/kg). Showed lysis of the internal lining layer and fibrosis in (Br) bronchile. (H & E). stain.
Fig (16): Section of rat lung of treated group with the suspension of *A.niger* (0,4 mg/kg). Showed (H) congested blood vessels, significant increase in the thickness of the alveolar walls with narrow lumen. (H & E) stain.
Fig (17): Semithin section in rat lung of treated group with the suspension of *A. niger* (0,4 mg/kg). Showed (A) alveoli with thick walls which is known as collapse phenomenon. Toluedin blue stain.
Fig (18): Electron section in rat lung of infected group with *Aspergillus niger* (0, 4 mg/kg). Showed increase number of P□ cells and decreas in P□ Cell.x1950.
Fig (19): Electron section in rat lung of infected group with *A.niger* (0, 4 mg/kg). Showed red blood cells (RBC) and an atrophy of the nucleus of P□ Cells. X1450. Fig (20): Electron section in rat lung of infected group with *A.niger* (0.4mg/kg).showed the macrophage with lysosomes(Ly). X5800.
Fig (21): Section in rat Lung of treated group with costus extract (0.4 mg / kg). Showed air bronchiole present in regular lung tissue (Br), muscular layer (SM) and (EP) epithelial layer. (H & E) stain.
Fig (22): Semi thin section in rat lung of treated group with costus extract (0, 4 mg / kg). Showed Clara cells (CC) in terminal bronchiole (T B).Toluedin blue stain.
Fig (23): Section in rat lung of treated group with costus extract (0, 4 mg / kg). Showed clara cells (CC) in terminal bronchiole (TB) and respiratory bronchiole (RB). (H & E) stain.
Fig (24): Semi thin section in rat lung of treated group with costus extract(0,4mg / kg), showed alveoli (A) and p□,p□ cells. (p□,p□). Toluedin blue stain.
Fig (25): Section in rat lung of infected group with the suspension of *A.niger* (0,4mg /kg). And costus exctract(0,4mg/ kg). and costus extract (0.2 mg/kg) Showed (Br) air bronchiole, Blood vessel (Bv) and alveolar sacs (AS) in regular structure.(H&E) stain.
Fig (26): Section in rat lung of infected group with suspension of *A. niger* (0, 4 mg/kg). And treated with costus extract (0,4 mg/kg). Showed air bronchiole (Br) in well formed and regular blood vessel (Bv) with regular wall and noncongested.(H & E)stain.
Fig (27): Semi thin section in rat lung of infected group, with suspension of A. niger (0, 4 mg/kg). And treated with costus extract (0, 4 mg/kg). Showed alveoli with clear p□ and p□ cells. Toluedin blue stain.
Fig (28): Electron section in rat lung of treated group with costus extract (0, 4 mg/kg) showed regularity of lung cells p□ and p□. X1450.
Fig (29): Electron section in rat lung of treated group with costus extract (0, 4 mg/kg) showed mast cell(Mc) x3400.
Fig (30): Electron section in rat lung of treated group with costus extract (0, 4 mg/kg).showed macrophage (Ma) with fatty drops (Ld) and lysosomes(Ly). X7900
Fig (31): Electron section in rat lung of treated group with *A.niger* (0,4 mg/kg) and treated with costus extract (0.2 mg /kg) showed macrophage(Ma). X5800.
Fig (32): Electron section in rat lung of infected group with *A.niger* (0,4 mg/kg) and treated with costus extract (0.2 mg /kg). Showed lung cells P□ a nd P□.x4600.
Fig (33): Electron section in rat lung of infected group with *A.niger* (0, 4 mg/kg) and treated with costus extract (0, 4 mg /kg). Showed lung cells P□ and P□. X 5800.
Fig(34,35): Section in rat Lung of infected group with suspension of *A.niger* (0,4 mg/kg) and treated with Amphotericin B. Showed irregular deformed air bronchiole (Br) with increased wall thickness of alveoli which filled with infiltration (0) and bleeding (H).(H & E) stain.
Fig (36): Section in rat lung of infected group with suspension of *A.niger* (0,4mg/kg) and treated with Amphotericin B. Showed distorted blood vessel filled with bleeding (Bvm). (H & E) stain.
Figs (37,38): semithin sections in rat lung of infected group with suspension of *A.niger* (0, 4 mg/kg) and treated with Amphotericin B. Showed clara cells (CC) secietions filled the terminal bronchiole lumen (T B). Toluedin blue stain.
Fig (39): Electron section in rat lung of infected group with *A.niger* (0, 4 mg/kg) and treated with Amphotericin – B showed increased wall thickness of alveoli. X1450.
Fig (40): Electron section in rat lung of infected group with *A.niger* (0, 4 mg/kg) and treated with Amphotericin – B showed lung cells P□ with increase number of objects laminate. X3400.
Fig (41): Electron section in rat lung of infected group with *A.niger* (0, 4 mg/kg) and treated with Amphotericin – B showed macrophages (Ma) around the capillaries (Ca) filled with red blood cells (RBC). X3400.

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Assessment of quality of life in hepatitis B patients compared with healthy people

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Abstract: The life quality in patients with hepatitis B is considered as a major concern in these patients. The aim of this study was to analyze the regression model of the life quality in patients with hepatitis B in comparison with healthy people from Mazandaran province.This cross sectional-descriptive study was carried out on 420 cases on two groups: hepatitis B chronically-infected patients and healthy peoples from six regions of Mazandaran province. The method of sampling was convenience in two groups. Measuring the quality of life carried out according to the world health organization questionnaire (WHOQOL-BREF). Data analysis was consisted of multiple regression method and for comparison one-sample test of Kologroph- Smirnoph was used.Statistical analysis showed that the average of public life quality in patients with hepatitis B was weak (1<1.76<5) and in healthy people was evaluated average (1<2.94<5). According to results, fully integrated of the care program of these patients in network system, easy access and facilitating in intervention to improve the life quality is offered.
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Keywords: analysis, quality of life, patients, hepatitis B, healthy people

1. Introduction

The scope of the quality of life assessment is not widespread in any time like today. Economists, social scientists and politicians look at this topic from the particular approach [1, 2, 3]. The indicators of life quality include the large range from food and clothing to health care and social- physical environment [4]. Although the life quality has been translated to life level in some resources, but life level and material development includes only one of the basics of life quality[5]. In fact, the concept of life quality is a composite variable that is influenced by several variables [6]. Despite different definitions of life quality, there has not been a consensus regarding the definition to enfold the various aspects of this concept. The World Health Organization (WHO) defines quality of life as; "Individuals' perception of their position in life in the context of the culture and value systems in which they live and in relation to their goals, expectations, standards and concerns[7]. Currently, the scope of life quality and its assessment in chronic diseases have been studied widely. In chronic disease, the main purpose of health care monitoring and treatment is life satisfaction and well-being feeling. However, the life quality of patients with chronic hepatitis B is often below the normal range[8]. Studies showed that with the progression of liver disease and ineffective anti-viral treatment, the physical and mental health of patients damage increasingly[9, 10]. These patients suffer from fatigue, loss of confidence, inability to work, anxiety, depression and other emotional problems that reduce severely their life quality[11]. According to results from previous studies and agreement about the reducing of life quality with regards to disease progression [20,21], however, in this study in terms of the cultures and value systems, the life situations have different goals, expectations, standards and priorities that is not clear with others. Therefore, current investigations have been compared the life quality of two groups of patients and healthy peoples with the new approach by public questionnaires with statistical methods. Perhaps research about the life quality at the group of patients in different situations leads to modern steps to compare with mathematical techniques to solve medicine problems and other problems. Quality of life is measured with likert scale in different area, but last studies showed that the score of quality life in some area is not acceptable. Therefore, non liner regression based on discrete-descriptive should be used. Thus, the aim of this study was analyzing of dimension of the life quality at patients with hepatitis B in comparison with healthy people using multiple regression in the Mazandaran province.

2. Material and Methods

This cross sectional- descriptive study included 420 individuals in two groups: healthy peoples (210person) that referred to health centers for another reasons and hepatitis B chronic patients (210person) who were passed six months from their first refer to urban and rural health care centers that

now are inactive from 6 regions of Mazandaran province in2011: Sari, Neka, Ghaemshahr, Amol, Tonekabon and Noor. The method of sampling was convenience in two groups. All studied groups were over 18 years old. In this study World health organization quality of life (WHOQOL) instrument was used. It is consist on 26 questions and four domains namely, physical, psychological, social relationships and environment. This study was performed with analysis hierarchical multiple regression (AHP). Two special specifications of multiple regression analysis (the mean estimation of "regression weights") and measuring of the "Barazesh model" were use for the analysis of quality. In this survey, firstly of all the independent variables were scored, and secondly, their proportional share estimated in dependent variables, then the score of each independent variable was done according to its effect on the dependent variable. These numerical values are called "regression weights" or efficiencies. Finally, after standardization of scores or efficiencies, ranking of independent variables were compared with each other[12].The experimental model of the measuring of life quality contains the hierarchical structures: criteria, sub- criteria and effective criteria in the process of measuring of life quality which make different levels of this model[13]. This model has been described in three levels. The first level is the life quality. The second level consists of 4 criteria including: physical health, mental health, social relations and environment health and the third level of the model is the analyzing of more sub-criteria . For analyzing of data, the multiple regressions and for comparing results the one-sample test of Kologroph-Smirnoph have been used.

3. Results

According to findings which were obtained from one-sample T test, the average of public life quality in patients with hepatitis B was weak (1<1.76<5) and in healthy people was evaluated as average (1<2.94<5). Also, for comparing the score average of life quality at six cities, one-sample test of Kologroph- Smirnoph was used. Mental health domain in second level for patients group was lower than other sub-criteria (1<1.40<5), on the other hand, in healthy people group, the environment health domain was lower than other sub- criteria (1<2.46<5).

4. Discussions

HBV leads to cirrhosis in up to 20% of those chronically infected and is a one of the most common indications for liver transplantation worldwide. This economic burden is compounded by the significant impact of HBV on health- related quality of life (HRQOL) resulting from complications of advanced liver disease, such as encephalopathy, variceal hemorrhage, ascites, and liver transplantation. After statistical analysis on quality of life in two groups, the mean of life quality and its domains in two groups in table 1 at two level and criteria ranking at table 2 and the mean of life quality of sub criteria in comparison form presented in table 3. According to results quality of life in patients with hepatitis B was weak (1<1.76<5) but at healthy people was average (1<2.94<5) that significant differences is between healthy people and patient group. This result is similar to Meltem study. In his study performed on 131 patients with HBV showed that the scores of life quality in patients in comparison with the control group were lower than healthy people. According to the study results, the authors reported that HBV carriers had significantly higher levels of depression and anxiety and lower level of functioning when compared with healthy controls [15]. Niederaun and his colleagues stated that the life quality of in patients with chronic hepatitis C (especially in treated patients with interferon) was lower than the normal range, and quality of life as well as mental health damage have been increasingly seen according to the progression of liver disease and ineffective antiviral treatment [16]. In the sub- criteria at the second level of patients group, mental health was lower than other sub-criteria (1<1.40<5); on the other hand, in healthy group, the environmental health was lower than other sub criteria (1<2.46<5). Bernstein hypothesized that attention to life quality was the main concerns of chronic hepatitis patients and patient care should be propelled to maintain life quality such as the ability to maintain the job and relationship with family and friends, and to continue their happiness and enjoyment of pleasant situation[17].

As shown in the table 2, the impact coefficient (β) of life quality for patient group was 0.253. Sub-criteria of the second level in this group which were effective on life quality included: mental health, social relations, and physical and environment health with impact factors (coefficient) of 0.272, 00. 0.244 and 0.242, respectively. Furthermore, Sub- criteria of the second level in the healthy group included mental health, social relations, and physical and environment health with impact factors (coefficient) of 0.558, 0.550, 0.537 and 0.438 respectively. In a previous study carried out by Ghanbariet al., age, gender, AST, clinical symptoms, mental and physical health were effective on life quality with β factors: 0.33, 0.18, 0.19, 0.35, 0.14 and 0.15, respectively. But physical health as an interface factor changes 95% of life quality score and mental health change it 78%. In total, 58% of changes in life quality fit with this model.

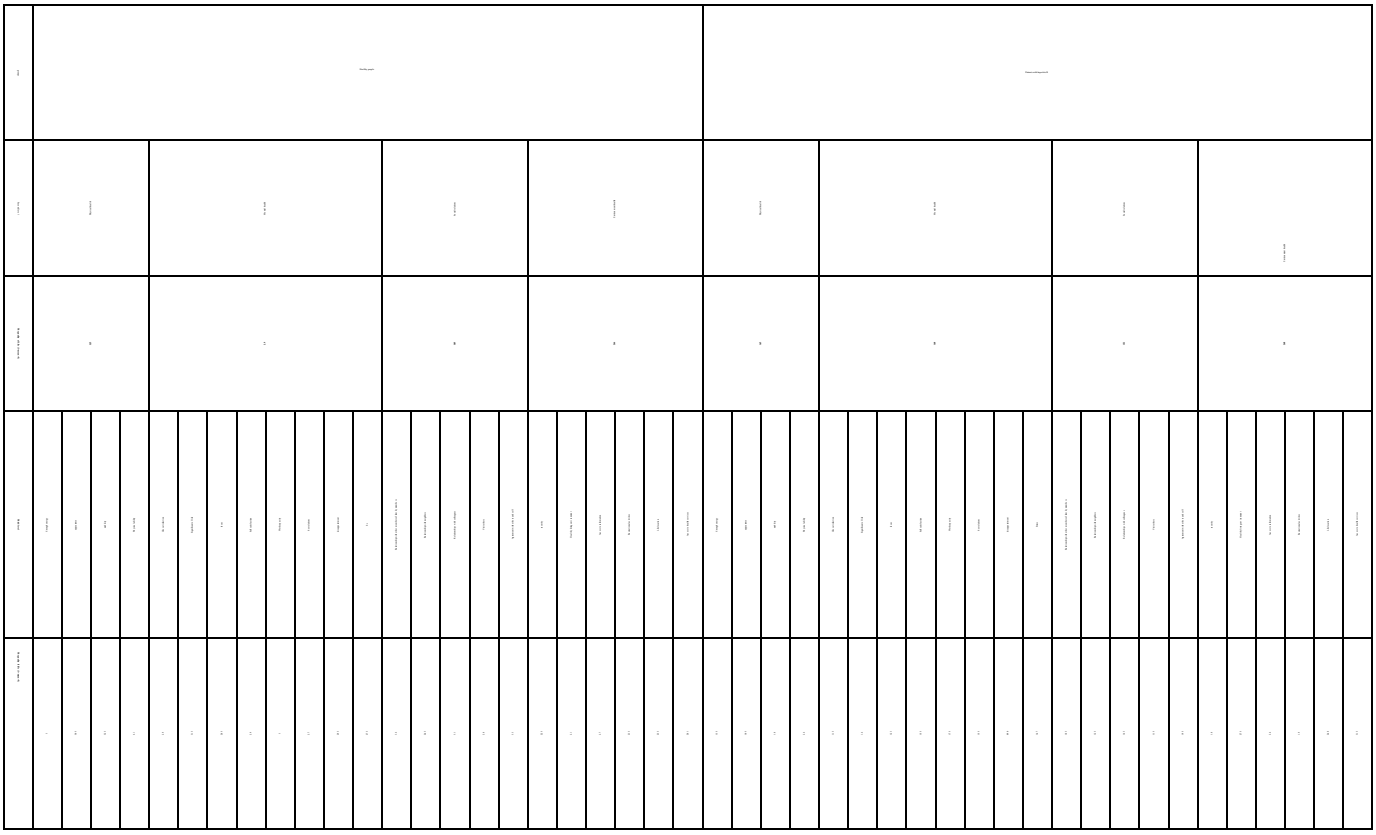
Table 1: The situation of life quality between hepatitis B patients and healthy people as criteria of the first and second levels

Groups	First level	The quality of life (1<mean<5)	Second level	Life quality (1<mean<5)
Patients with hepatitis B	The quality of life	1.76	Physical health	1.65
			Mental health	1.40
			Social relations	1.53
			Environment health	2.48
Healthy people	The quality of life	2.94	Physical health	3.35
			Mental health	2.9
			Social relations	3.05
			Environment health	2.46

Table2: β Coefficient of the importance of criteria and sub- criteria of hepatitis B patients and healthy people at the third and second levels.

Groups	First level	β coefficient	Second level	β coefficient
Patients with hepatitis B	The quality of life	0.253	Physical health	0.244
			Mental health	0.272
			Social relations	0.254
			Environment health	0.242
Healthy people	The quality of life	0.532	Physical health	0.537
			Mental health	0.550
			Social relations	0.558
			Environment health	0.483

Table 3: Situation of quality of life in hepatitis B patients compared with healthy people as criteria and sub-criteria of second and third levels.



The important issue arose from these results indicated that the disease associated with the impact factor (coefficient) of 0.36 had more effect on mental health in comparison with other independent variables and clinical symptoms (with 0.35 of the impact coefficients has a direct effect on life quality). Physical health and health with 0.15 and 0.14 of the impact coefficients, respectively affected the life quality[18]. In some studies on quality of life, the effective variables were individual features and diseases[19], however, in this study, we did not include those issues as we were not ascertain about the stage of chronic disease in Patient group.

In the end, sub- criteria of third level in hepatitis patients, disappointment with average (1<0.9<5) and environmental health with (1<2.1<5) in healthy people were the lowest level of life quality. Generally, according to findings quality of life in hepatitis patients is lower than healthy people and also, mental health in hepatitis patients is more important than social relations in healthy peoples. So, managers should be aware about promotion of life quality by good program and intervention with every group to prepare the appropriate level of life quality. All together, increasing the number of samples together with application of other interventions with comprehensive designation, the quality of these studies will be increased. One of the main steps for improving the quality of life of these patients is incorporation of patients cares in Primary health care (PHC) system.

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Comparison of the Low Dose Polyethylene Glycol with Lactulose and Magnesium Hydroxide in Constipated Children. A Multicentric Randomized Clinical trial.

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Abstract: This study was designed to compare the efficacy and safety of very low dose of polyethylene glycol 3350 (PEG) with magnesium hydroxide (MOM) and lactulose in functional constipation of children. A total number of 468 patients 1-15 year old with chronic constipation entered a randomized comparative multicenteric trial. Subjects were healthy outpatients who had hard, painful or $\leq$ 3 stools per week. Their parents were given a teaching pamphlet about constipation, diet and toilet training. The patients were allocated to 3 treatment groups; lactulose (70%) 1ml/kg/day/BID, MOM (400mg/5ml) 1ml/kg/day/BID, and PEG (40%) 1ml/kg/day/BID. The dose was adjusted up to three times depending on responding. Treatment scheduled for two months and data were collected on 2ed, 4th and 8th weeks. 354 patients completed the trial. After eight weeks, patients in the PEG groups had higher number of bowel movement (Pv< 0.001) and low straining at stool (Pv <0.001) than patients in two others groups. Patient's adherences with PEG were better than lactulose and magnesium hydroxide (Pv<0.001). Soiling and blood on stool declined significantly in three groups (Pv<0.001) without differences among. There were no serious adverse effects. Thus low dose of polyethylene glycol 3350 was more effective than lactulose and similar to M.O.M for the treatment of functional constipation and better tolerated without any significant adverse events in children.
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Key words: Constipation, Polyethylene glycol, lactulose, magnesium hydroxide, laxative, children.

1. Introduction:

Constipation, defined as a delay or difficulty in defecation, is a extremely common pediatric problem(Gordon et al. 2012, Ahmed, Pai and Reynolds 2012), estimated to occur in 3(Tabbers et al. 2011) to 10% of children (Leung, Chan and Cho 1996) and 12% to 35% of the general population.(Youssef 2007, De Giorgio et al. 2011) The incidence appears to be increasing, possibly because of changes in diet, reduced fluid intake and lack of exercise.(Hardikar, Cranswick and Heine 2007) Beyond the neonatal period, about 95% of constipations has a functional cause and can result in fecal impaction, fecal soiling and abdominal pain.(Dupont et al. 2006, Tabbers et al. 2011) Functional constipation most commonly is due to painful bowel movements with resultant voluntary withholding of feces by a child who wishes to avoid an unpleasant defecation.(Baker et al. 1999) Specific evidence-based standards for evaluation and treatment are lacking.(Miller, Dowd and Fraker 2007, Burgers et al. 2012) The recommended approach is to empty the constipated bowel and keep it empty.(Candy, Edwards and Geraint 2006, Biggs and Dery 2006) The successful management of chronic constipation involves several aspects, including an increase in dietary fiber(Schmulson Wasserman et al. 2008) and fluid intake(Bae, Son and Lee 2010), the introduction of regular toilet sits after the main meals,(Loening-Baucke 1993, Bautista Casasnovas et al. 2011) behavioral modification(Bell and Wall 2004), counseling and the use of various laxatives and stool softeners (Pashankar and Bishop 2001) and, above all, close follow-up.(Croffie JM 2004) The long-term outcome in children with chronic idiopathic constipation, with or without encopresis, is not well established. Many children do not respond to medication and continue to have chronic problems.(Nurko 2000) Treatment success rate reported between 50 to 90% with various laxatives.(Davidson, Kugler and Bauer 1963, Staiano et al. 1994) Recently, low dose polyethylene glycols have been suggested as safe and effective alternative treatments for constipation. Polyethylene glycols

(PEG) laxatives, whose osmotic properties enable softening of stools and promoting bowel transit (Andorsky and Goldner 1990), have clearly demonstrated their efficacy and tolerance for the treatment of chronic constipation, not only in adults but also in children.(Dupont et al. 2006, Wang et al. 2012, Horn, Mantione and Johanson 2012) Electrolyte-free PEG 3350 (MiraLax,) has been used as a laxative for short term(Pashankar and Bishop 2001, Gremse, Hixon and Crutchfield 2002, Dupont et al. 2005) and long term(Loening-Baucke 2002, Pashankar, Bishop and Loening-Baucke 2003, Pashankar, Loening-Baucke and Bishop 2003, Michail et al. 2004, Erickson et al. 2003, Loening-Baucke and Pashankar 2006, Voskuijl et al. 2004) in constipated children. The safety and efficacy of 0.63 to 1.5g/kg/day of electrolyte-free PEG to treat constipation have been recently demonstrated in children.(Erickson et al. 2003, Pashankar and Bishop 2001, Rafati et al. 2011) We have used small daily doses (0.4g/kg/day) of PEG 3350 solution in our clinical practice and have found it to be a safe, effective and palatable laxative. We then carried out a prospective, comparative, multicenteric, controlled trial examining efficacy and dosing of PEG in constipated children.

2. Materials and methods

This comparative, randomized and controlled trial was conducted between October 2007 and September 2008 in 5 tertiary care centers in I.R.Iran. Because we were not able to produce the three remedies in one shape, the study was not blinded. The trial was approved by the Shiraz University of Medical Science Ethics Committee. Sample size of 354 patients was calculated for showing a difference at least 15% between three groups. One hundred eighteen children were assigned randomly to receive one of medications. According to plurality of research centers and probability of high attrition we decided to enroll about 500 cases; 150 subjects in Shiraz, 100 patients in Tehran and Isfahan and 75 patients in Sari and Babul each one, but study was finished with completion of determined sample size. Systematic randomization was used for allocation of the patients and prepared dossiers and PEG solution were sending from Shiraz to other centers, all of which used the same investigational protocol (Figure.1). In spite of using systematic randomization for allocation of the patients, for ethical considerations we decided in each group, if the patients had been tried the same therapies previously, and had refractory problem, they were assigned to afterwards schedule, although we anticipated that the number of patients in three groups were not equal. Functional constipation was defined by a duration of ≥8 weeks and ≥2 of the following characteristics: frequency of bowel movements of <3 stools per week, >1 episode of fecal incontinence per week, large stools noted in the rectum or felt during abdominal examination, passing of stools so large that they obstructed the toilet, and retentive posturing (withholding behavior).

Exclusion criteria were impossibility to access the subject (no phone), hirschsprung's disease, anorectal malformations, history of abdominal surgery, no use medication and any systemic illness that could lead to constipation. Baseline evaluations included history, physical examinations and collection of demographic data before initiation of the study. Before the first appointment, parents have been expounding about the pathophysiology of constipation and the rationale of therapy. In addition to, we had made ready a teaching pamphlet consisting of five sections: 1) introduction to constipation, 2) diet, 3) toilet training, 4) enema and 5) therapy, and had given them. Children of appropriate developmental status were advised to sit on the toilet for 5-10 minutes after each meal. Written informed consent was obtained for all participating patients.

Then the children who fulfilled the selection criteria were randomly allocated to 3 therapeutic groups: lactulose (70%) 1ml/kg/d/BID, magnesium hydroxide (400mg/5ml) 1ml/kg/d/BID, and polyethylene glycol 3350 (40%) 1ml/kg/d/BID. The polyethylene glycol 3350 (solution 40% with no added salts) was supplied to the investigator in 40g/L. For preparation of 40% PEG (3350) solution; sodium benzoate (0.1%, w/v) and sugar (25%) were dissolved in an adequate amount of water preheated to 80°C. The required amount of PEG 3350 for preparing 40% (w/v) solution is added to the adequate amount of water preheated to 80°C, and the sodium benzoate/sugar solution is added to the PEG mixture during stirring and then stirred to complete dissolution. Adequate amount of orange essence and an orange color was added to the solution after complete dissolution of the mixture.

Study was scheduled for an 8-week period and treatment efficacies were evaluated at days 14, 28, and 56. Clinical efficacy and tolerance were assessed using a questionnaire which included the administered drug and dose, number of stools and soiling and presence of the following symptoms: straining at stool, blood on stool, abdominal pain, vomiting, bloating and flatus.

If the treatment was to be considered to be having insufficient effect (persisting symptoms), the administered dose could be increased 0.5 to 1ml/kg/d per visit up to 3ml/kg/d. However, if the diarrhea was reported (defined as; more than 3 stools/d) the original dose was reduced by 50%. If the subjects unable to return to clinic for follow up, history was obtained by

phone contact. After the initial phase, the patients with history of prolonged constipation duration beforehand were asked to continue cooperation with investigators for an additional 16 weeks. Symptoms recorded with outpatients clinical follow up at 16 and 24 weeks.

Primary Outcomes

Improvement was defined as ≥ 3 bowel movements per week, ≤ 2 episodes of fecal incontinence per month, and no abdominal pain, with or without laxative therapy.

Statistical analysis

It was estimated that a total sample of 354 patients would be adequate to show a difference of at least 15% more success at 2 months using PEG compared with lactulose and MOM (alpha risk= 0.05 and power=80%). For each patient the clinical efficacy and tolerance variables were analyzed for the first and second two weeks and the last four weeks as well as for the eight weeks of the study. Mean values were calculated for the number of stools, dosage of drugs taken and soiling per month between groups. Subjective criteria were using χ^2 with continuity correction and Pearson correlation. Quantitative mean data were compared by one-way ANOVA. A value $p<0.05$ was consider statically significant.

3. Result

A total numbers of 468 patients with functional constipation were enrolled the study in 5 centers in I.R. Iran and 354 (186 female and 168 male) patients completed the 8 week period of the study. The subjects were 164 patients in Shiraz because the study was designed here and started earlier, but the numbers of cases were 93 patients in Tehran and 80, 69 and 60 patients in Isfahan, Sari and Babul, respectively.

Since the most of patients had been used several different therapies (i.e. lactulose and M.O.M) previously, if the patients had refractory problem, they were assigned to afterwards schedule, thus the number of patients in three groups were unequal. Because lactulose and M.O.M had been used routinely in Iran formerly, and PEG were used for the first time, therefore there were 154 children in PEG group, 94 receiving lactulose and 106 receiving MOM.

The three treatments groups were well matched for all baseline clinical characteristics (table 1). Mean age was 4±2.26 (range, 1 to 13 years). Mean duration of constipation was 2.04±1.7 years (range, 6month to 7 years). Mean age at start constipation was 1/95±1.59 years (1 to 11 years) and in 76% start before 2 years. Mean weight was 15.43±5.56 kilograms.

The mean PEG treatment dose at the 2-month follow-up evaluation was 0.46 ± 0.11 g/kg body weight daily, the mean MOM and lactulose daily doses at the 2-month follow- up evaluation were 1.05 ± 0.38 ml/kg and 1.07 ± 0.33 mL/kg body weight, respectively. Results of the 8-week follow-up are shown in table 2. Throughout the eight week period, the mean stool frequency was higher in the PEG than MOM than lactulose groups (PV=0.0001).

Table 1. Baseline patient's characteristics

Groups / Variables	PEG (n=154)	Lactulose (n= 94)	MOM (n= 106)	Pv
Sex (F/M)	86/68	56/38	64/42	0.24
Age, mean ± SD (yr)	4 ± 2.26	3.77 ± 2.3	4.29 ± 2.36	0.24
Weight, mean ± SD (kg)	15.1 ± 4.7	14.76 ± 4.8	16 ± 7.3	0.22
Mean duration of constipation	1.82 ± 1.54	2.15 ± 1.42	2.27 ± 2.08	0.09
Mean age at start constipation± D(yr)	2.1 ± 1.49	1.6 ± 1.68	2 ± 1.62	0.06
Mean stool/week	2.08 ±0.98	1.8 ± 0.98	2.04 ± 1	0.28
Withholding (%)	118 (75.6%)	80 (85.1%)	86 (85.1%)	0.18
Abdominal pain(%)	55 (35.3%)	30 (31.9%)	38 (35.8%)	0.81
Painful defecation(%)	133 (85.3%)	86 (91.5%)	94 (88.7%)	0.33
Positive family history (%)	59 (37.8%)	40 (42.6%)	42 (39.6%)	0.76
Fecal impaction (%) (Disimpaction)	88 (56.4%)	54 (57.4%)	58 (54.7%)	0.92
Soiling (%)	33 (21.2%)	14 (14.9%)	28 (26.4%)	0.13
Blood on stool (%)	65 (41.7%)	36 (38.3%)	32 (30.2)	0.16

Abdominal pain, soiling and bloody stool decreased significantly within groups (PV=0.0001) but no significant differences between three groups were seen (PV=0.2, 0.13 and 0.16 respectively), and there were no new abnormal findings on follow up examination.

Straining at the stool was seen in the majority of patients before treatment (91.5% in lactulose, 90.4% in MOM and 85.3% in PEG) and significantly decreased within groups after treatment (lactulose 51.1%, MOM 28.3% and PEG 18.6%). There was significant differences between groups (P=0.0001).

As can been seen in table 2, this study demonstrates that PEG and MOM are more effective than lactulose in treating childhoods functional constipation over an 8-wk period and improvement rates were 90.4% with PEG, 88.7% with MOM and 70.2% with lactulose (PV=0.001). Adverse events leading to drug withdrawal were recorded during the study; twelve (12.5%) of children interrupted lactulose (because of abdominal pain, diarrhea and flatulence) whereas five (4.7%) cases discontinue MOM (because of diarrhea and intolerance) before to be cured, but fifteen (9.6%) cases refused PEG (because of worry) despite primary agreement and informed consent. Failure rate with intent to treat was 29.8% with lactulose and 9.6% and 11.3% with PEG and MOM respectively. (PV=0.0001).

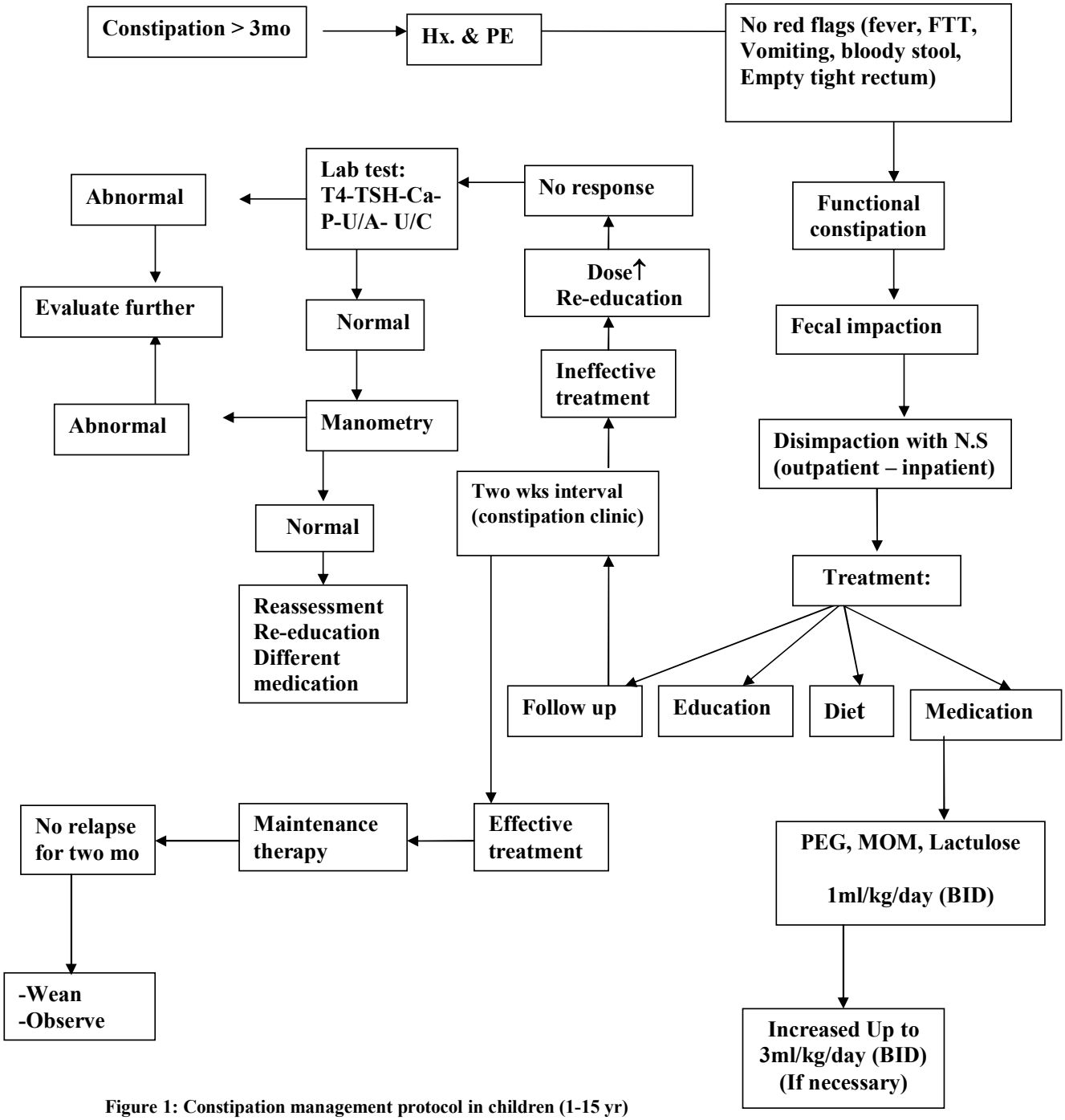

Figure 1: Constipation management protocol in children (1-15 yr)

Table 2. Comparing childhood constipation response to different medications.

Groups / Variables	Lactulose (n=94)	Magnesium Hydroxide (n=106)	Polyethylene glycol (n=154)	Pv
Mean doses (ml/kg)	1.07 ± 0.33	1.05 ± 0.38	1.08 ± 0.27	0.84
Mean stool/2nd wk	4.7 ± 2.06	5.7 ± 1.7	6.1 ± 1.7	0.001
Mean stool/4th wk	5.3 ± 2.03	5.79 ±1.77	6.15 ± 1.71	0.002
Mean stool/8th wk	5.09 ± 2.07	5.67 ± 1.77	6.07 ± 1.77	0.0001
Abdominal pain (%)	20 (21.3%)	34 (32.1%)	31 (19.9%)	0.05
Painful defecation (%)	48 (51.1%)	30 (28.3%)	29 (18.6%)	0.0001
Soiling (%)	8 (8.5%)	14 (13.2%)	8 (5.1%)	0.06
Blood on stool (%)	8 (5.1%)	10 (10.6%)	12 (11.3%)	0.13
Improvement: n (%)	66 (70.2%)	94 (88.7%)	141 (90.4%)	0.001
Failure /stop: n (%)	28 (29.8%)	12 (11.3%)	15 (9.6%)	0.0001

4. Discussion

This is the first large-scale prospective randomized and multicenteric study designed to compare PEG 3350 with two other commonly used laxatives (magnesium hydroxide and lactulose) in children with functional constipation. We found that all three resulted in a significant increase in defecation frequency and a significant decrease in encopresis frequency after eight weeks of treatments. However, PEG and MOM were more effective than lactulose.

PEG 3350 without added electrolytes is a new non-absorbable, tasteless, odorless(Humphreys and Reinberg 2005) and chemically inert polymer(Loening-Baucke and Pashankar 2006) and has been introduced as a new laxative in recent years.(DiPalma et al. 2000, Cleveland et al. 2001, Herve et al. 2001, Hudziak et al. 1996, Youssef et al. 2002, Benninga, Candy and Taminiau 2005)

Since the use of laxatives alone had low success rate,(Abrahamian and Lloyd-Still 1984, Loening-Baucke 1990) and 50% of treated patients experience a relapse within 1 year (van Ginkel et al. 2003, Staiano et al. 1994) it is suggested that toilet training, dietary advice and regular use of laxatives should be combined to prevent future impaction and to ensure a prolonged period of painless defecation, which is essential to provide the confidence necessary for promoting regular bowel habits,(Dupont et al. 2006) thus we made ready a teaching pamphlet about constipation, diet, toilet training, enema and therapy, and gave the patients parents.

The findings of this study are shown that PEG 3350 without electrolytes was safe and well tolerated, and significantly more effective than lactulose in the treatment of chronic constipation in children over an 8-wk period, but with similar efficacy to MOM. Similar to our study, previous studies could not demonstrate superior efficacy of PEG over MOM(Loening-Baucke 2002, Loening-Baucke and Pashankar 2006, Loening-Baucke 2005, Gomes, Duarte and Melo Mdo 2011), whereas in other controlled studies PEG has been shown to be more effective than lactulose (Candy et al. 2006, Voskuijl et al. 2004, Gremse et al. 2002, Rendeli et al. 2006, Dupont et al. 2005, Wang et al. 2012, Lee-Robichaud et al. 2010).

Because of multiplicity of research centers, we could not collecting data after approved two months period. Since, by reason of good results from PEG therapy and users satisfaction, after completion of research, some investigators change other medications of their patients to PEG, whenever, the majority of patients continued for months. No significant side effects or tolerances were reported by any researchers for PEG after six months, only one patient needs to rising the total dose of PEG up to 1gr/kg/day (2.5ml/kg/day) in Esfahan. The low adverse reaction of PEG may be due to low dose utilization comparing to other studies with high dose and high adverse reactions.(Youssef et al. 2002, Andorsky and Goldner 1990, Loening-Baucke 2002, Pashankar and Bishop 2001)

Limitations of this study include short follow up and choice of an open-label design; because we were not able to prepare three medications in a similar shape and color needy for an actual controlled trial. Also, although systematic randomization was used for allocation of the patients but if the patients had been used the ready records medication previously, and had refractory problem, they were assigned to receive the drug of subsequent ready file. Thus the numbers of patients in three groups were not equal, and because of widespread using of lactulose and MOM for pediatrics constipation beforehand, these groups making smaller population than PEG group.

In conclusion, we can say that low dose PEG is more effective and safe for treating childhood's functional constipation but double blinded clinical trial with long term follow up is required for confirmations.

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Human Activity Recognition System: Using Improved Crossbreed Features and Artificial Neural Network

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Abstract: In this paper, we present an intelligent method of human action recognition based on hybrid features. These features are calculated from the space-time cubes (interest points). The Blocks or cubes are derived from the difference of consecutive frames. These features include average number of blocks per frame, velocity of the blocks i.e. average angle and displacement, some of the kinematic features like divergence and vorticity, and hu features derived from motion energy images (MEI). Principal Component Analysis (PCA) has been used to reduce the dimensionality of the feature vector. For classification, we employ artificial neural networks (ANNs), in which each action video is represented by a bag of features.
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Keywords: Human activity recognition; PCA; ANNs

1. Introduction

Human activities recognition in videos is one of the most promising and emerging applications of computer vision. In the current era, the problem of human activity recognition has caught the attention of researchers from academia, industry, security agencies, consumer agencies and the general public as well [1-8]. In simple words the problem of human activity recognition can be defined as, in a sequence of images having one or more persons performing single or multiple activities, can a system be designed that can automatically recognize the activity (activities) being performed? As simple as the problem seems to be, the optimum solution has been that much difficult to find. The human activity recognition system typically follows a hierarchical approach. At the lower levels background foreground subtraction, tracking and object detection is performed. Action-recognition modules serve as mid-level functions. Reasoning engines are at the high-level. They encode the activity semantics based on the lower level action-primitives. Therefore, it is necessary to have an understanding of both these problem domains to enable deployment of these systems in real-life. Some of the application areas that highlight the potential impact of vision-based activity recognition systems are behavioral biometrics, content based video analysis, security and surveillance, interactive applications and environments, and animation and synthesis [9-12].

In general, approaches used for human activity recognition can be categorized on the basis of representation of the features [1]. Some well-known representations include learned geometrical models of human body parts, space-time pattern templates, region features, shape features, interest-point-based representations, and optical flow patterns.

2. Proposed Method

In this paper, we utilize the motion of human body for the activity recognition. The proposed method consists of three steps which are feature extraction, dimensionality reduction and classification. The system diagram of the proposed approach is given in figure 1.

2.1 Interest Points Based Feature Extraction

We use a set of features for human action recognition. All these features depend on the motion of an actor performing the action. Multiple methods are exploited to extract features. All of the features are computed from the interest points. The success of interest points in object detection, their sparsity, and robustness against illumination and clutter have inspired a number of researchers working in the area of motion analysis and activity recognition. In the first step, we compute interest points and then extract features from these computed interest points. Our method of interest points computation is different from [7, 12] in the way they are computed. We describe next our method of interest points computation in detail. First of all each frame of video is localized and a Gaussian filter is applied on it. The Gaussian filter firstly smoothes out the frame and hence the noise is reduced. Secondly the small changes are ignored due to Gaussian smoothing filters. Each frame is divided into blocks (cubes) of size 3×3 or 5×5. Then each corresponding block in

the two consecutive frames is matched if its difference value is greater than a predefined threshold then that block is considered as a spatial temporal cube (interest point) otherwise it is ignored. The advantage of the threshold is that it further reduces the small individual changes. This process is repeated for all the frames of the video.

In the next step these interest points are mapped to the interest points generated in the previous frame which reduces the complexity of the algorithm as compared to the computation of optical flow. The mapping process becomes feasible due to small number of interest points as compared to the all pixels of all frames. The mapping process evaluates the benefit of the direction followed by most of the interest points. The histogram of moving parts also increases the accuracy of matching interest points in two consecutive frames. The advantage of the block based correlation is also evaluated to increase the accuracy of matching.

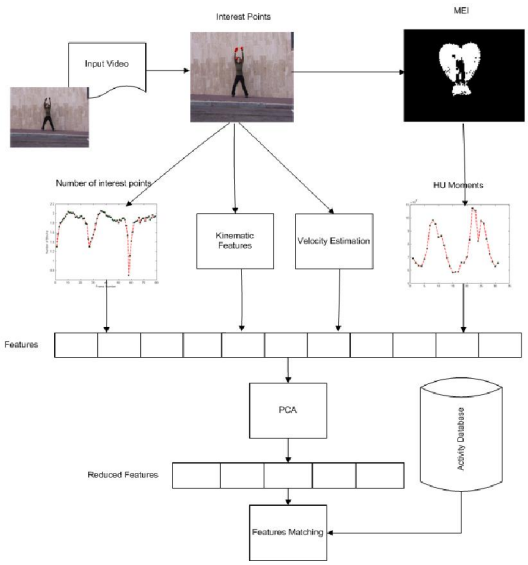

Figure 1. System diagram of human activity recognition

The outliers are removed before the matching of interest points. As shown in figure 1 rest of the features used for activity recognition are computed from these interest points. The first feature is the number of interest points generated for each frame and it is mostly different for various actions performed. Further, each type of activity has a great effect on the variation of interest points produced on each frame. The number of interest points computed for each frame is very close to each other in the running action while during performing the walking action the number of interest points varies regularly. Similarly all other actions have a close relation with the number of interest points generated on each frame of the video as shown in figure 2. Further, number of interest points generated for different actions are plotted against the frame numbers in figure 5. Number of interest points varies considerably for different actions.

2.1.1 *Kinematic Features Extraction and Reduction using PCA*

Kinematic features are those features that rely on the motion of the body parts and not on the forces responsible for motion. These kinematic features are different from the kinematic features used by Shah et al. [10]. We compute these kinematic features from the velocity of the interest points while they [10] have computed it from the optical flow vectors. We consider the interest points while they are using individual pixels of the flow vector. As the number of pixels are more than the number of interest points so our proposed approach reduces the complexity of the algorithm considerably. As a result of the mapping process the horizontal, vertical displacements and direction of these interest points are calculated. These components of velocity are then used for deriving the kinematic features. The kinematic features which we compute are divergence and vorticity. Divergence of a flow field is a scalar quantity which is defined at some point (x, ti) in space and time as the sum of the horizontal and vertical distances i.e. the partial derivatives of the point with respect to horizontal and vertical direction.

Divergence is calculated using equation (1). The importance of the divergence is that it has the ability to capture the amount of local expansion that is why it helps to discriminate between the independent and dependent motions of the different parts of the body. It means that the independent moving parts of the body will have a high value for divergence than the dependent motion of the body parts. The second kinematic feature used for human action recognition is the vorticity. It is a broader concept than rotation; still they are very closely inter-related. Vorticity also called rotation per unit area is the measure of local spin around the axis perpendicular to the plane of the flow field. It can be computed at a point (x, ti) as the difference between the partial derivative of the spatial interest points with respect to x, and y coordinates. It measures the rigidity in motion. It is computed using equation (2). It means that the divergence of bending or hand waving will be greater than that of walking which lack curl or rotation.

$$Dv(x,t_i) = \frac{\partial u(x,t_i)}{\partial x} + \frac{\partial v(x,t_i)}{\partial y} \quad (1)$$

$$Vr(x,t_i) = \frac{\partial u(x,t_i)}{\partial x} - \frac{\partial v(x,t_i)}{\partial y} \quad (2)$$

The velocity itself is also used as a feature for human action recognition because it varies for different actions. For instance, walking action has a less velocity than a running action.

Bobick et al. [13] use motion energy images (MEI) to recognize many types of aerobics exercises. The binary cumulative motion image of a sequence of frames is known as motion energy images. Let I(x, y, t) be a sequence of images and B(x, y, t) be a binary sequence of images showing the region of motion. Then the motion energy image ET (x, y, t) can be computed as given in equation (3). The MEI can be calculated using frame differencing. In equation (3) and equation (4) represents the temporal extent of an action.

$$E_T(x,y,t) = \bigcup_{i=0}^{T-1} B(x,y,t \quad i) \quad (3)$$

Obviously, if periodic motion exits in the video sequence, we can find similar poses in different frames. In other words, high correlation value can be obtained when comparing the foreground blobs in different frames within a specific period of time. In simple words the change of two consecutive frames is accumulated to form MEI. So in our algorithm we compute the MEI by accumulating the interest points of the frames till the last frame of the image sequence. Mathematically equation (4) summarizes this process. In Equation (4), 'A' represents the number of space time interest points of a particular frame. The MEI can also be achieved by thresholding the motion energy images above zero. In MHI pixel intensity is a function of temporal history of motion at that point. It is a scalar valued image. In MHI [14] more recently moving pixels are brighter than previous moved pixels. The MEI generated in our algorithm is shown in figure 3.

$$MEI(x,y,t) = \bigcup_{i=0}^{T-1} \bigcup_{j=0}^{A} I(x,y,t-i) \quad (4)$$

This MEI is then used to compute the statistical moment based features. We are using Hu moments as a part of our feature set. The use of moments as invariant binary shape representations was first proposed by Hu in 1961 [11]. He successfully used these features to classify handwritten characters. The regular moment of a shape in an M by N binary image is defined in equation (5).

$$u_{pq} = \sum_{i=0}^{M-1} \sum_{j=0}^{N-1} i^p j^q f(i,j) \quad (5)$$

In equation (5) i ε {1, . . . , M} and j ε {1, . . . , N} and p = 0, . . . , M – 1 and q = 0, . . . , N – 1. These MN moments ($u_{p,q}$) are sufficient to uniquely determine the discrete image f(i,j) with MN pixels, p+q is the order of moment. As this calculation is a function of the distance between shape pixels and the origin, so the measurements are taken relative to the shapes centroid to make it translational invariant. Therefore the coordinates of the centroid can be determined using equation (6).

$$\bar{i} = \frac{u_{10}}{u_{00}} \quad and \quad \bar{j} = \frac{u_{01}}{u_{00}} \quad (6)$$

Relative moments are then calculated using equation (7) designed for the computation of central moments.

$$u_{pq} = \sum_{i=0}^{M-1} \sum_{j=0}^{N-1} (i-\bar{i})^p (j-\bar{j})^q f(i,j) \quad (7)$$

These moments individually do not have the descriptive power to uniquely discriminate arbitrary shapes. Further they do have the invariance characteristics. In this experimental study, we derive Hu feature set of 7 invariant features by combining different moments. These features can be calculated using equations (8-14).

$$M_1 = (u_{20} + u_{02}) \quad (8)$$

$$M_2 = (u_{20} + u_{02})^2 + 4u_{11}^2 \quad (9)$$

$$M_3 = (u_{30} + u_{12})^2 + (3u_{21} + u_{30})^2 \quad (10)$$

$$M_4 = (u_{30} + u_{12})^2 + (u_{21} + u_{03})^2 \quad (11)$$

$$M_5 = (u_{30} + 3u_{12})(u_{30} + u_{12})((u_{30} + u_{12})^2 - 3(u_{21} + u_{03})^2) + (3u_{21} + u_{03})(u_{21} + u_{03})(3(u_{30} + u_{12})^2 - (u_{21} + u_{03})^2 \quad (12)$$

$$M_6 = (u_{20} \mid u_{02})((u_{30} \mid u_{12})^2 \quad (u_{21} \mid u_{03})^2 \mid 4u_{11}(u_{30} \mid 3u_{12})(u_{21} \mid u_{03}) \quad (13)$$

$$M_7 = (3u_{21} - u_{03})(u_{30} + u_{12})((u_{30} + u_{12})^2 - 3(u_{21} + u_{03})^2) - (u_{30} - 3u_{12})(u_{21} + u_{03})(3(u_{30} + u_{12})^2 - (u_{21} + u_{03})^2) \quad (14)$$

These moments provide reasonably discriminative shape based features. The Hu invariant moments include area, centroid, and information about its orientation. They are less sensitive to noise, rotation, and translation.

After extracting all the features, the feature vector has a high dimensionality which may increase the computational complexity of the classification. Considering of this fact, we employ Principle Component Analysis PCA to reduce the curse of

dimensionality. PCA is a technique that reduces data dimensionality by performing a covariance analysis between factors.

2.1.2 Classification using ANN

After extracting features and applying PCA to reduce the feature vector dimensionality, an Artificial Neural Network (ANN) is trained on a set of given action videos to recognize human activity. The whole action video data set is divided into training and test sets. The training data was used to train the network through back propagation algorithm. The testing data was used to check the performance of the trained neural network. The classification of testing videos gives an indication of how well the network generalizes the classification for new unseen data set. A 3-fold cross validation method was used in which the whole data was divided into 3-folds. Each fold is composed of equal number of videos of different actions. Out of the three folds, two were used for training and the rest one is used for testing the accuracy of the neural network. The training and testing process was repeated for maximum possible combinations of the three folds.

The number of input neurons in our experimentation depends on the number of inputs. The input vector is a combination of all features which we derive as mentioned in previous section. For a 25 frames of an action sequence the feature vector has 131 data elements. It contains average distance, angle, vorticity, and divergence of all interest points of each frame. It also includes the number of interest points generated in each frame and 7 Hu moments calculated from the average motion energy images. So without using PCA the input vector has length of 131 elements. So the input neuron will be 131. After extensive experimentations 5 hidden layers having 50 neurons each, 6 neurons in output layer which represents the number of classes of action videos. The experimentation also proves that the tansig works well as activation function. Using PCA 80 input features works considerably well for the recognition of different actions. So after feature vector dimensionality reduction through PCA, number of input neurons is taken 80. Besides, the experiments are conducted on the features of each frame as well. In this case the number of input neurons depends on the number of interest points, velocity i.e. magnitude and direction of each interest point, vorticity and divergence of all interest points, and Hu moments of the Motion Energy Image generated from each frame of action sequence. It increases the computational complexity a little bit.

Table 1. Confusion matrix for our algorithm

Activity	Hand Clapping	Hand Waving	Jogging	Boxing	Running	Walking
Hand Clapping	88.5	2.2	8.13	1	0	0
Hand Waving	2.2	92.1	1.2	2	1.9	1.1
Jogging	3.5	6.4	88.6	2	0	0
Boxing	0	2.1	1	88	5.34	3.2
Running	0	2.61	1.2	3	88.1	4.87
Walking	0	0	1.1	5	7.92	86.31

3 Results & Discussion

In order to verify the performance of our algorithm, we performed experiments on many datasets including KTH dataset and [2, 6]. The KTH dataset consists of six different actions which includes hand clapping, hand waving, boxing, running, walking and jogging. It is a challenging data set due to the zooming in and out, and change in the size of the actor. Confusion matrix obtained through our proposed method for the KTH data set is presented in table 1.

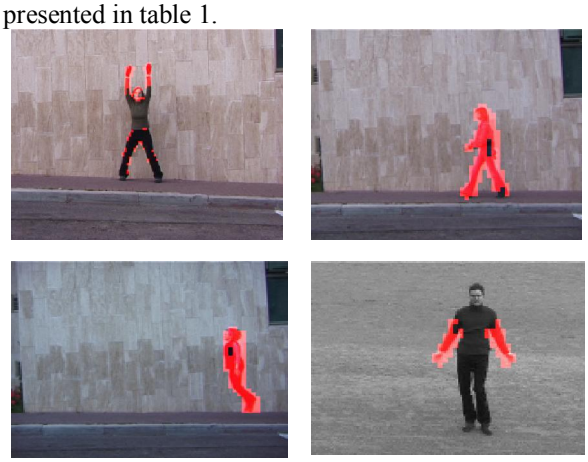

Figure 2. Interest points generated for different activity sequences

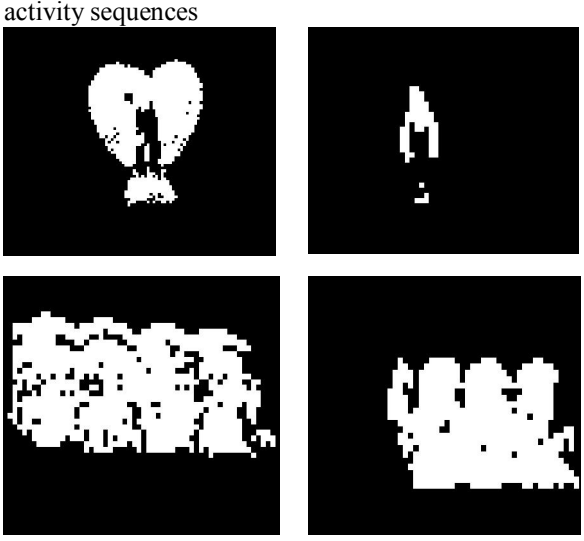

Figure 3. Motion energy images for different activity sequences

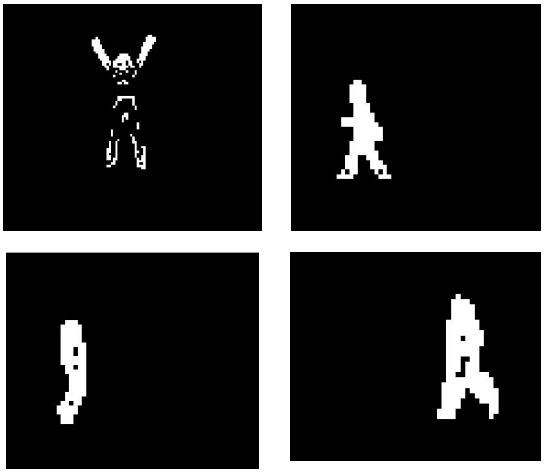

Figure 4. Interest points generated for a single frame of different activity sequences

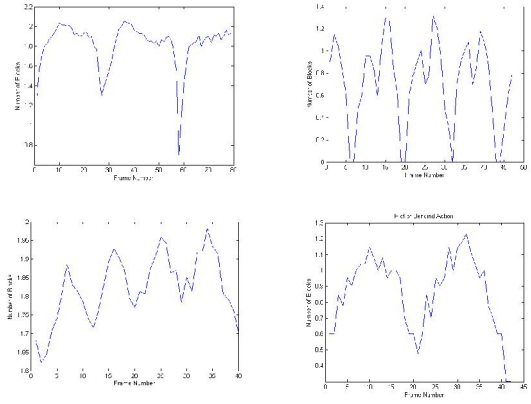

Figure 5. Number of interest points generated for 25 frames for different activity sequences

The figure 4 shows the foreground background subtractions and is achieved through the proposed method from space time interests' points. It also plays a vital role in recognizing different human actions. These images can be also used to form Motion Energy Histograms for extracting further discriminating features as well. The further effects of the number of space time interests points is displayed in figure 5. Our method of space time interest points form images that are very similar to the low resolution images. The main advantage of the proposed algorithm is that it is computationally less expensive. We have implemented the proposed technique in Matlab 7. Firstly, it processes 13 frames per seconds, which indicates that its processing speed is more than state of the art approaches. Secondly, our proposed approach is totally dependent on motion features which are computed from space time interest points generated through the proposed technique. The accuracy of our algorithm is 88.61%, which is quite good on the basis of that much compromise on computational complexity. This accuracy is a little bit good from state of the art approaches of human activity recognition. Its main advantage is the least computational complexity as compared to those methods.

4. Conclusion

Neural network classifier based human activity recognition in videos has been proposed. Providing the ability to see and understand as humans do has fascinated scientists, engineers and even the common man. In this paper we have explored the use of kinematic features, derived from motion information of the interest points [10] for the task of human activity recognition in videos. As motions in human activity videos become more complex the optic flow is more difficult to discern, while our features depends on the interest points instead of optical flow. The numbers of interest points are also playing an important role in the recognition of different human actions and we use appearance based technique. We can find out the continuity and repetition of an action using these interest points and this makes it computationally more feasible. The dominant features are selected by employing PCA on each feature set. The selected features are then used to train and query a neural network, and the recognition rate of the human activities is used as an evaluation measure of the system. In future work, we intend to explore new features sets which may further improve accuracy of human activity recognition system. Our method of interest points generation can be used for background foreground subtraction with some amendments. It has the ability to handle the background noise efficiently.

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Saudi Arabia Global Health Professional Students Tobacco Survey 2010–2011

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Abstract: The Saudi Arabia GHPSS Global Health Professional Students Survey assess the prevalence of cigarette smoking and other tobacco use, as well as it sheds the light on the role of health professional students and their school environment in prohibiting smoking. In addition to information about barriers of smoking cessation in health professional students such as early age of smoking, smoking urge especially within half to one hour of wake up, previous failed trials to tobacco cessation, and if they received help for tobacco cessation or not, insufficient knowledge of health professional students about smoking hazards and their inadequate training on tobacco cessation and it is treatment. It includes also attitude of health professionals towards tobacco cessation and anti –smoking campaign. Comparison also is made between health professional student's smokers and non smokers to Environmental Tobacco Exposures "negative smoking exposures" at home and outside home. The Saudi Arabia GHPSS is a Health Professional Students School based survey conducted for the studying year 2010-2011. A two stage sample design was used to produce representative data for Saudi Arabia. At the first stage a census was done for all health professional schools. At the second stage all health professional students within all schools were surveyed. All health professional students were eligible to participate in the survey. Health professional students were interviewed through using self administered questionnaires containing multiple choice questions. Data entered and analyzed using Epi info software. The response rate for schools was 100%, the response rate of health professional students reached 90.8% as most of them were willing to conduct the survey. The survey concludes that the prevalence of cigarettes and shisha smoking is considerably high in Saudi Arabia's health professionals students especially among dental and pharmacy students and among males compared to females. It also concludes that the National Saudi Tobacco Control Program apparently working effectively, but still program activities needs to be intensified further to reduce smoking in health professional students especially nurses and physicians because of their great role in advising and treatment of smokers patients.

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1. Introduction

Tobacco use a major public health problem causes considerable morbidity and mortality worldwide. Evidently, smoking cause's more than 5 million deaths per year and by the year 2020 will exceed 10 million a year.[7,24,33] Approximately, 100.000 youths use cigarettes and other tobacco products for the first time, and expectedly 80% of them will smoke in the future. Besides high prevalence of smoking and high mortality and morbidity of smoking; there is increase of tobacco marketing and promotional strategies of cigarettes manufacturing companies.[24] Governments' action to establish various tobacco control initiatives can prevent mortality and tobacco related diseases from happening and save a significant number of lives. Tobacco control measures include: Raising tobacco prices by imposing higher excise taxes, advertising and marketing bans and restrictions, and clean indoor air provisions.[23] A 10% increase in tobacco tax could lower tobacco consumption by 8% and save 10 million lives. It was found that this is the most effective tobacco control measure. It is estimated that most of the reduction in the number of deaths (about 90 percent) will occur in low- and middle-income countries.[31].

The fact that the majority of 5.1 million global tobacco deaths occur in the Asia, make that proper surveillance of tobacco related morbidity and mortality is important. Furthermore, since Asia is the one of the largest producer of tobacco and the largest consumer of tobacco and tobacco products, it is imperative that the work of the industry will also be monitored in an attempt to counter the ever growing menace of tobacco. Saudi Arabia is one of the countries in Asia with high tobacco consumption rates.[34]

Apart from the amount of disease, disability, and premature death that it causes, tobacco is unique among the preventable causes of disease because: It is

always dangerous, and it is highly addictive to many consumers, it is actively and energetically promoted; by one of the world's largest and most powerful industries, and its use harms not only those who consume it; but also other people who are exposed to their smoke. These important characteristics make that tobacco use is a particularly difficult public health problem, requiring urgent action from a wide range of sources, including political action.[37]

With the latest adoption of the Framework Convention on Tobacco control by all members of the WHO, there exists both an opportunity as well as a risk for developing countries. The opportunity is to strengthen its anti-smoking policies and collaborate with the international community towards smoking control. The risk is increase tobacco consumption by citizens if countries do not take advantage of the convention and progress aggressively towards protecting their citizens. While the prevalence of tobacco use has declined in some high-income countries, it is increasing in some of developing low and middle-income countries.[17] In Eastern Mediterranean countries the prevalence of tobacco use is impressively rising especially among youths and women attributed to in-effective tobacco control measures, economic boom and globalization, and strong marketing plan of tobacco industry; as tobacco industry focus on developing nations, where the awareness of tobacco dangers is lower and where enforcement of anti-smoking laws are not as strict.[2,3,15,22]

As indicated before, there is a wide acceptance of the need for more tobacco control activities on an international level. Tobacco control requires efficient and systematic surveillance mechanisms to monitor the trend of consumption. With this in mind, the World Health Organization, the Centers for Disease Control and Prevention; in collaboration with other partners, are developing a Global Tobacco Surveillance System (GTSS). The Global Tobacco Surveillance System (GTSS) using standard methodology and design among specific groups: Youths, school personnel and health professional students through three surveys, which is; the Global Youth Tobacco Survey (GYTS), the Global School Personnel Survey (GSPS), and the Global Health Professional Students Survey (GHPSS).[12,13,32] This system will provide a standardized and reliable structure and capacity to track and assess the tobacco situation within and across countries and to disseminate this huge epidemiological information. With this information, national authorities can critically evaluate their own tobacco control situation in light of the experiences of other countries and use "lessons learned" to enhance their own tobacco control efforts.[4,6,12-14,28-30,36]

Several studies in the Gulf Cooperation Council (GCC) countries have confirmed alarming figures of tobacco use among boys, and shisha smoking especially among females.[2,3,15,22] In Bahrain secondary school smoking survey showed that 25% of male students in intermediate grade were current smokers.[3] In Saudi Arabia's many studies have been conducted to estimate tobacco use among different groups within Saudi population. These studies show an alarming expansion of tobacco use as follow:

In 2001:

The first round of GYTS (Global Youth Tobacco Survey) which was conducted in the **Riyadh region** shows that one fifth of male students were current smokers of any tobacco products (20%).[15,16]

In 2006:

The first round of GTSS (Global Tobacco Surveillance System) shows that more than one third of males (35.8%) versus 5.8% of females were current smokers.

In 2007:

The second round of GYTS shows that 19.3% of students were current users of any tobacco products (males 24.2%, females 11.2%).[1]

In 2009:

The third round of GYTS shows that there is decrease in the number of students as current users of any tobacco products to 14.9% (male 21.2%, female 9.1%).[18]

In order to continue monitoring the prevalence of smoking in Saudi young people, prevention and control of tobacco use among them, it is necessary to run new rounds. Two rounds were conducted in 2010-2011. One of them is GHPSS "Global Health Professional Students Survey", and the other is GSPS "Global School Personnel Survey.

Objectives

- To estimate the prevalence of tobacco use (cigarettes and other tobacco use) among health professional students.
- To determine the role of health professional students and school policies on prevention and control of tobacco use.
- To identify barriers and obstacles faced by health professional student's in their tobacco cessation trials.
- To assess the needs for training of health professional students on tobacco cessation and treatment options available.
- To determine the attitude of health professional students towards tobacco cessation and anti-smoking campaigns.
- To measure the exposure of smokers and non smokers' health professional students to environmental tobacco smoke (negative smoking), both at home and outside.

2. Methodology

About Global Health Professional Students Survey (GHPSS)

It is the world's largest public health surveillance system among Health Professional schools. It is school-based Health Professional Students survey designed to enhance the capacity of countries to monitor tobacco use among health professional students, enforce policies and regulation on health professional students and the school environment to prohibit smoking and tobacco use, and guide the implementation and evaluation of tobacco prevention and control programs. The GHPSS uses a standard methodology for constructing the sampling frame, selecting schools and health professional students, preparing questionnaires, following consistent field procedures, and using consistent data management procedures for data processing and analysis. The information generated from the GHPSS can be used to stimulate the development of tobacco control programs and can serve as a means to assess progress in meeting programmed goals. In addition, GHPSS data can be used to monitor Articles in the WHO Framework convention on tobacco control.[8,11,32]

Sample Description of 2010 -2011 study

All Medical schools, Pharmacy, Dental, Medical Sciences, Applied Medical Sciences, and Nursing schools having 3rd year students were included in the sampling frame. A census was taken of all these schools. All students within all schools were approached for the survey (census).

Table 1: Number of Health Professionals Schools and Students Enrolled and Their Response Percentage

Type of School	Schools No.	Students No.	No. Respond	Response %
Medical schools	5	205	188	91.7
Pharmacy	4	143	124	86.7
Dental	5	154	128	83.1
Medical Sciences	4	196	189	96.4
Applied Med.Scien	1	36	34	94.4
Nursing	4	174	162	93.1
Total	23	908	825	90.8

Data collection procedures and analysis

Survey procedures were designed to protect the privacy of the health professional students by allowing for anonymous and voluntary participation. The self-administered questionnaire was administered in venues in the health professional schools. Participants recorded their responses directly on an answer sheet that could be scanned by a computer.

The questionnaires used in the GHPSS consisted of 49 multiple-choice questions. The questions included solicited information on the tobacco use; role of health professional students and school policies in prohibiting smoking; factor related to health professional students smoking behavior (such as age of smoking initiation, smoking urge after waking up, and previous trials for smoking cessation, and help received for smoking cessation); attitude about tobacco cessation and antismoking campaigns; knowledge about harmful effect of smoking; training on tobacco cessation and treatment options; and the exposures of health professional student's to negative smoking (environmental tobacco smoke) for both non-smokers and smokers. All data were analyzed and tabulated by statisticians from the WHO using Epi-Info software. It should be noted that in the case of missing answers for any question, the percentage of calculation is done on the total answered questions.

3. Results and Discussion

The results can be used to make important inferences concerning tobacco use risk behaviors in Saudi Arabia for 3rd year of medical students 22%, pharmacy students 15%, dental students 16%, medical sciences students 23%, applied medical sciences students 4% "the least contributor", and nurse students 20% (Figure 1).

The GHPSS was implemented to provide baseline data on prevalence of tobacco use among health professional students. According to this survey in Saudi the prevalence of cigarettes smoking ranges from 2.9% in applied medical science students to above 25% (25.9%, 27.3%) in dental and pharmacy students respectively. The prevalence of shisha smoking ranges from 2.9% in applied medical science students to 34.3% in dental and 28.6% pharmacy students (Table2, Figure 2). While the findings results in Yemen GHPSS 2009 show lesser smoking prevalence. Among Yemen nursing students 16.4% currently smoke cigarettes, in dental students 14.4%, 14.3% for pharmacy, and 10.9% for medical students. For shisha, among Yemen dental students 13.5 % smoke shisha, 12.9% among nursing students, 11.5% for medical and 9.1% for pharmacy students.[11] In Bahrain GHPSS 2009 show lesser smoking percentage than Saudi and Yemen as the prevalence of current smoking in medical students 10.9% and 9.4% in nursing students, also the prevalence of other tobacco use than cigarettes were in medical students 16.3% and in nursing students 10.8%.[8] This higher prevalence percentage of smoking among some groups of the health professionals is alarming and negatively influence the future health professions workforce to deliver anti-tobacco counseling as they act as role models for their patients and the public in general, in addition it endangers their health as health professional smokers are liable of tobacco related diseases and premature deaths as general smokers.[20] The overall currents tobacco users in this survey

range from 5.9% in applied medical science students to 39.5% in dentist students. The percentage of ever smokers (who tried smoking even for one or two puffs) ranges from 17.6 % to 53.3%, and highlight the importance of never starting smoking (Table 2). There are 2 to 6.4 times more male current cigarette smokers than females. For shisha smoking this is 1.2 to 5.7 (Figure 3). In Yemen GHPSS 2009; the prevalence of ever and current tobacco use among males is also significantly higher than females students as Saudi GHPSS.[11] The reasons of females smoking in Saudi and Middle East countries in general should be investigated 'as female smoking considered as strange and new habits in these countries', and health education messages should be directed towards these findings.

Health professions students have been found to play important role in cessation and prevention of tobacco use among their patients as counseling by health professions students has shown an increase in smoking cessation.[5,25] In the survey health professional students stated that their roles in tobacco cessation programs is mainly to advise patients that smoke to quit smoking and tobacco use (ranged 90.2% - 96.3%), followed by inform smokers about tobacco cessation and how to stop (84.9% -94.4%), and lastly to be a role model for their patients (83.3% -85.3%). Note that these percentages not include applied medical students as most of them are not smokers, hence it is not surprising that they are more agreement with the stated roles: 97.1% to advice patients that smoke to quit and inform them how to stop and 94.1% act as a role model (Table3, Figure 4). The Yemen GHPSS data 2009 shows that 91.8% of nursing students and only 88.5% of medical students recognize that they are role model in their society; besides over 93.9% of the dental students, 95.4% of the medical students, 93.7% of the nursing students, and 95.3% of the pharmacy students thought health professionals have a role in giving advice about smoking cessation to patients.[11] The Bahrain GHPSS data 2009 show near result of Saudi as over 80% (82.4%- 86.3%) of both medical and nursing students think that health professions serves as a role model for their patients and public, and over 91.5% of the medical students and 93.5% of the nursing students thought health professionals have a role in giving advice about smoking cessation to patients.[8] In Tunisia medical students 2010 show lesser percentage in their both thoughts about health professionals serves as a role models for their patients (73.8%) and thoughts that health professionals have a role in advising patient on smoking cessation (86.4%) than previous stated surveys.[10]

In Saudi GHPSS, the percentage of cigarettes smoking or other tobacco use at schools of health professionals is ranging from 3.7% (medical) to 15.8% (pharmacy) for cigarettes smoking and the percentage is ranging from 7.1% (medical) to 13% (dental) for other tobacco use (Table5). The presence of policies in clinics and schools of health professionals is ranging from 44.1% (applied medical science) to 68.2% (dental). This rather low when comparing the results with the enforced school policies, as that ranges from 85% (medical) to 98.7% in medical science students (Table4, Figure 5) . It found in Yemen GHPSS that enforcement of school policies banning of smoking is low as it ranges from 14.8% in nursing students to 60.6% in dental students.[11] In Bahrain more than 40% of the students reported their schools have an official policy banning smoking in their buildings and clinics; which considered less than moderate, while enforcement of school policies reached to 88% in medical and nursing Bahrain schools. So from Saudi, Yemen, and Bahrain GHPSS we found that the actual presence of school policies is not enough to ensure that they are really applied. Educational institutions of health professions students should be encouraged to provide smoke free work and study areas by banning smoking in their buildings and clinics. A smoke free work environment has shown to improve air quality, reduce health problems associated with exposure to tobacco smoke, support and encourage cessation attempts among smokers trying to quit.[26,27] Furthermore, the creation of smoke free areas by health education institutes send a clear message to educators, students, patients, and clinicians about the impact and hazards of tobacco. [26,27]

Of those that are smoking, up to 33.3% pharmacy students smoke a cigarette within 10 minutes of waking up, and this is 100% within 30 minutes (Figure 6). Early initiation of smoking at age 10 from no one smoked in applied medical science students to 22.6% pharmacy students already smoked. This is 16.7% in applied medical science to 48.7% in medical students smoked at age 16 (Figure 7). This means that a considerable number of Saudi people start smoking at a young age. The highest prevalence of early age of initiation of smoking was in China, Poland, and Zimbabwe, and 33% of ever smoker students started tobacco use before age of 10. Initiation of smoking before age of 10 was lowest in Venezuela (12.1%) and Costa Rica (10.9%).[30] A large number of students 33.3% for the medical science students up to 89.5% for pharmacy students tried cessation before (and failed). The number that received help to stop with tobacco use ranges from zero in (medical students) to 81.5% in medical science students (Figure 8). Notably, a considerable number of smokers desired to quit but were experiencing difficulties in quitting, though nearly

80% of those smokers receive help to quit smoking (Figure 8). This attributed to ineffective or inadequate tobacco cessation services, and persistent nicotine addiction.[29] All the above factors highlight obstacles in tobacco cessation among health professional students that use tobacco.

67.7% (applied medical science) to 86.3% (medical) of students agree that patient tobacco cessation depends on the health professional advice to quit smoking and tobacco use. However, 74.8% (dental) to 90.5% (pharmacy) of students agree with the statement that health professionals that use tobacco themselves will less often (or even not) advise patients to quit tobacco use (Table6). Attitudes of health professional students towards antismoking campaigns is strongly to enforce the banning of smoking in enclosed restaurants (84.5% - 97.1%) and public areas (87.1% - 100%), enforce the banning of smoking advertisements (82.6% - 94.1%), to prohibit purchasing cigarettes and tobacco by adolescence (81.1% - 94.1%), and they also recommend to a lesser extent banning of smoking in cafes: 54.9% - 80.6% (Table7, Figure 9). In Bahrain 2009 more than 90% of both medical and nursing students thought that smoking should be banned in all enclosed public places and more than 85% of all students (medical & nursing) thought that sales to adolescents should be banned.[8] In Tunisia 2010 93.5% of medical students thought smoking should be banned in all enclosed public places; 83.3% thought tobacco sales to adolescents should be banned and 86.2% thought there should be a complete ban on advertising tobacco products.[10] In Egypt 2005 91.4% of medical students thought smoking should be banned in all enclosed public places; 80.7% thought tobacco sales to adolescents should be banned and 83.9% thought there should be a complete ban on advertising tobacco products.[9] So it's clear from above that health professions students either in Gulf or other Arabic countries strongly agree with banning smoking in enclosed areas, banning of smoking advertisement and prohibit selling of tobacco to adolescents.

Regarding knowledge of health professional students; the study highlight low knowledge of tobacco hazards and recording patient history specific in nursing students: 65%, 40.5% respectively (Table8, Figure 10) and defective training in nursing school on tobacco treatment (24% - 26.4%) except for nicotine therapy (68%). For training on anti-depressants therapy these figures are low in nursing as well (25.4%) to 44.6% in pharmacy students (Table9, Figure 11). In another study in U.S. about a qualitative assessment of perceptions and practices of nurse practitioners in the delivery of tobacco-use interventions and tobacco cessation services; little is known about the tobacco intervention strategies of nurse practitioners, as well as knowledge deficit related to national guideline. These findings may be reflective of a gap in the tobacco-related curricular content of nursing programs.[21] Low knowledge of tobacco hazards, treatment and cessation services among nursing students is contrary to the important role of nursing in tobacco control. Nurses actively involved in directing resources and talents toward public awareness and tobacco prevention programs, formulating public policy initiatives to control youth access to tobacco, and initiating cessation programs for youth and adults. Efforts can be targeted at the schools, community, and health care system, as well as the public policy arena and media campaigns. [19] In Bahrain 2009 and Tunisia 2010 surveys nearly, the same low percentage of medical students reported that they had ever received of formal training in their college on cessation approaches to use with their patients (37.4 %, 37.9% respectively).[8,10] Also, in this survey the percentage that learned the importance to provide educational materials to support patients who want to quit smoking was low ranged from 20.2% in dental students to 41.3% in medical science students, although these are a bit higher for nicotine replacement therapy: 61.4% for pharmacy to 81.8% for dental students (Table9, Figure 11). The training on tobacco cessation and provision of educational materials for smokers to help them to quit smoking and treatment therapy in tobacco cessation in general need more enforcement and should be included in the school curriculum as basic courses.

Environmental tobacco smoke "second-hand smoke or negative smoking exposure" poses a danger for non smokers and as well as for smokers, with more additive hazard for smokers. The adverse effects of second-hand smoke are both immediate and long-term and are felt by both children and adults. Globally, WHO estimates that nearly 700 million, or almost half the world's children, breathe air polluted by tobacco smoke! In adults, second-hand smoke increases the risk of lung cancer by 20% to 30% and the risk of coronary heart disease by 25% to 30%. In children, exposure to second-hand smoke increases the risk of lower respiratory tract illnesses, asthma, middle-ear infection and sudden infant death syndrome.[35] The results show that exposure of non-smokers at home and outside home are lower than exposure of smokers (which can be expected as smokers will probably have other family members that smoke or people within their social network that smoke, and/or visit places where smoking is allowed). The exposure to tobacco smoke by non smokers at home is between 10.6% in dental students to 24.4% in nursing students. For outside home these figures are higher ranging from 20.4% in medical students to

42.5% in pharmacy students. Exposure of smokers to environmental tobacco smoke at home ranges from 59.9% in medical students to 91.5% among medical science students. For outside home these figures are 55.1% among nursing students and 94.5% among pharmacy students (Table10, Figure 12). In Bahrain 2009 and Tunisia 2010 the results of exposure to second hand smoke was quit similar. In Bahrain among medical students 27.9% to 33.9% of nursing students reported that they had been exposed to second hand smoke in their home in the past 7 days. Among Medical students 50.4% to 46.6% of nursing students reported that they had been exposed to second hand smoke in public places in the past 7 days.[8] In Tunisia; 32.7% of medical students were exposed to second-hand smoke at home, during the past week and 51.7% of them were exposed to second-hand smoke in public places during the past week. [10] The results of exposure to second hand smoke in Yemen 2009 and Egypt 2005 was quit similar and higher than Bahrain and Tunisia results. In Yemen, 43.3% of medical students and 58.6% among nursing students reported that they had been exposed to second hand smoke in their home in the past 7 days. Among 70.1% pharmacy students, 74% of medical students, and 77.5% among nursing reported second hand smoke exposure in public places.[11] In Egypt; 45.6% of medical students were exposed to second-hand smoke at home, during the past week and 78.4% of them were exposed to second-hand smoke in public places during the past week. [9]

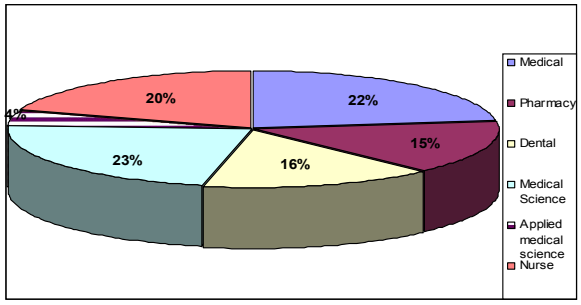

Figure 1: % of Participating Students by Health Profession

Table 2: Prevalence of Cigarettes and Shisha Smoking & Users of any Tobacco Products among Health Professional Students

	Ever Smoker	Current Smoker	Ever Shisha Smoker	Current Shisha Smoker	Ever any Tobacco user	Current any Tobacco user
Medical	40.6	8.2	26.3	6.9	55.8	17.1
Pharmacy	48.8	27.3	46.4	28.6	57.1	31.8
Dental	47.4	25.9	51.3	34.3	58.5	39.5
Medical Science	38.6	14.7	23.2	5.5	50.5	20.5
Applied medical science	17.6	2.9	15.2	2.9	29.4	5.9
Nursing	53.3	19.9	31.0	20.4	58.0	28.2

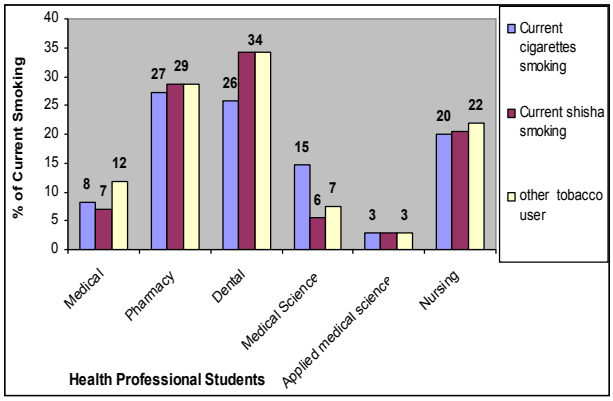

Figure 2: Health Professional Students Current Smoking

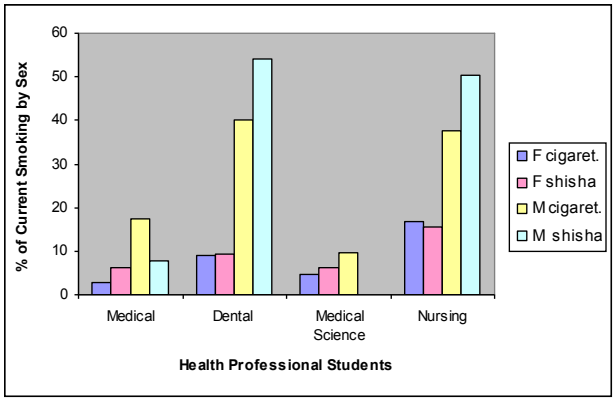

Figure 3: Health Professional Students Current Smoking by Sex

Table 3: Role of Health Professional Students(% that Agree with the statement)

	Medical	Pharmacy	Dental	Medical Science	Applied medical Science	Nursing
Role Model	85.3	84.2	83.3	83.9	94.1	83.9
Advise smokers to quit	93.3	90.2	94.5	96.3	97.1	93.7
Inform smokers about tobacco cessation	94.4	84.9	89.6	88.9	97.1	88.9

Table 4: Role of Health Professionals Schools

	Medical	Pharmacy	Dental	Medical Science	Applied medical Science	Nursing
Policies in school &clinics	63.1	55.7	68.2	53.6	44.1	64.3
Enforced school policies	85.0	95.2	86.5	98.7	92.9	98.5

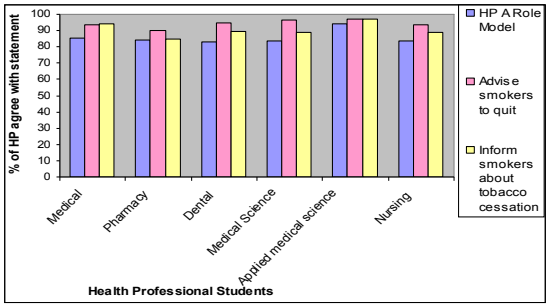

Figure 4: Role of Health Professional Students (% Agree with the Statement)

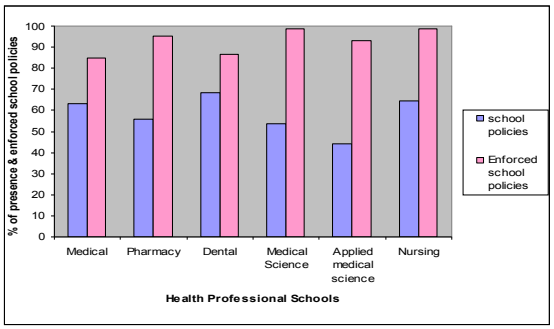

Figure 5: Role of Health Professionals Schools

Table 5: Percentage of Health Professional Students Smoking in School Building

	Medical	Pharmacy	Dental	Medical Science	Nursing
% smoke in school building	3.7	15.8	10.9	8.5	9.7
% use other tobacco in school building	7.1	-	13.0	7.6	8.7

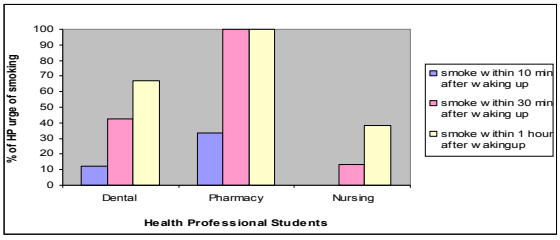

Figure 6: Health Professional Students Urge of Smoking

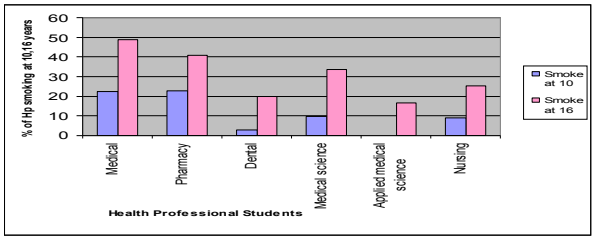

Figure 7: Health Professional Students (% Smoking at 10, 16 years)

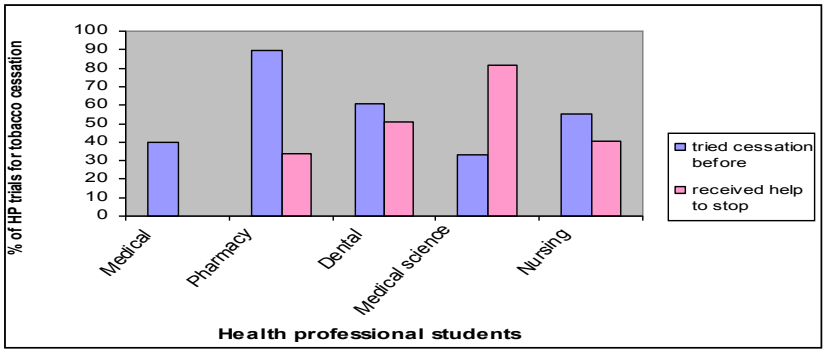

Figure 8: Health Professional Students Trials for Tobacco Cessation

Table 6: Health Professional (HP) Students Attitude about Tobacco Cessation

	Medical	Pharmacy	Dental	Medical science	Applied medical science	Nursing
Patient tobacco cessation depends on HP advise	86.3	81.0	84.0	75.0	67.6	76.4
A smoker HP less or not advise patient to quit smoking	80.5	90.5	74.8	88.2	79.4	81.2

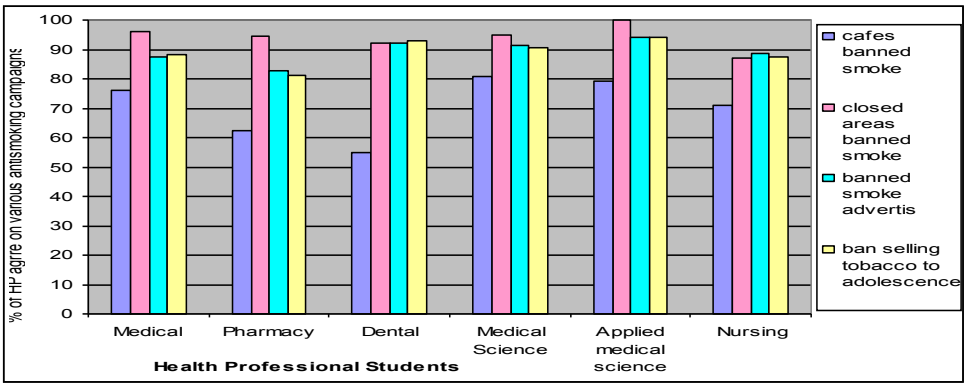

Figure 9: Health Professional Students Attitude towards Antismoking Campaigns (% Agreeing)

Table 7: Health Professional Students Attitude towards Antismoking Campaigns (% Agreeing)

	Medical	Pharmacy	Dental	Medical Science	Applied medical Science	Nursing
Banned smoking in Restaurants	93.4	97.0	86.7	95.6	97.1	84.5
Banned smoking in enclosed public places	96.2	94.7	92.1	95.0	100	87.1
Banned smoking in cafes	76.2	62.5	54.9	80.6	79.4	71.1
Banned smoking advertisements	87.6	82.6	92.0	91.4	94.1	88.7
Prohibit selling tobacco to adolescents	88.4	81.1	92.9	90.6	94.1	87.3

Table 8: Health Professional Students Knowledge of Smoking Hazards

	Medical	Pharmacy	Dental	Medical Science	Applied medical Science	Nursing
Hazards of Tobacco	84.6	81.1	85.8	74.1	79.4	65.0
Tobacco related diseases (record patient tobacco history)	46.2	47.9	72.6	52.7	35.3	40.5

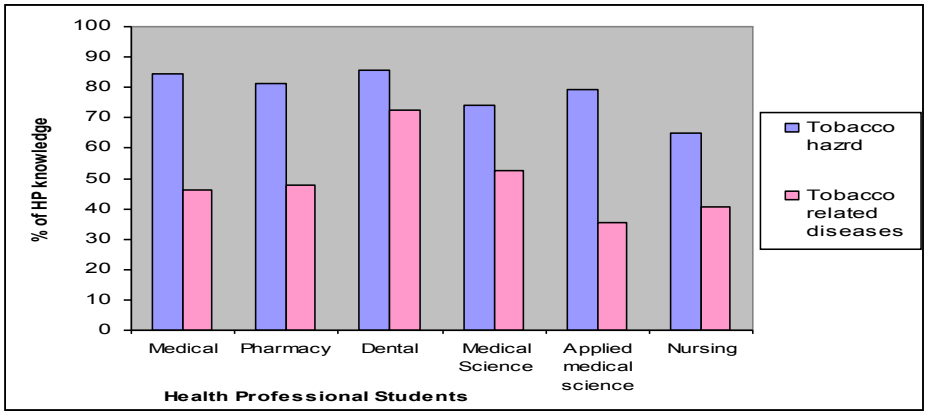

Figure 10: Health Professional Students Knowledgeof Smoking Hazards

Table 9: Health Professional Students Training on Treatment of Tobacco Users

	Medical	Pharmacy	Dental	Medical Science	Applied medical Science	Nursing
Tobacco Cessation	21.8	30.5	19.3	35.2	29.4	24.0
Educational materials	24.8	37.9	20.2	41.3	35.3	26.8
Nicotine replacement therapy	69.3	61.4	81.8	66.1	79.4	68.1
Anti-depressant therapy	31.6	44.6	32.2	37.0	38.2	25.4

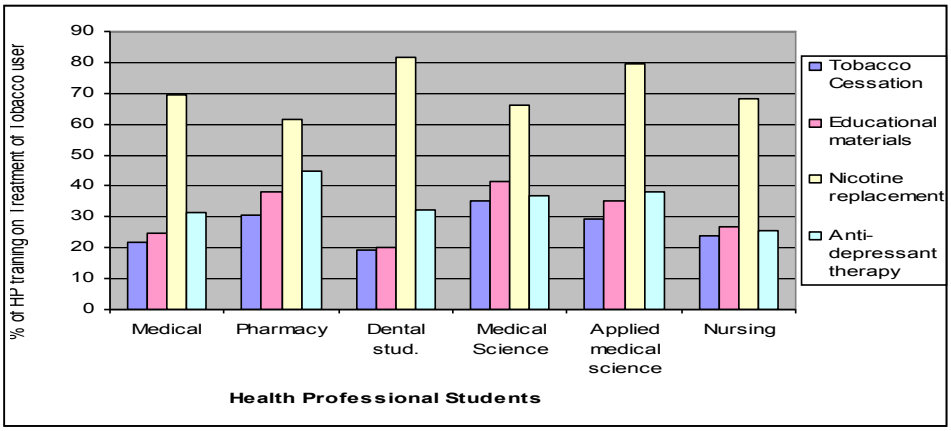

Figure 11: Health Professional Students Training on Treatment of Tobacco User

Table 10: Health Professional Students Negative Smoking Exposure (Exposed to someone smoking in their presence at home and outside their home)

A. Exposed to smoking at home	Medical	Pharmacy	Dental	Medical Science	Nursing
% of exposed (non - smokers)	16.3	21.5	10.6	21.5	24.4
% of exposed who smoke cigarettes	56.9	82.9	69.2	91.5	63.1
% of exposed	29.0	33.6	37.2	40.6	39.8
B. Exposed to smoking out-home	Medical	Pharmacy	Dental	Medical Science	Nursing
% of exposed (non - smokers)	20.4	42.5	34.0	34.0	27.8
% of exposed who smoke cigarettes	61.7	94.5	94.3	83.1	55.1
% of exposed	31.5	58.0	61.4	46.3	39.9

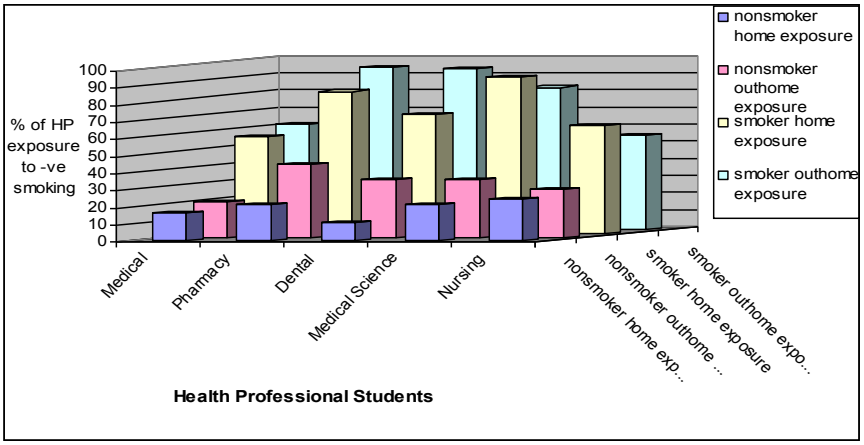

Figure 12: Health Professional Students Negative Smoking Exposure (Exposed to someone smoking in their presence at home and outside home)

Conclusion

The survey concluded that tobacco prevalence, especially cigarettes and shisha, is higher among dental and pharmacy students, followed by nursing, medical science and medical students, and the lowest prevalence is found in applied medical students. The prevalence of cigarettes and shisha smoking is considerably higher among males compared to females.

Health professional students roles are considered to advise the patients that smoke to stop smoking, followed by informing smokers about tobacco cessation and lastly to be a role model for their patients. The role of schools of health professionals is to encourage tobacco cessation through setting rules and policies prohibiting smoking in schools. However, the percentage of enforcing schools policies was high, but the actual presence of these policies was much lower. Efforts should be undertaken to implement the policies that are present.

Barriers for health professional student's with respect to tobacco cessation are the considerable numbers of students that already smoke at a young age, the high number of smokers that start smoking soon after waking up, the large number that tried cessation before (and failed) and the fact that quite number of students did not receive help to stop tobacco use. Adding that Health professional knowledge of tobacco hazards was not as high as expected, and it was lower in nursing students. Also training of health professional students was lower than expected – all below 50% except for nicotine replacement therapy, which may explain why their knowledge was lower. It can therefore be concluded that health professional students need to learn and know more about smoking hazards and tobacco cessation and how to quit smoking through more training sessions specifically for nursing and medical students because of their important role in tobacco cessation, which should be incorporated in school curriculum.

Regarding the attitude of health professional students towards tobacco cessation, more than two thirds agreed that patient tobacco cessation depends on the advice of the physician, and more than three quarters regard the smoking behavior of health professionals themselves a barrier for advising and informing their patients about tobacco cessation. Health professional students also agreed on antismoking campaign through banning smoking in restaurants and enclosed public areas, as well as banning smoking advertisements and prohibition of purchasing tobacco to adolescence (all more than 81%) and to a lesser extent banning of smoking in cafes.

The exposure of health professional students that don't smoke to environmental tobacco smoke, i.e. "negative smoking exposure" at home and outside home is lower than that of health professional students that smoke. For the non-smokers, exposure at home is generally lower compared to outside home, while for the smokers this more similar.

Recommendations

All countries in the Gulf Region must adopt strategies to avoid the increase of tobacco consumption among the public, especially among health professional students and to facilitate decreasing in tobacco use. This should be directed to Saudi Arabia as one country in the Gulf region with high tobacco prevalence and consumption. The following recommendations found useful within the context:

1. Increase information and knowledge about the hazards of smoking and all forms of tobacco use among the public and health professional students to build a negative attitude towards smoking and tobacco use.
2. Direct health education messages and awareness to prohibit and prevent all forms of tobacco use for health professionals (and the public) as health professionals use both cigarettes and other forms of tobacco on an equal basis.
3. Stigmatize female use of tobacco to prevent and prohibit female use of all forms of tobacco as female smoking nearly one third from male smoking.
4. Prevent tobacco use and smoking of health professionals because they act as a role model and have great influence on their patients, especially the young patients.
5. Enhance the role of physicians, nurses and health professionals in tobacco cessation programs as they act as a role model, they advise patients that smoke to quit and inform them how to stop tobacco use.
6. Prevent tobacco use especially in the health professional's school environment through the following: Enact and set legislations, policies and rules prohibiting tobacco use among schools of health professionals, and enforce use of these rules, add health education, teaching, learning materials and schools awareness campaigns that directed on the harmful effects of smoking, conduct training sessions, activities and programs to health professionals for prevention of smoking initiation and tobacco use, design and implement tobacco cessation programs in health professional's schools; which must be integrated in the school curriculum and should not be done on an ad hoc basis and include nicotine replacement therapy and anti-depressants therapy and new drugs help in

tobacco cessation programs for health professionals.
7. Formulate policies and strategies to restrict or ban smoking and tobacco use through banning of tobacco advertisements, banning purchasing tobacco to adolescents, prohibition of smoking in schools; governments buildings and public areas, such as; restaurants and cafes.
8. Cut off the urge of smoking and desperate desire of smoking in smokers through delaying age of smoking initiation.
9. Decrease when possible the harmful effect of negative smoking exposure indoors and outdoors for smokers and non smokers.

Acknowledgements

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Study of Leucocyte Esterase Reagent Strips as a screening test for Spontaneous Bacterial Peritonitis

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Abstract: Background: Spontaneous bacterial peritonitis (S B P) is a severe and frequent complication of cirrhosis with a high mortality rate. A bacteriological laboratory is not always available for all departments admitting cirrhotic patients with ascitis. It follows that alternative methods for rapid diagnosis of SBP are an urgent requirement. **Objective**: This study was planned to compare between leucocyte esterase reagent strips as a bed side test and standard manual polymorphnuclear leucocytes (PMN) counting in the ascitic fluid as regards efficacy and rapidity in diagnosis of SBP. **Subjects & methods**: The study included 100 patients with liver cirrhosis resulting from chronic hepatitis C infection complicated by ascites and SBP. All patients were subjected to complete clinical examination, laboratory investigation that include CBC, PT, INR, LFT, s., creatinine and blood urea, pancreatic enzymes (amylase & lipase), ESR, tuberculin test, radiological investigations including CXR, pelvi-abdominal US and CT, in addition to specific investigations that include cytological, bacteriological and biochemical examination of ascitic fluid as well as examination of ascitic fluid by leucocyte esteras strips. **Results**: There was a highly statistically significance of leucocyte esterase reagent strips in diagnosis of SBP with high specificity 91.5%, sensitivity 83% and accuracy 88%. There was positive correlation (r = + 0.56) between ascitic fluid PMN counts and the corresponding result of leucocyte esterase reagent strip test which was statistically of high significance (*P* value= 0.000). **Conclusion**: It's concluded that the reagent strip testing of ascitic fluid is a very sensitive and specific method for diagnosis of SBP in cirrhotic patients with ascites. It can be used at the patient's bedside and is rapid, easy, inexpensive and results are available within a maximum of 120 seconds.
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Keywords: Spontaneous bacterial peritonitis (SBP), leucocyte esterase reagent strips.

1. Introduction

Cirrhosis is an irreversible alteration of the liver architecture. It consists of diffuse fibrosis of the hepatic parenchyma resulting in nodule formation[(1)].

The World Health Organization defined cirrhosis as a diffuse process characterized by fibrosis and the conversion of normal liver architecture into structurally abnormal nodules which lack normal lobular organization[(2)].·

Liver cirrhosis is a disease found all over the world, affecting all races, ages, sexes. However, there are geographic differences regarding the most important factors as rate of alcohol consumption and frequency of viral? hepatitis [(3)].

Cirrhosis is often symptoms-free, autopsy examination suggest a prevalence of 4-10%. The incidence is about 240 per million inhabitants per year with high incidence of morbidity and mortality. Liver cirrhosis is the final stage of a hepatic disease which has generally run a chronic course for several years[(4)].

The mechanisms able to elicit and sustain liver fibrogenesis may be classified in three main groups: chronic activation of the wound healing reaction, oxidative stress, derangement of epithelial-mesenchymal interactions and epithelial-mesenchymal transition in cholangiopathies[(5)].

Ascites is the most common complication in decompensated cirrhotic patients. Approximately 50% of patients with compensated cirrhosis will develop ascites over a 10-years period[(6)].

Spontaneous Bacterial Peritonitis (SBP) is bacterial infection of the ascitic fluid without any intraabdominal source of infection[(7)].

Patients with cirrhosis and ascites show a higher susceptibility to bacterial infections mainly because of the inadequate defence mechanisms. The most frequent and the most severe one being SBP[(8)].

SBP occurs in 8-30% of hospitalized patients with ascites[(9)].

SBP pathogenesis in patients with cirrhosis is considered to be the main consequence of bacterial translocation (BT). The bacterial translocation is the process through which viable or non-viable bacteria and bacterial products (bacterial deoxyribonucleic acid (DNA) or endotoxins) cross the intestinal lumen and come into the mesenteric lymph nodes or extraintestinal. The BT is a disturbance of the equilibrium between the normal intestinal flora and the

organism, leading to an inflammatory reaction and finally producing infection. Bacterial translocation also is involved in increasing the hyperdynamic state of cirrhosis and in aggravation of haemostasis disorders[8].

There are some mechanisms that are being proposed to explain BT in cirrhosis: the intestinal bacterial overgrowth, the structural and functional alterations of the intestinal mucosal barrier and the deficiencies of the local immune response[10].

The intestinal bacterial overgrowth plays a key role in BT in cirrhosis and is the result of the delayed intestinal transit existing in these patients. It seems that the sympathoadrenal stimulation, increased nitric oxide (NO) synthesis and the oxidative stress of the mucosa are the main causes for decreased intestinal motility[10].

Besides, although normally in the small intestine there is a more reduced microbial density compared to that of the colon, in cirrhotic patients an increase of the colonization process of the small intestine with bacteria from the colon (approx.30-50%) is recorded [11].

In hepatic cirrhosis two processes that alter the intestinal mucosa barrier occur: increased mucosal permeability (especially in patients with sepsis) because of the mucosa oxidative stress, enterocyte mitochondria malfunctioning, endotoxaemia, increased nitric oxide (NO) and proinflammatory cytokine level and the mucosal structural changes. The latter include the intercellular spaces enlargement, vasodilatation, oedema, firbromuscular proliferation, decreased villi/crypts ratio, thickened muscularis mucosa and inflammation[12].

The structures that form the gut-associated lymphoid tissue (GALT) react to the presence of germs from the intestinal lumen by intraepithelial lymphocyte proliferation, germinative center appearance in the lymphoid follicles and in the lamina propria and an increase of the secreted immunoglobulin (Ig) level. In return, bacteria that form the commensal intestinal flora interact with the intestinal epithelium and can start up the primary immune response as well as the adaptative one[13].

In cirrhosis, because of the local and systemic immune deficiencies, the BT process is followed by bacteremia and ascitic fluid inoculation. If the ascitic fluid complement level is low, this will determine a low bactericidal activity and thus a higher risk of ***SBP*** [14].

For SBP diagnosis, the number of polymorpho-nuclear leucocytes (PMN) from the ascitic fluid obtained by paracentesis must exceed 250 cells/mm^3 and from bacteriological cultures only one germ must be isolated [15].

Culture-negative neutrocytic ascites (probable SBP) exists when the ascitic fluid culture are negative yet the neutrophil (PMN) count is >250 cells/mm^3. This may happen in as many as 50% of patients with SBP and may not actually represent a distinctly different disease entity. It may be the result of poor culturing techniques or late-stage resolving infection[16].

Bacterascites exists when a positive culture coexists with a non elevated ascites PMN count. Although this often may be the result of contamination of bacterial cultures, this may represent an early form of the disease. All patients described that eventually developed SBP were symptomatic, making this a valuable aid in establishing a diagnosis [17].

However, because of the organization of facilities in many hospitals, a bacteriological laboratory is not always available for all departments admitting cirrhotic patients with ascites. It follows that alternative methods for rapid diagnosis of SBP are an urgent requirement[18] .

Use of reagent strip testing for leucocyte esterase has been proposed to reduce the time from paracentesis to a presumptive diagnosis of SBP from a few hours to a few seconds[18].

The aim of this study is to compare between leucocyte esterase reagent strips as a bed side test and standared manual PMN counting in the ascitic fluid as regards efficacy and rapidity in diagnosis of SBP.

2.SUBJECTS AND METHODS

This study was carried out in intensive care unit of Internal Medicine Department, Zagazig University Hospital.

The study was conducted on 100 patients with liver cirrhosis resulting from chronic hepatitis C infection complicated by ascites and spontaneous bacterial peritonitis (SBP) which diagnosed by:

*__Clinical presentation:__ Fever, generalized abdominal pain and tenderness.

*__Laboratory investigation:__ (PMN in ascitic fluid > 250/mL).

Exclusion Criteria:

I. Ascites due to renal, cardiac, tuberculous, malignant pathology and pancreatic ascites.

II. Secondary peritonitis.

III. Patients with focal lesion or hemoperitoneum complicating hepatocellular carcinoma were also excluded.

All patients were subjected to the following:

I- Full medical history and physical examination:

II- Laboratory investigations:

1. Complete blood count (CBC).
2. Prothrombin time (PT). Normal PT was regarded as 12 seconds and International Normalized Ratio (INR).
3. Liver functions tests (total plasma proteins, serum albumin, alanine amino transaminase (ALT),

aspartate aminotransferase (AST), total and direct serum bilirubin, and alkaline phosphatase.
4. Kidney function test [serum creatinine, blood urea nitrogen (BUN)].
5. Pancreatic enzymes (amylase &lipase).
6. Erythrocyte Sedimentation Rate (ESR).
7. Tuberculin test.

III- Radiological investigations:

1 - Chest x-ray
2- Abdominal & Pelvic ultrasonography
3- Computed tomography Scanning of the abdomen and pelvis: Abdominal ultrasonographic examination and Computed Tomography scanning were done in fasting state evaluating:
*The liver for size, echo pattern and presence or absence of focal lesions.
*Presence or absence of hepatic periportal fibrosis.
*The spleen for size, hilar varices and dilatation of the splenic vien.
*Presence of ascites.
*Portal vein diameter (normally regarded as 9-12 mm) and presence or absence of thrombosis.
*Abdominal lymph node enlargement (para aortic & porta hepatis).
*The ovary (masses were excluded from the study)

IV-Cytological, Bacteriological, and biochemical examination of ascitic fluid.

V-Examination of ascitic fluid by leukocyte esterase strips.

Specimen collection:

The site of an ascitic tap is away from the midline, at the point of maximal dullness, and ideally in the left iliac fossa, two fingerbreadths medial and two ventral to the anterior superior iliac spine ("Runyon's spot")[19]. Equipment required for the tap comprises: 10-mL syringe; 1.5-inch, 22-gauge metal (or 18-gauge) needle; pack of sterile gloves and a galipot with skin disinfectant[20].

Data processing and analysis:

*The collected data were reviewed and coded.
*SPSS statistical software version 10.0 (SPSS Inc., Chicago, IL, USA) was used for data analysis.
*Quantitative data were represented as arithmetic means and standard deviations (means ± SD); the "t test" was used for comparison between two groups and Mann-Whitney U test (for not normally distributed data)
*Qualitative data were represented as frequencies and percents, Chi square test (x^2) was carried out for calculating significant relations between the groups.
*The results were considered statistically significant when the significant probability was less than 5 % ($p < 0.05$).
*Exact 95% confidence interval (C.I) for each statistic was calculated from the binominal distribution.

***Sensitivity:** was defined as the proportion of patients with a positive reagent strip divided by the number of those with SBP diagnosed by criteria previously defined.
***Specificity:** was defined as the proportion of patients with a negative reagent strip divided by the total number of patients without SBP.
***Positive predictive value (PPV):** was defined as the proportion of patients with a true-positive reagent strip divided by the total number of patients with a positive reagent strip.
***Negative predictive value (NPV):** was defined as the proportion of true-negative reagent strips divided by the total number of patients with a negative reagent strip.
***Accuracy** was defined by dividing the sum of the true positives and true negatives by the total number of samples evaluated.

Ethical consideration:

The consent of patients who are studying them was taken and the confidentiality of consumers' data were kept and used only for this study.

3. Rusults

Table (1): Comparison between patients with SBP and those without SBP as regard to age, sex and Child- Paugh classification: There is no statistical significant difference between both groups as regard age, sex and Child-Paugh classification.

Table (2): Distribution of the study population according to leukocyte estrase reagent strips result (n =100): This table shows results of leukocyte esterase reagent strips in which 56% test negative and 25% test +3.

Table (3): Distribution of the study population according to SBP which diagnosed laboratory by (PMN ≥250/ml) (n. = 100): This table shows results of S.B.P diagnosed laboratory by (PMN>250/mL) in which 41% positive (+ ve) and 59% negative (- ve).

Table (4): Validity scores of leukocyte estrase reagent strips in diagnosis of SBP (PMN ≥ 250/ ml) using cut off value ≥ +2 of the strips: It shows that:
True positive cases = 34
False positive cases = 5
True negative cases = 54
False negative cases = 7
Total cases =100
So the validity of the test is:
Sensitivity = 34/41* 100- 83%.
Specificity = 54/59* 100= 91.5%.
Positive predictive value (PPV) = 34/39* 100=87.2%.
Negative predictive value (NPV) = 54/61* 100- 88.5%.
Accuracy = 34 + 54/100* 100 = 88%.

Table (5): Sensitivity, Specificity, Positive predictive value and Accuracy for the reagent strips to

diagnose correctly SBP: This table shown validity and accuracy of the reagent strips to diagnose correctly SPB.

Table (6) and Fig (1): Correlation between ascitic fluid PMN counts and the corresponding result of leucocyte esterase reagent strip test: There is positive correlation (r = +0.56) between ascitic fluid polymorphonuclear (PMN) counts and the corresponding result of leukocyte esterase reagent strip test which is statistically high significant (*p* value = 0.000).

Table (7): Culture of SBP positive cases: Shows that the common organism of SBP was *E. coli* (34.14%) and Klebsiella (21.95%).

Table (1): Comparison between patients with SBP and those without SBP as regard to age, sex and Child-Paugh classification

Variable	SBP(n.=41)	Non-SBP (n.= 59)	Significant test	*P* value
1- Age (years) - mean ± SD	54.61±8.24	54.85±9.7	0.128*	0.89
2-Sex				
- male	29	44	0.18**	0.67
- female	12	15		
3-Child-Paugh Classification				
- A	1	7		
- B	18	30	4.403**	0.11
- C	22	22		

*** T test. ** X^2 (Chi square test).**

Table (2): Distribution of the study population according to leukocyte esterase reagent strips results (n. =100).

Strip result	N.	%
0 (negative)	56	56%
+ 1	5	5%
+ 2	14	14%
+ 3	25	25%

Table (3): Distribution of the study population according to S.B.P which diagnosed laboratory by (PMN ≥ 250/mL) (n. =100)

S.B.P diagnosed laboratory (PMN > 250/mL)	N.	%
- ve	59	59%
+ ve	41	41%

Table (4): Validity scores of the leukocyte esterase reagent strips in the diagnosis of spontaneous bacterial peritonitis (PMN ≥ 250/mL) using cut off value ≥ + 2 of the strips.

Leukocyte esterase reagent strips	Spontaneous bacterial peritonitis		Total
	Positive	Negative	
Test +ve	34	5	39
Test -ve	7	54	61
Total	41	59	100

Table (5): Sensitivity, Specificity, Positive predictive value (PPV), Negative predictive value (NPV) and Accuracy for the reagent strips to diagnose correctly spontaneous bacterial peritonitis (SBP)

Variable	Value	95% C.I
Sensitivity	0.83	0.75-0.91
Specificity	0.915	0.89-0.95
Positive predictive value (PPV)	0.87	0.84-0.9
Negative predictive value (NPV)	0.885	0.86-0.92
Accuracy	0.88	0.85-0.91

Table (6): Correlation between ascitic fluid polymorphonuclear (PMN) counts and the corresponding result of leukocyte esterase reagent strip test

Variable	Pearson correlation (r)	*P* value
Polymorphonuclear (PMN)	0.56	0.000
Test	1.000	0.000

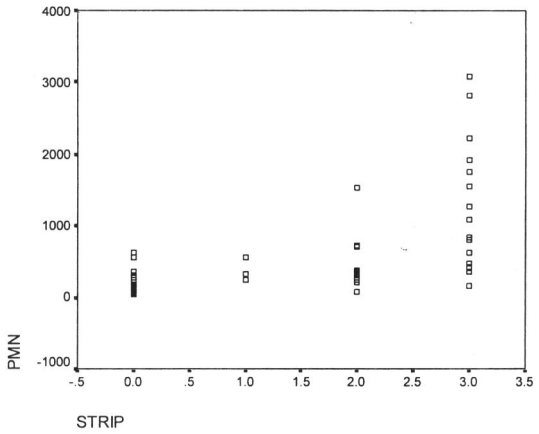

Fig. (1): Correlation between ascitic fluid polymorphonuclear (PMN) counts and the corresponding result of leukocyte esterase reagent strip tests

Table (7): Culture of spontaneous bacterial peritonitis positive cases

Culture result	N. = 41	%
Negative	3	7.31%
Staph. coagulase Negative	4	9.8%
E. coli	14	34.14%
Klebsiella	9	21.95%
Proteus	5	12.2%
Citrobacter	6	14.6

4. Discussion

Cirrhosis which is considered the third leading cause of death after cardiovascular disease and cancer in the Western world results in major complications such as portal hypertension with variceal bleeding,

ascites, or liver failure leading to renal failure and coma [21].

It has been said that cirrhosis is the most common form of immunodeficiency, exceeding even AIDS. Infectious complications in cirrhotic patients can cause severe morbidity and mortality[22].

Spontaneous bacterial peritonitis is a severe and frequent complication of cirrhosis with a high mortality rate. Spontaneous bacterial peritonitis is probably related to several impaired defense mechanisms, such as depressed reticuloendothelial system phagocytic activity, leukocyte dysfunction, reduced serum complement, and low bacterial activity of ascitic fluid [23].

Use of reagent strip testing for leucocyte esterase has been proposed to reduce the time from paracentesis to a presumptive diagnosis of SBP from a few hours to a few Seconds [18].

The test is based on the esterase activity of granulocytes. 3-Hydroxy-5-phenyl-pyrrole esterified with an amino acid is used as the substrate. Hydrolysis of this ester by the esterase releases 3- hydroxy-5-phenyl-pyrrole, which in turn reacts with a suitable diazonium salt, yielding a violet azo dye, the intensity of which correlates to the leukocyte count [24].

The aim of this study is to compare between leucocyte esterase reagent strips as a bed side test and standard manual PMN counting in the ascitic fluid as regarding efficacy and rapidity in diagnosis of SBP. The study was conducted on 100 patients with liver cirrhosis complicated by ascites and spontaneous bacterial peritonitis (SBP).

In this work, the test of ascitic fluid by leukocyte esterase reagent strips showed that 39 patients were positive (+ve) and 61 patients were (-ve) , while laboratory examination of ascitic fluid showed that 41 patients were (+ve) and 59 patients were negative (-ve) with Specificity =91.5% and Sensitivity = 83%

These results were in agreement with that of **Castellote *et al.,*** [25], who demonstrated sensitivity (96%) and specificity (89%) for detecting SBP in cirrhotic patients with ascites. Also, these results were in consistent with that of **Vanbiervliet *et al.,*** [26] study that showed the Multistix 8SG rapid urine screening test had 100% sensitivity and specificity for SBP diagnosis.

In another study, the reagent Combur-2 test® LN, was tested and showed a sensitivity of 89% and a specificity of 100% [27]. In the same direction, **Sithara *et al.,*** [28] showed results of LER strip using the more stringent purple-color cut off to diagnose SBP had a sensitivity of 92% and specificity of 100%.

In contrast, **Nousbaum *et al.,*** [29] study showed that sensitivity was only 45.3%. Several explanations are possible for this poor sensitivity. First, published studies were limited to a small number of patients with SBP. Second, the strip was initially designed for detection of urinary tract infections in which the number of leukocytes is significantly higher than in SBP.

In this work, Positive predictive value (PPV) and negative predictive value (NPV) of leukocyte esterase reagent strips in the diagnosis of S.B.P were 87.2%.and 88.5%. This findings were in accordance with that of **Tarsila *et al.,*** [30] who showed that positive and negative predictive value were 91% and 98%. With a positive reagent strip results taken as grade 2 or more.

This study showed the accuracy of the reagent strips to diagnose correctly spontaneous bacterial peritonitis (SBP) was 88%. In the same context, **L'opez *et al.,*** [31] who studied a group of non selected paracentesis performed on a cohort of cirrhotic patients, at admission, when an SBP was suspected or was clinically indicated, by use of a reagent strip for leukocyte esterase designed for the testing of urine (Aution sticks; A. Menarini Diagnostics, Firenze, Italy), the accuracy of the reagent strips was 0.91 (0.87-0.94).

In this study, the commonest causative agents isolated from infected ascetic fluid in our stud^ were *Escherichia coli* (34.14%), Klebsiella spp. (21.95%), Citrobacter (14.6%), Proteus (12.2%).

This results were in consistent with that of **Koulaouzidis *et al.,*** [32] who showed that the commonest causative agents isolated from infected ascitic fluid were *Escherichia coli* (70%), Klebsiella spp. (10%), Proteus spp. and *Enterococcus faecalis* (4% each), Pseudomonas spp. (2%) and others (6%). This difference in prevalence might be explained by the difference in study size.

This study showed that there was positive correlation (r= +0.56) between ascitic fluid polymorphonuclear (PMN) counts and the corresponding result of leukocyte esterase reagent strip test which was statistically high significant (*p* value = 0.000)

Although, this results were in consistent with that of **Castellote *et al.,*** [33] study in which a group of non selected cirrhotic patients were undergone diagnostic paracentesis performed on a cohort of cirrhotic patients were studied at admission, there was a very good correlation between the reagent strip test result and the PMN count, using Aution sticks (Arkray Inc., Edina, Minnesota,USA) , it was against with that of **Daniel *et al.,*** [34] who reported that there was a lack of correlation between the degree of reagent strip positivity and the ascitic PMN counting (Bedside leucocyte esterase reagent strips with spectro-photometric analysis to rapidly exclude spontaneous bacterial peritonitis [35, 36].

This difference in this result may be explained by the reagent strip results were read spectrophotometrically using the clinitek status, thus removing operator subjectivity or indeed error (if colour blind). This removes interobserver variability from the diagnostic algorithm. Another explanation that in our study we selected highly suspected patients with SBP from intensive care unit of internal medicine department while others showed non selected cirrhotic patients for diagnosis. In addition, these strips have only been validated for urine by the manufacturers and numerous factors in ascites, not present in urine, could affect that colorimetric reaction[37].

In summary,

There was a highly statistically significance of leukocyte esterase reagent strips in diagnosis of SBP with highly Specificity 91.5%, Sensitivity 83% and Accuracy 88%.

There was positive correlation (r= +0.56) between ascitic fluid polymorphonuclear (PMN) counts and the corresponding result of leukocyte esterase reagent strip test which was statistically of high significance (*p* value =0.000).

Conclusion:

It's concluded from this study that the reagent strip testing of ascitic fluid is a very sensitive and specific method for diagnosis of SBP in cirrhotic patients with ascites. It can be used everywhere at the patient's bedside and is rapid, easy to use, inexpensive and results are available within a maximum of 120 seconds. A positive result should be an indication for empirical antibiotic therapy, and a negative result excludes SBP and may be useful as a screening test in patients on large-volume paracentesis.

Recommendation:

- There must be much more work about the leukocyte esterase reagent strips as a bed side test in cirrhotic patients with ascites.
- The work must include the efficacy and the rapidity of leukocyte esterase reagent strips for the early detection of the possibility of developing SEP.

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Evaluation of Some Fibrinolytic Factors for Assessment of Lower Extremity Arterial Disease (LEAD) in Diabetic Patients

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Abstract: This study was carried out in the Clinical Pathology and Internal Medicine Departments, Faculty of Medicine, Zagazig University Hospitals. The study included 57 subjects classified into 3 groups. Group I: It included 13 apparently healthy subjects. Group II: It included 22 insulin-dependent diabetic patients. They were sub-classified into 15 NON Lower Extremity Arterial Disease (LEAD) and 7 LEAD. Group III: It included 22 non-insulin-dependent diabetic patients. They were sub-classified into 15 NON LEAD and 7 LEAD. All patients and control subjects were subjected to the followings: 1- Full history taking. 2-Complete clinical examination 3-Complete blood picture (CBC). 4-Prothrombin time (PT) and Partial thromboplastin (PTT). 5-C reactive protein (CRP). 6-Liver and kidney functions tests fasting and two hours postprandial serum glucose.7-Lipid profile (total cholesterol, triglycerides, HDL- cholesterol).8- Specific laboratory investigations: Glycosylated hemoglobin (HbA1c). Assay of fibrinogen. Assay of tissue plasminogen activator (t-PA). The results revealed the following: - There was no significant difference between all studied groups as regard age of subjects and duration of clinical diabetes. There was a significant increase in BMI in group III compared to groups I and II but no significant difference was found between group II and group I. t-PA was significantly increased in group II compared to group I but significantly decreased in group III compared to groups I and II. There was a significant increase in HbA1c in group II and group III compared to group I with no significant difference was found between group II and group III. There was a significant increase in fibrinogen in groups II and III compared to group I with no significant difference was found between group II and group III. There was a significant increase in CRP in in groups II and III compared to group I. CRP was also significantly increased in group III compared to group II. There was a significant decrease in the levels of cholesterol In groups II and III compared to group I and significantly lower in group II compared to group III. The level of triglyceride was significantly higher In groups II and III compared to group I, also triglyceride in group III was significantly higher compared to group II. The level of HDL-cholesterol was significantly higher in group II compared to group I and significantly lower in group III compared to groups I and II. There was no significant difference between NON LEAD and LEAD in group II as regards t-PA but in the group III, t-PA was significantly higher in LEAD compared to NON LEAD. There was statistically positive correlation between t-PA and age and diabetic duration in total, NON LEAD and LEAD group II. There were statistically positive correlations between t-PA and age in total, NON LEAD and LEAD group III. There was a statistically positive correlation between t-PA and HbA1c in total group III. There were statistically positive correlation between t-PA and diabetic duration in total, NON LEAD and LEAD group III.

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1. Introduction

Diabetes mellitus is a group of disorders characterized by hyperglycemia and associated with microvascular (i.e., retinal, renal, neuropathic) and macrovascular (i.e., coronary, peripheral vascular) complications. Hyperglycemia results from lack of endogenous insulin or resistance to the actions of insulin in muscle, fat and the liver. In addition to an inadequate response by the pancreatic beta cell **(Wolfs *et al.*, 2009).**

Diabetes leads to a hypercoagulable state. It is associated with the increased production of tissue factor by endothelial cells and vascular smooth muscle cell (VSMC), as well as increased plasma concentrations of factor VII. Hyperglycemia is also associated with a decreased concentration of antithrombin and protein C, impaired fibrinolytic function, and excess production of PAI-1 **(Beckman *et al.*, 2004).**

The etiology of atherosclerosis, including lower extremity arterial disease (LEAD), is multifactorial. Major risk factors are hyperglycemia, smoking and hypertension **(Dieter *et al.*, 2002).**

The fibrinolytic system includes a broad spectrum of proteolytic enzymes with physiological and pathophysiological functions in several processes

such as hemostatic balance, tissue remodeling, tumor invasion and angiogenesis **(Fay *et al.*, 2007).** The main enzyme of the plasminogen activator system is plasmin, which is responsible for the degradation of fibrin into soluble degradation products. The activation of plasminogen into plasmin is mediated by two types of activators, urokinase–type plasmingen activator (uPA) and tissue-type plasminogen activator (t-PA).The activity of both is regulated by specific plasminogen activator inhibitors (PAIs) **(Esther *et al.*, 2008).**

The fibrinolytic system is primarily an interaction between plasminogen activators, and inhibitors and one response to vascular injury is an activation of t-PA. Increased t-PA-activity may therefore be a potential indicator of an early ongoing vascular damage and, possibly, a compensatory mechanism. Both t-PA and PAI-1 mass levels have been suggested as indicators of vascular damage **(David Sahli *et al.*, 2009).**

In diabetic patients, vascular endothelial cells exposed to high glucose level leading to elevation of t-PA in the plasma accompany impaired fibrinolysis **(Maiello *et al.*, 1992).**

Tissue plasminogen activator level elevates in the plasma of the diabetic patients with lower extremity arterial disease (LEAD) and can be use as an early marker for diagnosis of these cases **(Raffetto *et al.*, 2005).**

The **aim** of the present study is to evaluate the plasma level of tissue plasminogen activator (tPA) as an early predictor marker of asymptomatic lower extremity arterial disease (LEAD) in patients with diabetes mellitus. Also correlation of this marker with diabetic atherosclerosis was done.

2. Subjects and methods

Subjects:

This study was carried out at Clinical Pathology and Internal Medicine Departments, Faculty of Medicine, Zagazig University Hospitals.

Fifty seven subjects were included in the study (13 healthy controls and 44 diabetic patients). They were divided into three groups as follows:

Group I: It included 13 apparently healthy subjects. They were 7 males and 6 females, with a mean age 45.8 ± 8.1 years. They matched well with patients as regard age and sex.

Group II: It included 22 insulin-dependent diabetic patients. They were 10 males and 12 females. Their ages ranged from 30-70 years with a mean of 48.1 ± 5.7 years. They were sub-classified into 15 NON LEAD and 7 LEAD.

Group III: It included 22 non- insulin-dependent diabetic patients. They were 10 males and 12 females. Their ages ranged from 30-70 years with a mean 50.3 ± 8.0 years. They were sub-classified into 15 NON LEAD and 7 LEAD.

All patients were subjected to the followings:

1- Full systemic history of hypertension, cardiovascular disease, tobacco use and diabetic duration.
2- Full history of arterial disease (atherosclerosis) taking along a spectrum of severity ranging from no symptoms, intermittent claudication, rest pain, and finally to non-healing wounds and gangrene.
3- Physical local examination by inspection of the foot and palpation of peripheral pulses thorough:
   - Inspection for signs of vascular insufficiency such as dependent rubor, pallor on elevation, absence of hair growth, dystrophic toe nails and dry fissured skin. Also interdigital spaces inspected for fissures, ulcerations, and infections.
   - Assessment of pulsation of femoral, popliteal and pedal vessels.
4-Determination of Ankle Brachial Index (ABI): is the ratio of the systolic blood pressure in the ankle divided by the systolic blood pressure at the arm.
5- Determination of Body mass index (BMI): is a measure of body weight based on a person's weight and height.

Methods:

All members of this study were subjected to the following:

1. Routine laboratory investigations:

- Complete blood picture (CBC) by (Sysmex-KX 21N – Sysmex Corporation).
- Prothrombin time (PT) and Partial thromboplatin (PTT) by (Sysmex –CA 1500, Japan).
- C reactive protein (CRP) by (Cobas-Integra).
- Liver and kidney functions tests, fasting and two hours post prandial serum glucose by dimension RXL MAX autoanalyser (Seimens Medical Solution Diagnostics, UL, USA).
- Lipid profile (total cholesterol, triglycerides, HDL-cholesterol) by dimension RXL MAX autoanalyser (Seimens Medical Solution Diagnostics, UL, USA).

2. Specific laboratory investigations:

- Assay of Glycosylated hemoglobin (HbA1c).
- Assay of Fibrinogen by fibrin-timer BFT II analyzer (Siemens, Germany).
- Assay of tissue plaminogen activator (t-PA) by ELISA processor II (Dade Behring, USA).

Specimen collection:

After 12 hours, 10 ml venous blood sample was collected from all subjects by venipuncture aseptically and divided as follow:

1-1.5 ml of blood was delivered into a tube containing 1.2 mg/ml EDTA used for complete blood picture and glycosylated hemoglobin (HbA1c).

2-3.6 ml of blood was transferred into a tube containing 0.4 ml Na citrate with a ratio of 9:1 used for PT, PTT, fibrinogen and tissue plaminogen activator (t-PA).

3-The remainder of blood was transferred into a plain tube, allowed to clot at 37 °C followed by centrifugation at 3000 r.p.m. for 15 minutes the serum was collected and used for determination of liver function, kidney function, lipid profile and fasting serum glucose level. Another blood sample was withdrawn from Groups II and III 2 hours postprandial for determination of postprandial serum glucose level.

Assay of glycosylated hemoglobin (HbA1c)

Principle:

Whole blood was mixed with a lysing reagent containing a detergent and borate ions. Elimination of the labile Schiff's base was thus achieved during the hemolysis.

A preparation of hemolysed whole blood was mixed with a weakly binding cation–exchange resin. The non-glycosylated hemoglobin (HbA0) binds to the resin leaving (HbA1) free to be removed by means of resin separator in the supernate. The percent of HbA1 was determined by measuring the absorbance values at 415 nm of the HbA1 fraction and of the total Hb fraction **(Nuttall, 1998).**

Reagents:

1. Glycohemoglobin ion–exchange resin contained: cation – exchange resin (borate 150 mmol/l), imidazole buffered at pH 7.6.
2. Glycohemoglobin lysing reagent contained: 1 mol/l borate and detergents 0.25%.
3. Glycohemoglobin standard (freeze dried hemoglobin): 1 vial prepared from packed human erythrocytes.

Reagent preparation:

- One ml distilled water was added to reconstitute the standard vial and allowed standing for minutes at room temperature. The vial was shaked gently to hasten the reconstitution.
- Glycohaemoglobin ion–exchange resin and Glycohaemoglobin lysing reagent are ready to use

Procedure:

Hemolysate preparation

- 0.5 ml of lysing reagent was pipetted into tubes labeled standard (s) and unknown (u).
- 0.1ml of each well mixed blood sample was pipetted into appropriately labeled tube and mixed and leaved for 5 minutes.

Glycohemoglobin separation and assay:

- 0.1ml of the prepared hemolysate was pipetted into appropriately labeled resin tubes.
- A resin separator was positioned in tube and rubber sleeve was approximately 1-2 cm above liquid level then mixed on hematology rocker for 5 minutes.
- A resin separator was pushed into tube until resin was firmly packed in bottom of this tube.
- Each supernatant was directly poured into cuvette for absorbance reading.
- Absorbance of standard and unknown was read with water blank at 415 nm within 60 minute.

Total hemoglobin assay:

- 5.0ml of deionized water pipetted into tubes labeled standard (s) and unknown (u).
- 0.02ml of hemlysate was pipetted into approximately labeled tube, mixed well and transferred to cuvette for absorbance reading with water blank at 415nm.

Calculation of the HbA1c content

Factor F Determination by Use of (STD):

The glycohemoglobin percentage **(**% HbA1c (STD)**)** is stated on the label under %.

Atotal Hb (STD) X % HbA1c (STD)

F= AHbA1 (STD)

Glycohemoglobin Content of the sample:

$$\% \text{ HbA1c sample} = F \times \frac{\text{A Hb1c sample}}{\text{A total Hb sample}}$$

Clinical Interpretation:

Patients with controlled metabolism or stabilized diabetics

HbA1c 4.5 - 7.0 %

Diabetics, insufficiently controlled or with metabolic Imbalance HbA1c ≥ 8.5 %

Assay of fibrinogen

Principle of the method:

Quantitative measurement by bringing citrated plasma to full coagulation with a large excess of thrombin. So, the coagulation time will depend largely on the fibrinogen content of the specimen and can be measure by fibrin timer **(Cooper and Douglas, 1991).**

Preparation of Reagents:

Multifren dissolved with 2 ml of distilled water.

Procedure:

1-Multifren brought into a test tube warmed to 37°C.

2-One hundred µl of sample was pipette in reaction tube cuvette.

3-Sample was incubated for 60 seconds at 37°C.

4- Two hundred µl of Multifbren (37°C) was pipette in reaction tube cuvette.

5-The coagulation time was determined by fibrin timer.

Calculation of the results:

The results evaluated with the enclosed value table.

Expected Values:
180-350 mg/L.

Assay of tissue plasminogen activator (t-PA)

Principle:

In a first step, the diluted tested plasma is introduced into a microwell coated with a highly purified monoclonal antibody specific for human t-PA. When present, this protein is captured into the solid phase. Following a washing step, the immunoconjugate, which is a monoclonal antibody coupled to horse radish peroxidase (HRP), is introduced, and bound to another free epitope of immobilized t-PA. Following a new washing step, the peroxidase substrate, Tetramethylbenzidine (TMB) in presence of hydrogen peroxide (H2O2), is introduced and a color develops. The amount of color developed is directly proportional to the concentration of human tPA-Ag in the tested sample **(Stein *et al.*, 1997)**.

Reagents Preparation:

- Control I: 1 vial (plasma control I High) restored with 1 ml distilled water.
- Control II: 1 vial (plasma control II Low) restored with 1 ml distilled water.
- Tissue plasminogen activator (t-PA) standard: 1 vial was dissolved with1 ml distilled water.
- The immunoconjugate: (a monoclonal antibody coupled to HRP): each vial was dissolved with 7.5 ml of conjugate diluent.
- Wash Solution: incubated 15-30 min. in a water bath at 37°C until complete dissolution of solids, the vial then diluted 1:20 in distilled water.
- F-Sample Diluent: ready to use.

Specimen collection:

Blood sample collected on Na citrate anticoagulant, plasma is sequestrated following a 20 min. centrifugation or stored frozen at –20°C or below until to use, and thawed for 15 min. at 37°C just before use.

Preparation plasma or sample or controls:

- The sample diluted two fold (1:2) by the F-Sample Diluent. For expected t-PA concentrations > 20 ng/ml, plasma or samples can be tested at a higher dilution 1:5, or 1:10 or more**.**
- Controls I and II diluted two fold (1:2) with F-Sample Diluent.
- t-PA Standard diluted as follows:

STD	Dilution guide	t-PA concent. (ng/ml)
1	1 ml t-PA standard	C
2	0.5 ml t-PA standard + 0.5 ml F-Sample Diluent.	C/2
3	0.25 ml t-PA standard + 0.75ml F-Sample Diluent.	C/4
4	0.1 ml t-PA standard + 0.9 ml F-Sample Diluent.	C/10
5	0.05 ml t-PA standard +0.95 ml F-Sample Diluent.	C/20
6	1 ml F-Sample Diluent.	0

Procedure:

1- The strips were removed from the aluminum pouch. Then the strips were put in the frame.
2- Two hundred µl from each of 6 tubes of t-PA Standard, diluted controls and diluted samples (1:2) were pipetted in the corresponding micro ELISA plate well.
3- The plate was covered and incubated for 1 hour at room temperature.
4- After incubation, the plate washed 5 times by 300 µl wash solution in each well.
5- Two hundred µl of immunoconjugate was pipetted into each well and the plate was covered, incubated for 1 hour at room temperature. Then the plate washed 5 times by 300 µl wash solution in each well.
6- Two hundred µl of Tetramethylbenzidine (TMB) is delivered immediately after the washing in each well.
7- The plate was incubated 5 min. exactly at room temperature.
8- The reaction was stopped by adding 50 µl of stopping reagent to each

Well; absorbance was read at 450 nm after 10 minutes.

Calculations of results:

A standard curve was constructed by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis on log-log graph and a best fit line was drawn through the points on the graph.

Expected range:

The t-PA:Ag concentration in normal human plasma is usually < 10 ng/ml.

Statistical analysis:

Data were entered, checked and analyzed using SPSS for windows version 10.

3. Results

This study included three groups : group I (13 normal individuals as control), group II (22 patients with insulin-dependent diabetes, they were sub-classified into 15 NON LEAD and 7 LEAD) and group III (22 patients with non-insulin-dependent diabetes, they were sub-classified into 15 NON LEAD and 7 LEAD). The results statistically analyzed and came to the following:

Demographic characteristics as regard sex, male and female account respectively for 53.8% and 46.2% in group I, 45.5% and 54.5% in group II and 45.5%,

and 54.5% in group III. No statistically significant difference was found between the three studied groups (X^2=0.28, p=0.86).

As regard smoking (tobacco use), it is positive in 38.5% in the group I, 27.3% in group II and 27.3% in group III. No statistically significant difference was found between the three studied groups (X^2=0.6, p =0.74).

As regard hypertension, it is positive in 30.8% in the group I, 59.1% in group II and 68.2% in group III. No statistically significant difference was found between the three studied groups (X^2=4.7,p =0.1).

As regard cardiovascular disease, it is positive in 13.6% in group II and 18.2% in group III. No statistically significant difference was found between the three studied groups (X^2=2.56, p =0.27).

There was a significant difference between the three groups as regards SBP (p <0.05).

There was a very highly significant difference between the three groups as regards BMI and Ankle Brachial Index (p <0.001).

There was non-significant difference between the three groups as regards age and DBP (p >0.05).

BMI was significantly increased in group III compared to groups I and II (p <0.001) but no significant difference was found between groups II and I (p >0.05).

SBP was significantly increased in groups II and III compared to group I (p <0.05) with no significant difference was found between group II and group III (p >0.05).

ABI was significantly lower in group II and group III compared to group I (p <0.001) with no significant difference was found between group II and group III (p >0.05).

There was a significant difference between the three groups as regards triglycerides (p <0.05).

There was a highly significant difference between the three groups as regards fibrinogen and CRP (p <0.01).

There was a very highly significant difference between the three groups as regards HbA1c, t-PA, cholesterol and HDL- cholesterol (p <0.001).

HbA1c was significantly higher in group II and group III compared to group I (p <0.001) with no significant difference was found between group II and group III (p >0.05).

t-PA was significantly higher in group II compared to group I (p <0.001), but significantly lower in group III compared to group I and group II (p <0.001).

There was a significant increase of fibrinogen in group II and group III compared to group I (p <0.05 and p <0.001 respectively) with no significant difference was found between group II and group III (p >0.05).

There was a significant increase of CRP in group II and group III compared to group I (p <0.05 and p <0.001 respectively). CRP was also significantly higher in group III compared to group II (p <0.05).

Cholesterol was significantly lower in group II and group III compared to group I (p <0.05) and significantly lower in group II compared to group III (p <0.05).

Triglyceride was significantly higher in group II and group III compared to group I (p <0.05 and p <0.001, respectively)), Also triglyceride in group III was significantly higher compared to group II (p <0.05).

HDL- cholesterol was significantly higher in group II compared to group I (p <0.001), but significantly lower in group III compared to group II and group I (p <0.001).

There was significant difference between LEAD and NON LEAD as regards CRP and cholesterol (p < 0.05).

There was a very high significant difference between LEAD and NON LEAD as regards Ankle Brachial Index (p <0.001). There was non-significant difference between LEAD and NON LEAD as regards age, BMI, SBP, DBP, HbA1c, t-PA, fibrinogen, diabetic duration, triglycerides, HDL cholesterol, FSG and PPSG (p >0.05).

There was significant difference between LEAD and NON LEAD as regards HbA1c, t-PA and Fibrinogen (p < 0.05).

There was a very highly significant difference between LEAD and NON LEAD as regard age, SBP, CRP and Ankle Brachial Index (p <0.001).

There was non-significant difference between LEAD and NON LEAD as regard BMI, DBP, cholesterol, triglycerides, HDL- cholesterol, diabetic duration, FSG and PPSG (p >0.05).

There were statistically positive correlations with age and diabetic duration (r = 83.0, p = 0.000, r = 0.8, p = 0.000) ; (r = 0.84, p = 0.000, r = 0.81, p = 0.001) and (r = 0.92, p = 0.002,r = 0.82, p = 0.02) in total, NON LEAD and LEAD respectively .

There were statistically positive correlations with age (r = 0.77, p = 0.000), (r = 0.8, P = 0.000), (r = 0.73, P = 0.05) in total, NON LEAD and LEAD respectively.

There was statistically positive correlations with HbA1c (r = 0.65, p = 0.001) in total.

There were statistically positive correlations with diabetic duration (r = 0.59, p = 0.004), (r = 0.75, p = 0.001), (r =0.53, p = 0.05) in total, NON LEAD and LEAD respectively.

Table (1): Demographic characteristics of the studied groups:

	Group I n = 13	Group II n = 22	Group III n = 22	X^2	P
Sex	n	n	n		
M	7 53.8%	10 45.5%	10 45.5%	0.28	0.86
F	6 46.2%	12 54.5%	12 54.5%		
Smoking	n	n	n		
-ve	8 61.5%	16 72.7%	16 72.7%	0.6	0.74
+ve	5 38.5%	6 27.3%	6 27.3%		
Hypertension	n	n	n		
-ve	9 69.2%	9 40.9%	7 31.8%	4.77	0.1
+ve	4 30.8%	13 59.1%	15 68.2%		
Cardiovascular	n	n	n		
-ve	13 100.0%	15 86.4%	18 81.8%	2.56	0.27
+ve	0	3 13.6%	4 18.2%		

Table (2): Comparison of clinical and demographic data in the three studied groups as regards age, BMI, SBP, DBP and Ankle Brachial Index.

	Group I (n =13) Mean ± SD	**Group II (n=22) Mean ± SD**	**Group III (n=22) Mean ± SD**	**F**	***P***
Age (years)	45.8± 8.1	48.1±5.7	50.3±8	1.6	0.21
BMI(kg/m^2)	24.5±2.4	25±1.8	29.6±3	24.7	0.000
SBP(mmHg)	128.8±14.4	143.8±15.4	142.7±18.6	3.8	0.028
DBp(mmHg)	76.5 ± 7.7	78.6±8	81.1+7.5	1.4	0.234
Ankle Brachial Index	1.29±0.28	0.96±0.33	0.90±0.40	5.2	0.0001

Table (3): LSD for comparison of BMI, SBP, and ABI between the three groups.

LSD for comparison of BMI		
	Group I	Group II
Group II	>0.05	
Group III	<0.001	<0.001
LSD for comparison of SBP		
	Group I	Group II
Group II	<0.05	
Group III	<0.05	>0.05
LSD for comparison of ABI		
	Group I	Group II
Group II	<0.001	
Group III	<0.001	>0.05
LSD for comparison of CRP		
	Group I	Group II
Group II	<0.05	
Group III	<0.001	<0.05
LSD for comparison of cholesterol		
	Group I	Group II
Group II	<0.05	
Group III	<0.05	<0.05
LSD for comparison of triglyceride		
	Group I	GroupII

Group II	<0.05	
Group III	<0.001	<0.05
LSD for comparison of HDL- cholesterol		
	Group I	Group II
Group II	<0.001	
Group III	<0.001	<0.001
LSD for comparison of BMI		
	Group I	Group II
Group II	>0.05	
Group III	<0.001	<0.001
LSD for comparison of SBP		
	Group I	Group II
Group II	<0.05	
Group III	<0.05	>0.05
LSD for comparison of ABI		
	Group I	Group II
Group II	<0.001	
Group III	<0.001	>0.05

Table (4): Comparison of laboratory data in the three studied groups as regards HbA1c, t-PA, fibrinogen, CRP, cholesterol, triglycerides and HDL- cholesterol.

	Group I (n =13) Mean ± SD	Group II (n=22) Mean ± SD	Group III (n=22) Mean ± SD	F	*P*
HbA1c (%)	4.46 ±0.4	7.2 ±1.1	6.9±1.1	33.9	0.000
t-PA(ng/ml)	10.0 ± 3.7	14.5±4.0	6.4±3.4	25.9	0.000
Fibrinogen(mg/dl)	263.9±69.9	340.4±85.4	357.1±86.2	5.52	0.01
CRP(mg/l)	2.7±1.5	17.7±6.7	33.6±37	7.34	0.002
Cholesterol(mg/dl)	181.8±20.5	160.6±17.8	178.6±36.4	33.6	0.000
Triglycerides(mg/dl)	73.2±8.5	116.8±70.8	156.7±55.1	3.5	0.03
HDL- cholesterol(mg/dl)	36.0±4.6	42.6±5.3	31.1±4.1	9.2	0.000

Table (5): Comparison of different parameters between NON LEAD and LEAD in group II.

Group II	NON LEAD (n = 15)	LEAD (n = 7)	t	*P*
Age	47.0±5.3	50.7±6.0	1.4	0.16
BMI	25.0±2.0	25.1±1.5	0.16	0.87
SBP	141.3±14.8	149.2±16.4	1.13	0.27
DBP	80.3±8.5	75.0±5.7	1.4	0.15
HbA1c	7.0±0.92	7.8±1.4	1.6	0.10
t-PA	14.3±3.0	14.9±5.8	0.32	0.74
Fibrinogen	320.6±96.6	383.0±26.1	1.6	0.11
C.R.P	20.0±4.0	12.5±8.7	2.7	0.03
Ankle Brachial Index	1.15±0.22	0.56±0.06	6.6	0.000
Cholesterol	155.4±15.7	171.8±17.6	2.19	0.04
Triglycerides	133.8±80.2	80.4±15.3	1.72	0.10
HDL- cholesterol	41.4±5.4	45.4±3.9	1.75	0.09
Diabetic duration	29.6±3.8	32.5±9.7	1.0	0.32
Fasting serum glucose	279.1±95.2	251±68	0.69	0.49
Post prandial serum glucose	310.7±94.8	275.2±65.4	0.89	0.38

Table (6): Comparison of different parameters between NON LEAD and LEAD in group III

Group III	NON LEAD (n= 15)	LEAD (n = 7)	t	*P*
Age	50.0±8.3	57.4±4.2	2.1	0.000
BMI	30.0±3.3	28.8±2.2	0.80	0.42

SBP	138.0±18.8	152.8±14.6	1.8	0.000
DBP	80.8±8.4	83.5±4.7	1.0	0.3
HbA1c	6.6±1.01	7.7±0.9	2.4	0.02
t-PA	5.2±2.6	8.9±3.8	2.5	0.02
Fibrinogen	329.3±85.6	416.7±53.2	2.4	0.03
C.R.P	13.1±13.2	77.5±33.0	6.6	0.000
Ankle Brachial Index	1.1±0.32	0.48±0.07	5.01	0.000
Diabetic duration	8.0±4	8.5±4.5	0.2	0.7
Fasting serum glucose	159.0±69.9	200.8±69.8	1.3	0.2
Post prandial serum glucose	188.9±98.3	244.2±87.4	1.2	0.21
Cholesterol	170.2±35.7	196.7±32.9	1.65	0.11
Triglycerides	144.5±57.3	182.8±43.1	1.57	0.13
HDL- cholesterol	31.2±4.1	30.7±4.4	0.28	0.77

Table (7): Correlation between t-PA and other clinical and laboratory findings in total, NON LEAD and LEAD in GroupII.

t-PA Group II	Total (n = 22)		NON LEAD (n = 15)		LEAD (n = 7)	
	r	*p*	r	*p*	r	*P*
Age	83.0	0.00	0.84	0.00	0.92	0.002
BMI	0.06	0.78	-0.22	0.43	0.50	0.25
SBP	0.03	0.87	0.26	0.33	-0.25	0.58
DBP	0.19	0.37	0.24	0.37	0.30	0.51
HbA1c	-0.14	0.54	-0.13	0.64	-0.20	0.65
Fibrinogen	-0.17	0.45	-0.25	0.36	-0.40	0.37
C.R.P	0.22	0.32	0.15	0.57	-0.39	0.38
Ankle Brachial Index	-0.19	0.39	-0.29	0.28	-0.49	0.26
FSG	-0.06	0.80	0.24	0.37	-0.55	0.19
PPSG	-0.06	0.78	0.21	0.44	0.52	0.23
Diabetic duration	0.8	0.00	0.81	0.00	0.82	0.02
HDL- cholesterol	0.11	0.62	-0.27	0.33	0.72	0.06
Cholesterol	0.001	0.99	0.000	1.00	-0.07	0.87
Triglyceride	-0.07	0.73	-0.21	0.43	0.8	0.03

Table (8): Correlation between t-PA and other clinical and laboratory findings in total, NON LEAD and LEAD in GroupIII

t-PA Group III	Total (n = 22)		NON LEAD (n = 15)		LEAD (n = 7)	
	r	*P*	r	*P*	r	*P*
Age	0.77	0.00	0.80	0.000	0.73	0.05
BMI	0.007	0.97	0.33	0.22	-0.37	0.41
SBP	0.30	0.17	0.03	0.91	0.37	0.40
DBP	0.24	0.27	0.29	0.28	-0.17	0.70
HbA1c	0.65	0.001	0.45	0.08	0.72	0.06
Fibrinogen	0.25	0.26	0.09	0.73	-0.02	0.66
C.R.P	0.54	0.008	0.39	0.14	0.21	0.85
Ankle Brachial Index	-0.35	0.124	0.124	0.66	-0.08	0.86
FSG	0.60	0.003	0.55	0.034	0.60	0.15
PPSG	0.58	0.005	0.53	0.04	0.57	0.17
Diabetic duration	0.59	0.004	0.75	0.001	0.53	0.05
HDLcholesterol	-0.17	0.45	-0.5	0.04	0.39	0.38
Cholesterol	0.08	0.71	-0.29	0.28	0.19	0.68
Triglyceride	0.05	0.80	-0.16	0.54	-0.09	0.84

4. Discussion

Diabetes mellitus is defined as the dysregulation of glucose metabolism characterized by chronic hyperglycemia resulting from defects in insulin secretion, decreased insulin sensitivity or a combination of both **(Jochen and Werner, 2006).**

Diabetes leads to a hypercoagulable state. It is associated with the increased production of tissue factor by endothelial cells and VSMC, as well as increased plasma concentrations of factor VII. Hyperglycemia is also associated with a decreased concentration of antithrombin and protein C, impaired fibrinolytic function, and excess production of PAI-1 **(Beckman *et al.*, 2004).**

The fibrinolytic system is primarily an interaction between plasminogen activators and inhibitors and one response to vascular injury is an activation of t-PA. Increased t-PA-activity may therefore be a potential indicator of an early ongoing vascular damage **(David Sahli *et al.*, 2009).**

Tissue plasminogen activator level elevates in the plasma of the diabetic patients with lower extremity arterial disease (LEAD) and can be use as an early marker for diagnosis of these cases **(Raffetto *et al.*, 2005).**

Peripheral arterial disease is a major risk factor for lower-extremity amputation, especially in patients with diabetes. Moreover, even for the asymptomatic patient, PAD is a marker for systemic vascular disease **(Nathaniel, 2003).**

Diabetes mellitus increases the risk for PAD via deleterious effects on the vessel wall e.g., (derangement of nitric oxide bioavailability in endothelial cell) as well as effects on blood cells e.g., (enhanced platelet aggregation and hypercoagulable state) and rheology e.g., (increased blood viscosity and fibrinogen levels) **(American Diabetes Association, 2003).**

Tissue plasminogen activator (t-PA) is synthesized and released by vascular endothelial cells into the circulating blood as the single-chain form and is the predominant activator in plasma. The main function of t-PA is in the dissolution of fibrin in the vasculature, helping to maintain vessel patency. Tissue plasminogen activator (t-PA) acts by forming a ternary complex with fibrin and plasminogen and catalyzes the conversion of inactive plasminogen to plasmin (**Suzanne *et al.*, 2006).**

Increased t-PA occurs in association with endothelial cell dysfunction and damage; elevated levels may reflect the presence of underlying endothelial damage **(Steins *et al.*, 2000).**

The objective of the present study was to evaluate the plasma level of tissue plasminogen activator (t-PA) as an early predictor marker of asymptomatic lower extremity arterial disease (LEAD) in patients with diabetes mellitus. This study was carried out on 57 subjects classified into 3 groups: Group I: - the control group included 13 subjects, Group II: - 22 insulin –dependent diabetic patients, they were sub-classified into NON LEAD and LEAD and Group III: - 22 non-insulin– dependent diabetic patients, they were sub-classified into NON LEAD and LEAD.

In this study BMI was highly significantly increased in group III compared to group I and group II but no significant difference was found between group II and group I. These results coincide with the results reported by **Andreas *et al.*, (2002)** who found a significant increase in BMI in diabetic patients compared to control group, furthermore the results of the study reported by **Ali *et al.* (1995)** are also in agreement with our results. On other hand **Morishita *et al.* (1996)** found no significant difference in BMI between control group and diabetic patients.

The results of this study revealed that there was no significant difference as regard diastolic blood pressure among all groups. These results coincide with the results in the study reported by **Ali *et al.* (1995)** who found no significant difference in DBP between control group and diabetic patients. SBP was significantly increase in group II and group III compared to group I with no significant difference was found between group II and group III, while the study by **Ali *et al.*(1995)** found no significant difference in SBP between control group and diabetic patients.

Moreover, Ankle Brachial Index (ABI) was significantly lower in group II and group III compared to group I with no significant difference was found between group II and group III. These results coincide with the results reported by (**Premanath and Raghunath, 2008)** who found significant decrease in ABI in diabetic patients with and without PAD compared to control group.

In the present study HbA1c was significantly higher in group II and group III compared to group I with no significant difference was found between group II and group III. These results coincide with the results of study reported by **Elizabeth *et al.* (2006)** who found significant increase in levels of HbA1c in type II DM with PAD compared to control group due to chronic elevation of the blood glucose level.

These results suggested that these patients with diabetes were exposed to hyperglycemia over long periods, with increased and accelerated glycosylation of hemoglobin A within the red blood cell throughout its 120 days life span in the circulation.

The results of this study, revealed that t-PA was significantly higher in group II compared to group I, but significantly lower in group III compared to group I and group II. These results coincide with the results reported by **David *et al.*(2009)** who showed that type 2 diabetes had significantly lower levels of t-PA and type 1 diabetes had significantly higher levels of t-PA than non-diabetic subjects**.** These results are explained by detection of elevated levels of the fibrinolytic inhibitor, PAI-1 which is generated from fat-laden

insulin-resistant adipocyte and lead to suppression of the fibrinolysis in diabetic patients due to negative feedback on tissue plasminogen activator (t-PA) **(Lange *et al.*, 2003)**.

In accord with our results, **Bastard *et al.* (2000)** reported that increased PAI-1 secretion due to interaction of a number of metabolic and inflammatory factors in type 2 diabetes can lead to decrease secretion of the tissue plasminogen activator (t-PA).

Our results are also in agreement with **Stegenga *et al.* (2006)** who found that t-PA was significantly lower in type II diabetes because fluctuating hyperglycemia lead to protein glycation that induce the oxidative stress, endothelial cell dysfunction, extracellular matrix formation and apoptosis. This lead to vascular damage that increase thrombotic formation, stimulation the fibrinolysis system, increase of (PAI-1) and decrease t-PA in the late stages.

Our study revealed a significant increase of fibrinogen in group II and group III compared to group I with no significant difference was found between group II and group III. These results coincide with the results reported by **Andreas *et al.* (2002)** who reported that fibrinogen level in diabetic patients were higher than those in the control group due to increased synthesis and turnover of fibrinogen in diabetes that is related to insulin deficiency. These results explained by **Meigs *et al.*(2000)** suggested that in diabetic patients complicated with vascular disease, there are multiple vascular damages which are responsible for the high fibrinogen level. On the other hand, the study of **Pandolfi *et al.*(2001)** found no significant difference in fibrinogen between control group and diabetic patients.

In the present study there was a significant increase of CRP in group II and group III compared to group I, also CRP was significantly higher in group III compared to group II. These results agree with the results of study reported by **Andreas *et al.* (2002)** who reported that CRP levels in diabetic patients were higher than those in the control non-diabetic group, suggesting that the hyperglycemia affects the CRP levels, the study of **Ridker *et al.* (2000)** found that in prolonged exposure to hyperglycemia there is an inflammatory process which lead to elevation of CRP level that leads to vascular damage. This stimulate endothelial production of procoagulant tissue factor, leukocyte adhesion molecules, and chemotactic substances and inhibits endothelial cell nitric oxide (NO) synthase, resulting in increased local production of compounds impairing fibrinolysis, such as plasminogen activator inhibitor (PAI-1) which inhibit the secretion of tissue plasminogen activator .

The results of this study also revealed that there was no significant difference between NON LEAD and LEAD in group II as regards t-PA. These results coincide with the results reported by **David *et al.* (2009)** who found that t-PA was not significantly different in type 1 diabetes with LEAD compared to NON LEAD.

Also, there was no significant difference between NON LEAD and LEAD in group II in the levels of HDL and triglyceride. These results coincide with the results reported by **Tzoulaki *et al.* (2006)** who found that there were no significantly differences in HDL and triglyceride in type 1 diabetes with or without PAD.

Otherwise, cholesterol was significantly increased in group II LEAD compared to NON LEAD. These results not matched with the results reported by **Tzoulaki *et al.* (2006)** who found that there was no significant difference in cholesterol in type 1 diabetes with or without PAD.

Moreover, there was a significant decrease in ankle brachial index in LEAD compared to NON LEAD in group II. These results coincide with the result showed by (**Premanath and Raghunath, 2008)** who found significant decrease in ABI in diabetic patients with PAD compared to diabetic patients without PAD.

On the other hand, there were no significant difference in age, body mass index, systolic blood pressure, diastolic blood pressure, glycosylated hemoglobin, fibrinogen, fasting serum glucose, post prandial serum glucose and diabetic duration between LEAD and NON LEAD of group II.

In the group III, tPA was significantly increased in LEAD compared to NON LEAD .These results coincide with the results reported by **David *et al.* (2009)** who found that tPA was significantly elevated in type 2 diabetes with LEAD than NON LEAD.

These results were explained by **(Kooistra *et al.*, 1994)** who said that peripheral ischemia stimulate the compensatory mechanism to sustain circulation in the vessels in the early stage of the disease and during this stage the endothelium still contact and secretory moreover, it is the major source for secretion of tPA so, the tPA secretion increase after vascular insufficient caused by peripheral vascular disease in type 2 diabetes.

Also there was no significant difference between NON LEAD and LEAD in group III as regards cholesterol, HDL-cholesterol and triglyceride. These results coincide with the results reported by **Tzoulaki *et al.* (2006)** who found that cholesterol, HDL-cholesterol and triglyceride not significantly different in type 2 diabetes with or without PAD.

Moreover, there was a significant decrease in ankle brachial index in LEAD compare to NON

LEAD in the group III. These results coincide with the result showed by **(Premanath and Raghunath, 2008)** who found significant decrease of ABI in diabetic patients with PAD compare to diabetic patients without PAD. This study found that atherosclerosis stimulate formation of the atheromatic plaque which affect the blood flow in the blood vessels simultaneously the change in the endothelial wall of the blood vessels which loss its elasticity and thickened leading to decrease the sound wave that transmitted through it as it become more flattened and rounded and lead to decrease the ankle systolic pressure.

Otherwise, there was a significant increase in glycosylated hemoglobin in LEAD compare to NON LEAD in the group III. These results coincide with the results of study reported by **Elizabeth *et al.* (2006)** who found significant increase in levels of HbA1c in type II DM with PAD compared to type II DM without PAD suggested that these patients with uncontrolled diabetes were exposed to hyperglycemia over long periods, with increased and accelerated glycosylation of hemoglobin A within the red blood cell throughout its 120 days life span in the circulation

On the other hand, there were no significant difference in body mass index, diastolic blood pressure, fasting serum sugar, post prandial serum sugar and diabetic duration between LEAD and NON LEAD of group III.

In the present study, tissue plasmingen activator was positively correlated in group II with age and duration of diabetes.

Furthermore, in our study tissue plasminogen activator was positively correlated in group III with age, duration of diabetes and with HbA1c in (total).

In our study, there was no correlation between tPA and body mass index in group II and group III, While another study by **Ali Keskin *et al.* (1995)** show positive correlation of t-PA with BMI, this observation further supports documented decreased fibrinolytic activity in obesity. These results were explained as an elevation in BMI predisposes to insulin resistance and hyperinsulinism rather than to insulin deficiency.

In our study we found a significant correlation between tissue plasminogen activator and glycosylated hemoglobin in group III (total). These results were agree with the result shown by **Thomas *et al.* (2009)** who found that there was a significant correlation between t-PA and HbA1c, on the other hand the study reported by **Ali Keskin *et al.* (1995)** who found no correlation between t-PA and HbA1c was against our study. So, the concentration of the tissue plasmingen activator according to our study seems to be dependent on diabetic control.

Otherwise, our study in group II found no correlation among t-PA and glycosylated hemoglobin, systolic blood pressure, diastolic blood pressure, fibrinogen, C-reactive protein, ankle brachial index, fasting serum sugar,post prandial serum sugar, HDL- cholesterol, cholesterol and triglyceride .

our study in group III found no correlation among t-PA and systolic blood pressure, diastolic blood pressure, fibrinogen, C-reactive protein, ankle brachial index, fasting serum sugar,post prandial serum sugar, HDL- cholesterol, cholesterol and triglyceride.

In **conclusion** Tissue plasminogen activator (tPA) present in the early stage after vascular damage so, measurement of t-PA is a reflection for increased activity of the fibrinolytic system and can potentially be useful as an early predicator marker for the measure of activated coagulation in diabetic patients with asymptomatic lower extremity arterial disease (LEAD) . In our study there was no significant difference between NON LEAD and LEAD in type 1DM (groupII) in levels of t-PA. Otherwise, in type 2DM (group III) levels of t-PA were significantly increase in LEAD compared to NON LEAD These results were explained by a compensatory mechanism to sustain circulation that present in the early progression of the disease when the endothelium still is a major source of t-PA so, the t-PA secretion increase in the early stage of vascular insufficient in type 2 diabetes. Although this mechanism present in type1DM but the difference in the results can be explained by the hypothesis that provide the deterioration of this mechanisms by the long duration.

There is correlation between tissue plasminogen activator and glycosylated hemoglobin in type 2 DM; these results reveal that in uncontrolled type 2 DM there was an increase in incidence of lower extremity arterial disease.

In conclusion, measurement of tissue plasminogen activator can potentially be useful as an early marker for prevention of vascular complications before they become clinically evident.

We recommended that tissue plasminogen activator (t-PA) can be used as an early marker for diagnosis and follow up in diabetic patients. More studies on large numbers of patients are needed to confirm these results. Also, further studies should be done as PAI-1 and factor VII in diabetic patients.

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Evaluation of Cystatin C, Fibronectin and Alpha-Feto Protein as Biochemical Markers in Patients with Liver Diseases

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Abstract: Objective: We investigate the serum level of cystatin C as biochemical marker that can determine the severity of tissue injury resulting from chronic liver diseases. Also we investigate other two parameters - alph-fetoprotein & fibronectin- serum levels as biomarkers of chronic liver disease progression. **Study design:** This study was carried out on 66 patients (50 males & 16 female). We assessed serum levels of cystatin C, alpha-fetoprtotein, albumin,ALT,AST,creatinine, urea, plasma level of fibronectin and HCV genotype. **Results:** We found that (a positive & a negative) correlations between serum levels of cyctatin C and plasma level of fibronectin (respectively) and progression of liver diseases. Also we found that a positive correlation between plasma level of fibronectin and both serum level of albumin and prothrombin time. We found a positive correlation between serum level of alpha-fetoprotein and liver bilharzial hepatic fibrosis. In addition we found that HCV genotype 4 was the prevalent type in Egyptian patients. **Conclusion:** Serum levels of cystatin C, alpha-fetoprotein and plasma level of fibronectin can be useful markers in long-term monitoring of the progression of liver diseases.

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Keywords: Evaluation; Cystatin C, Fibronectin; Alpha-Feto Protein; Marker; Patients

1. Introduction

Cystatins are endogenous inhibitors of lysosomal cysteine proteinases, such as cathepsin, L, H, and S **(Zahran etal., 2007).** Cystatin C, belonging to the type II Cystatin gene superfamily, is the most abundant extracellular inhibitor of cysteine proteinase (**Merz et al., 1997**). Mature cystatin C is a 13 kD, positively charged, secreted protein composed of 120-122 amino acids expressed by many cell types. It is present in large quantities in cerebrospinal fluid, seminal plasma, serum and other body fluids **(Newman, 2002)**. One of the most prominent functions of cystatin C is related to the inactivation of the cathepsin family members of cysteine proteinase, which are involved in the cleavage of membrane and extracellular matrix proteins among others and thus is disease-related tissue remodeling. The diagnostic value and prognostic significance of cystatin C have been reported for several diseases **(Koeing et al., 2005)**. The correlations have been found between cystatin C expression, mutations, and clinical status of patients with cerebral amyloid angiopathy, hereditary brain hemorrhage **(Palsdottir et al., 2006, Levy et al., 2006)**, atherosclerosis, aortic aneurysms **(Shi et al., 1999, Mares et al., 2003)** and macular degeneration **(Zurdel et al., 2002).** Diseases of the liver have been an important health issue since severe hepatic diseases could lead to persistent inflammation and necrosis, and even liver cirrhosis and hepatoma. Thus indicators for the long- term monitoring on the progress of hepatitis are of great clinical importance. Liver fibrosis has been shown to be a result from an imbalance between degradation and synthesis of extracellular matrix. Cysteine proteinases and metalloproteinases, have been found to be involved in the degradation of extracellular matrix **(Chu et al., 2004)**. Infections caused by specific hepatitis C virus (HCV) genotypes, such as 1a and 1b, are more refractory to antiviral therapy than those caused by other genotypes **(Layden et al., 2002)**. For this reason, HCV genotyping has become a critical component of the standard of care of HCV-infected patients **(Richter, 2002).**

2. Patients and Methods

Patients:

Our study was carried out on 86 individuals (patients& healthy). 66 patients (50 males & 16 females) were selected from

outpatient and inpatient clinic of gastroenterology & hepatology department at Al-Azhar university hospital (Assiut branch).Their ages ranged from 31 – 65 years. They are classified as follows;

Group I: 28 patients with chronic viral hepatitis (HBV: 7 patients& HCV: 21 patients). Group II: 27 patients with bilharzial hepatic fibrosis. Group III: 11 patients with primary Hepatocellular carcinoma.

Group IV: 20 healthy subjects as a control group Patients were subjected to the following clinical examination and laboratory investigations: Complete history taking, full clinical examination including liver and spleen in details, routine laboratory investigations; (urine analysis, stool analysis and complete blood picture), sigmoidoscopy and rectal snip biopsy with fresh examination for detection of Schistosoma Mansoni ova for group II

Abdominal ultrasonography, ultrasonography - guided liver biopsy for group III, HBV markers (HBsAg & H B e A g) for all groups to exclude HBV infection in other groups and HCV-Ab.).

Sample collection:

Blood samples were collected from all participant by vein puncture under complete aseptic conditions and divided into three parts: First part was mixed with sodium citrate (9 parts of fresh blood+ 1 part sodium citrate). Plasma was separated by centrifugation and transferred to a clean tube. Plasma was tested for fibronectin and PT.

Second part was mixed with ethylenediamine tetra acetic acid (EDTA) (2 mg/ml blood). Plasma was separated by centrifugation and transferred to a clean tube. Plasma was tested for HCV genotyping (real time PCR). Third part was left to be clotted and serum was separated by centrifugation. Serum was divided into separate aliquots and stored at- 70°C until used for cystatin C, AFP, ALT, AST, albumin, blood urea and creatinine, determination.

Methods:

●Measurement of serum cystatin C concentration

Cystatin in serum samples was quantified using a human cystatin C ELISA kit supplied by(BioVendor, Czech Republic) following manufacturer's instructions. Human cystatin standards were provided in the kit (200-10,000 ng/mL), and test serum samples were diluted 1:400 in the dilution buffer supplied. One hundred microliter aliquots of the diluted standards and test samples were added in duplicate to the wells of a microtiter plate coated with antihuman cystatin C antibody. Dilution buffer alone was added to a pair of duplicate wells to serve as blank. After incubation at room temperature for 30 min on an orbital shaker, the plate was washed thrice with the wash solution and 100 µL of antihuman cystatin antibody labeled with horseradish peroxidase was added to the wells.

The plate was incubated for 90 min at room temperature followed by washes as before and addition of 100 µL of substrate solution containing hydrogen peroxide and tetramethylbenzidine to the wells.

The plate was covered with aluminum foil to protect from light and incubated for 10 min to allow for color development. The reaction was stopped by the addition of 100 µL of stop solution, and the optical densities were determined by reading absorbance at 450 nm. A standard curve of concentration of cystatin versus absorbance was plotted using the four-variable function, and the test values were derived from the measured absorbance using this curve (fig.1). **(Tian et al., 1997).**

●HCV typing:

Serum samples were obtained from 21 patients chronically infected with HCV, genotype-specific primers were designed to detect nine genotype 1a, 1b, 1d, 2a, 2b, 3a, 3b, 4, 5a, or 6a. As there were nine different subtypes that we tried to detect, the detection primers are divided into two different types on the basis of differences in the sizes of the different bands, so that no genotype-specific bands are of similar molecular size in the same gel. All samples were analyzed twice by PCR with either mix 1 or mix 2. Mix 1 will allow for the specific detection of PCR products for HCV genotypes 1b, 2a, 2b, and 3b. Mix 2 will allow for the specific detection of HCV genotypes 1a, 3a, 4, 5a, and 6a. The detection of genotype-specific products in mix 1 and mix 2 was designed so that the differences in the sizes of PCR products could be evaluated on gels easily (fig.2). In the analysis of results, one should look for the strong specific bands seen on either gel. Faint bands are nonspecific, may be generated through weak priming. **(Ohno, et al., 1997)**

●Serum alpha-fetoprotein concentration (AFP)

Serum alpha-fetoprotein concentration (AFP) was measured by ELISA (Engall et al., 1980) using human AFP ELISA kit supplied by (Europa Bioproduct Ltd, United Kingdom). The serum AFP was determined from calibration curve (fig.3).

●Plasma fibronectin concentration

Plasma fibronectin concentration was

measured by ELISA (Ruoslahiti et al., 1981) using human fibronectin kit supplied by (MyBiosource, USA). The plasma fibronectin was determined from calibration curve (fig.4).

●Serum albumin concentration

Serum albumin concentration was estimated colorimetrically by bromocresol green (Doumas et al., 1971) using kit supplied by (Spectrum Diagnostics, Egypt)

●Serum ALT and AST concentration

Serum ALT and AST concentration were estimated by colorimetric method (Reitman and frankel, 1957) using kit supplied by (Spectrum diagnostics, Egypt)

●Serum of creatinine concentration

Serum of creatinine concentration was determined by kinetic colorimetric method (Jaffe', 1986) by using kit supplied by (human

●Serum of urea concentration

Serum of urea concentration was determined by enzymatic colorimetric method (Rock, et al.,.1987) using kit supplied by (Vitro-Diagnostic

●Determination of Prothrombin Time (PT) and International Normalized Ratio (INR):

Tissue thromboplastin, in the presence of calcium ions, is an activator which initiates the extrinsic pathway of coagulation. When a mixture of tissue thromboplastin and calcium ions is added to normal anticoagulated plasma, the clotting mechanism is initiated leading to formation of a fibrin clot. If a deficiency exists within the extrinsic pathway, the time required for clot formation will be prolonged depending on the severity of the deficiency **(Hull et al., 1982).**

Statistical Analysis:

Statistical analysis was performed using SPSS-16 for windows.All results are expressed as mean ± standard deviation (SD), median values and ranges. Data were analyzed by one- way analysis of variance in addition to the student –Newman post-hoc test. Coefficient correlation was evaluated using Pearson correlation. Statistical significance was established at $p < 0.05$.

3. Results:

As regard to cystatin C serum levels (mg/L) the results revealed high statistically significant difference in the serum cystatin C levels between the control, chronic hepatitis, H.bil.F and HCC groups ($p < 0.001$ for all, table 1, fig. 5). Also serum cystatin C has a positive correlation with ALT serum levels (U/l) in chronic hepatitis group ($r= 0.74$, $P<0.01$, fig.6), hepatic bilharzial fibrosis group ($r=0.64$, $P<0.01$, fig.7) and in HCC group ($r=0.8$, $P<0.01$, fig.8). There are significant increase in serum cystatin C levels in control and three classes of different severity of hepatic disease based on serum level of ALT **(Chu et al., 2004).** Where class that has ($ALT\leq35$ U/l) considered mild, that has ($35<ALT<105$ U/l) considered moderate and that has ($ALT \geq105$ U/l) considered severe hepatic diseases respectively, based on three times reference value (35U/l) .The results revealed high statistically significant increase in the serum cystatin C levels between the control and three classes ($P<0.01$ for all, tabl.2,fig.9).There are a significant increase in fibronectin plasma levels (µg/ml) in chronic viral hepatitis, and hepatocellular carcinoma groups when compared with cntrols ($P<0.01$ for all table 3, fig. 10). There are a significant increase in the serum alpha -feto protein (AFP) levels in chronic viral hepatitis, hepatic bilharzial fibrosis and hepatocellular carcinoma groups when compared with controls ($P <0.01$ for both, table 4, fig.11).

AST and ALT serum levels revealed statistically very high significant increase in all groups when compared with controls ($P<0.0001$ for all, table 5). Serum albumin levels revealed statistically high significant increase in hepatic bilharzial fibrosis and hepatocellular carcinoma groups ($P<0.001$ for both, table 5). But in chronic viral hepatitis group there are no significant differences. Plasma prothrombin times revealed very high significant increase in hepatic bilharzial fibrosis and hepatic cellular carcinoma groups ($P< 0.0001$ ror both, table 5), but insignificant differences in chronic viral hepatitis group. Percentage of each genotype of HCV. Type 4 represents most cases infected with HCV (90.4 %) while type 1 only represents (4.8 %) of cases.

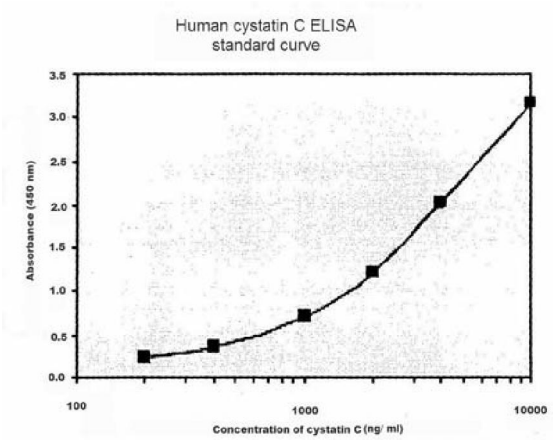

Fig. 1: Human cystatin C ELISA standard curve.

Table (1): Comparison between serum cystatin C levels in control versus chronic viral hepatitis, hepatic bilharzial fibrosis and hepatocellular carcinoma groups.

	Controls	CVH	HBF	HCC
Cysatin C (mg/L) ± S.D	0.66± 0.018	0.8±0.11	0.82±0.12	0.89±0.12
P value		P<0.001	P<0.001	P<0.001
Significance		H.S	H.S	H.S
N.	20	28	27	11

H.bil.F= hepatic bilharzial fibrosis, H.S= high significant, HCC=hepatocellular carcinoma; N= number of cases.

Table (2): Comparison between cystatin C and the severity of liver diseases, patients were regrouped into three groups, mild (ALT≤35 U/L), moderate(35 <ALT < 105) and severe (ALT≥105), hepatic diseases respectively based on an increase more than three times reference value of ALT serum level (U/I).

	Controls	Mild H.D (ALT<35 U/I)	Moderate H.D (35<ALT<105 U/I)	Severe H.D (ALT > 105 u/I)
Cysatin C (mg/L) ± S.D	0.66± 0.018	0.76±0.09	0.87±0.09	1.02±0.08
P value		P<0.01	P<0.01	P<0.01
Significance		H.S	H.S	H.S
N.	20	30	28	10

H.D= hepatic disease, H.S= high significance, N.= number of cases.

Table (3): Comparison between plasma fibronectin levels (µg/ml) in control versus chronic viral hepatitis, hepatic bilharzial fibrosis and hepatocellular carcinoma groups.

	Controls	CVH	HBF	HCC
Fibronectin (µg/ml) ± SD	398.2±57.06	371.8±68.6	234.4±122	322.6±80.57
P value		P<0.01	P<0.01	P<0.01
Significance		H.S	H.S	H.S
N.	20	28	27	11

H.bil.F= hepatic bilharzial fibrosis, H.S= high significant, HCC=hepatocellular carcinoma, N= number of cases.

Table (4): Comparison between serum alpha-fetoprotein levels (ng/ml) in controls versus chronic viral hepatitis, hepatic bilharzial fibrosis and hepatocellular carcinoma groups.

	Controls	CVH	HBF	HCC
AFP (ng/ml) ± SD	2.23 ± 1.05	2.47 ± 1.7	41.7 ± 17	174.9 ± 92.4
P value		0.599	< 0.001	< 0.001
Significance		N.S	H.S	H.S
N.	20	28	27	11

AFP= alpha-fetoprotein, CHV=chronic viral hepatitis, HBF= hepatic bilharzial fibrosis, H.S= high significant, HCC=hepatocellular carcinoma, N= number of cases, NS= non-significant.

Table (5): Comparison between serum ALT, AST, creatinine, urea, albumin and prothrombin time in controls versus chronic viral hepatitis, hepatic bilharzial fibrosis and hepatocellular carcinoma groups.

parametrs	Controls	CVH	P value	HBF	P value	HCC	P value
ALT(U/I)± SD	14.6±3.9	50±25.9	<0.0001 VHS	37.9±21.6	<0.0001 VHS	39.4±24.1	<0.0001 VHS
AST(U/I)± SD	21.3±3.8	64.3±31.9	<0.0001 VHS	48.8±24.3	<0.0001 VHS	50.3±24	<0.0001 VHS
Creatinine (mg/dl)±SD	0.76±0.09	0.81±0.13	0.420 NS	0.79±0.1	0.420 NS	0.76±0.12	0.601 NS
Urea (mg/dl)±SD	15.6±1.1	16.3±1.5	0.0970 NS	14.8±2.4	0.1618 NS	14.95±0.47	0.1694 NS
Albumin (g/L) ± SD	4.19±0.24	4.1±0.16	0.2063 NS	3±0.41	<0.001 HS	2.9±0.36	<0.001 HS
PT(sec.)	12.26±0.9	12.61±1.31	0.3021 NS	18.8±4.1	<0.0001 VHS	19±1.78	<0.0001 VHS

CHV=chronic viral hepatitis, HBF= hepatic bilharzial fibrosis, HS= high significant, HCC=hepatocellular CHV=chronic viral hepatitis, HBF= hepatic bilharzial fibrosis, HS= high significant, HCC=hepatocellular carcinoma, N= number of cases, NS= non- significant, VHS=very high significant, ALT=alanine transferase, AST= aspartate transferase, sec= seconds.

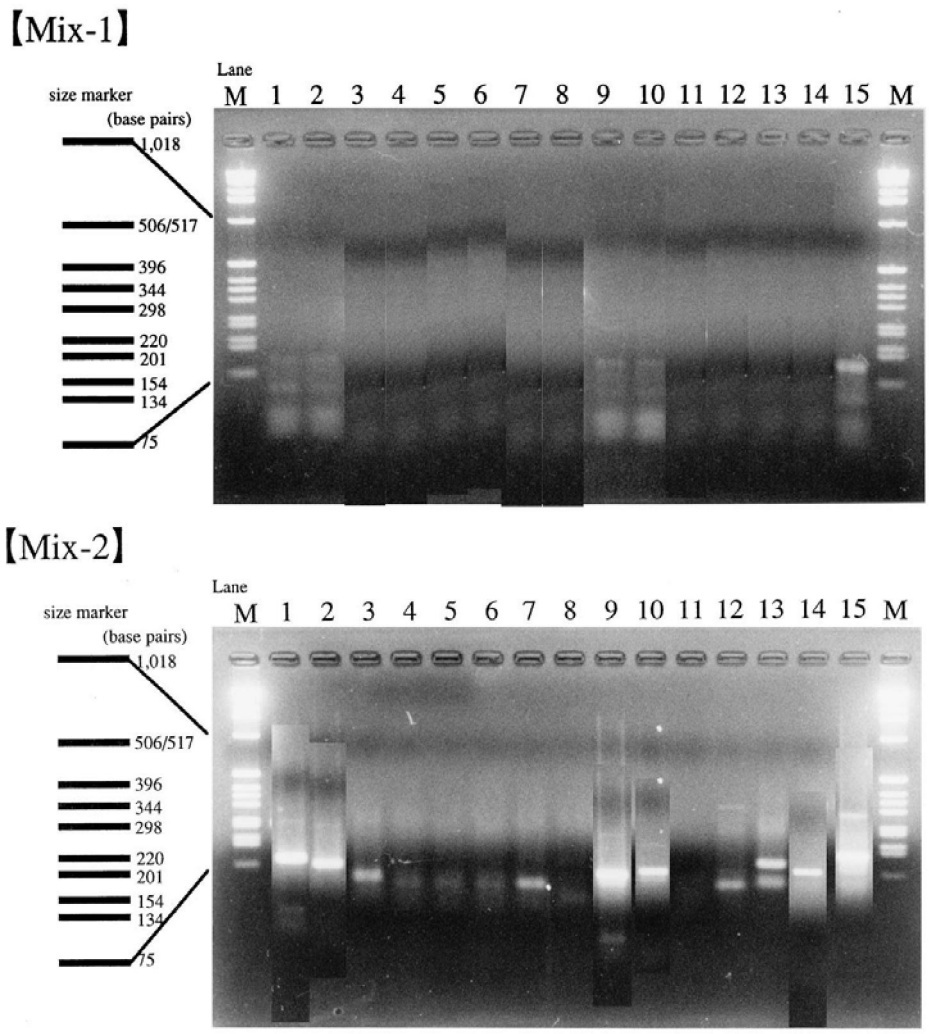

Fig. 2: Typical electrophoresis patterns of PCR products from 15 cases. All samples were analyzed twice by PCR with either mix 1 or mix 2. Mix 1 will allow for the specific detection of HCV genotypes 1b, 2a, 2b, and 3b.Mix 2 will allow for the specific detection of HCV genotypes 1a, 3a, 4, 5a, and 6a. The figure shows that fifteen cases give no specific bands in mix 1(except one at lane 15 give a specificband for 1b genotype, but fourteen cases give in amix2 a specific bands for 4 genotype.

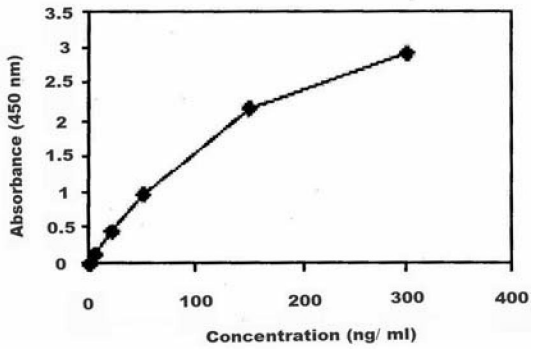

Fig. 3: Alpha-fetoprotein (ng/ml) standard curve.

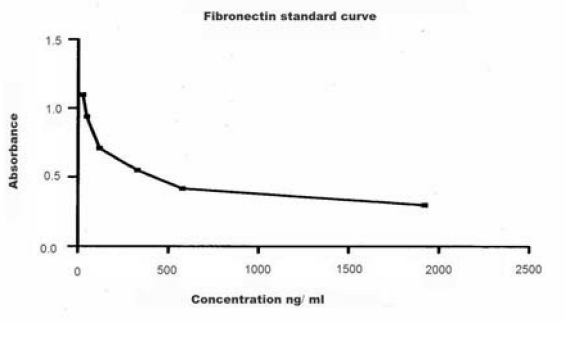

Fig.4: Fibronectin (ng/ml) standard curve.

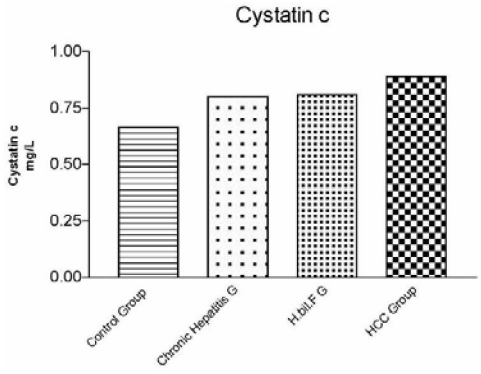

Fig. 5: Comparison of cystatin c levels (mg/L) incntrols and diseased groups.

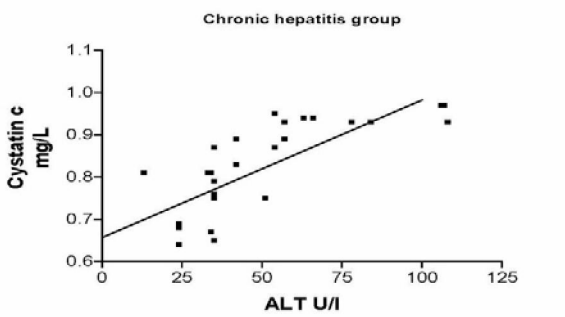

Fig.6: Positive correlation between serum cystatin C and ALT inchronic hepatitis (r=0.74, P<0.001)

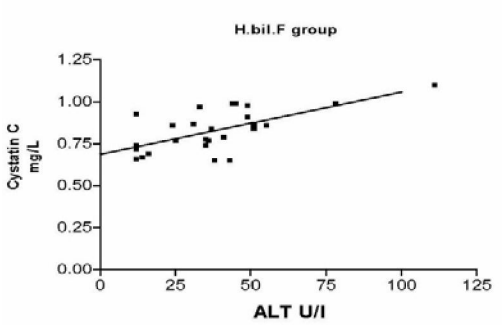

Fig.7: Positive correlation between serum cystatin C and ALT in hepatic bilharzial fibrosis (r=0.64, P<0.01)

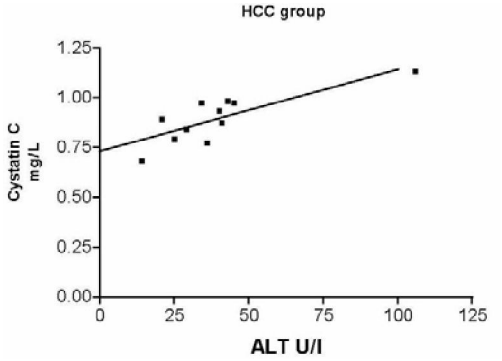

Fig.8: Positive correlation between serum cystatin C and ALT in hepatocellular carcinoma (r=0.8, P<0.01)

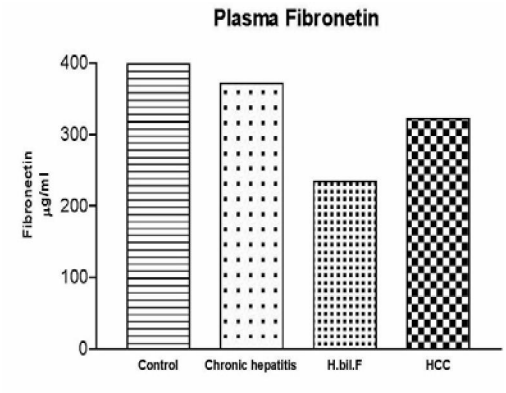

Fig.9: comparison between serum cystatin c levels In controls three different severity of hpatic disease diseased groups (accordin to ALT serum levels)

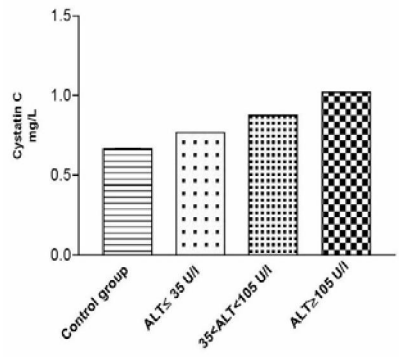

Fig.10: Comparison between plasma levels of fibronectin in controls and

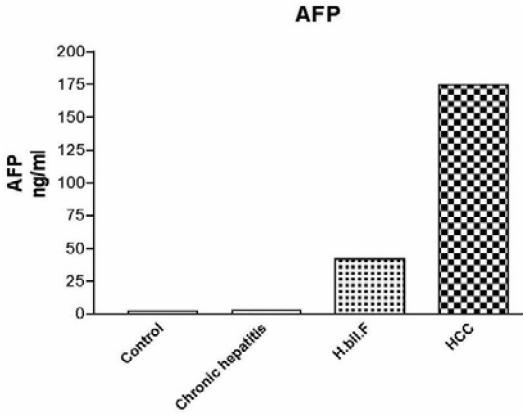

Fig.11: Comparison between serum levels of alpha-fetoprotein In controls and diseased groups

4. Discussion

A further highly relevant function of cystatin C has been recently reported, which is concentrated on the inhibitory effect on transforming growth factor-β (TGF-β)-Signal transduction in normal and malignant cells **(Sokol and Schiemann, 2004).** In vitro, TGF-β1 is a potent inducer of cystatin C secretion in vascular smooth muscle cells **(Shi et al., 1999).** Furthermore, expression of cystatin C mRNA in astrocyte precursor cells is directly linked to the activity of TGF-β **(Solem et al., 1990).** TGF-β is known to be the fibrogenic master cytokine in human due to its ability to stimulate the expression and inhibition of degradation of extracellular matrix (ECM) proteins and to promote the trans-differentiation of profibrogenic hepatic stellate cells (HSCs) to ECM-producing hepatic myofibroblasts (MFBs) **(Gressner et al., 2002).** The phenotypic and functional trans-differentiation of HSCs is initiated in acute and chronic inflammatory liver diseases, ultimately leading to organ fibrosis and cirrhosis **(Takeuchi et al., 2001).** Significantly elevated serum concentration of cystatin C have been recently described in chronic liver diseases showing a strong correlation between the degree of elevation and the severity of disease **(Chu et al., 2004).** Based on these new findings, we tried to figure out the potential function relevance of cystatin C in various liver diseases.

Diseases of the liver have been an important health issue since severe hepatic diseases could lead to persistent inflammation and necrosis, and even liver cirrhosis and hepatoma. Thus indicators for the long- term monitoring on the progress of hepatitis are of great clinical importance. Liver fibrosis has been shown to be a result from an imbalance between degradation and synthesis of extracellular matrix. Cysteine proteinases and metalloproteinases, have been found to be involved in the degradation of extracellular matrix **(Chu et al., 2004).** Since an increased activity of cystein proteinase in liver fibrosis may be accompanied by increased activity of cystatin C, a very potent inhibitor of lysosomal cysteine proteinases. So, in this study serum cystatin C concentration of 66 patients with liver diseases of various severities and 20 healthy controls were analyzed. Since renal diseases are common complications of hepatic diseases, we have eliminated the possibility of any renal disease complications by screening serum creatinine and blood urea nitrogen levels. Fourteen patients have been excluded from this study due to their abnormal kidney function tests.

In the present study, our results showed that the levels of serum cystatin C were statistically significant increased progressively with the progression of liver disease ($P<0.001$), in chronic viral hepatitis, hepaic bilharzial fibrosis hepatic bilharzial fibrosis and hepatocellular carcinoma groups in comparison with the control group. Our results also showed that serum cystatin C concentration was significantly higher in patients with liver fibrosis and hepatocellular carcinoma than in control or chronic hepatitis These results were in agreement with **Chu et al., (2004) and Chen et al., (2005)** who found that serum cystatin C concentrations significantly and progressively increased with the progression of liver disease and that is a potential marker for liver fibrosis.

ALT value is considered as a direct indicator for liver inflammation or fibrosis. To look at the correlation between cystatin C and the severity of liver diseases in our study, patients were regrouped into three groups, mild ($ALT \leq 35$ U/L), moderate ($35 < ALT < 105$) and severe ($ALT \geq 105$), hepatic diseases respectively based on a moderate increase or an increase more than three times reference value **(Chu et al., 2004).** We have found a positive correlation between serum cystatin C concentrations and the severity of liver diseases which determined by the increase in serum ALT ($P<0.01$). So, serum cystatin C was progressively increased with the progression increase of ALT. These findings were in agreement with **Chu et al., (2004)**. The previous results indicate that serum cystatin C concentration was closely related to the progression of liver diseases and chronic liver inflammation.

This study also was conducted on 28 Egyptian patients chronically infected with viral hepatitis; 75% with HCV and 25% with HBV. Chronically infected patients with HCV have been studied for HCV genotyping. HCV genotype 4 was detectable in 90.4 % of HCV patients. These reveals that the most prevalent genotype among Egyptian patients is type 4. These results were in agreement with **(Omran et al., 2009)** and **(Abdel-Hamid et al., 2007).** They

found that most prevalent type in Egyptian patients was type 4 (94.3%) and (95.9%) respectively.

Our data showed that plasma fibronectin concentrations were statistically significantly decreased in hepatic bilharzial fibrosis group (P<0.001) and non- significant decrease in HCC groups. Whereas, there was non-significant difference in chronic viral hepatitis group. These finding were in agreement with **Simon et al., (1995)** , **Jitoku et al., (1996)** and **Fortunato et al., (2001)** who found significant lower values {(P<0.001), (P<0.001) and (P<0.01) respectively} in liver cirrhosis and non-significant lower values in chronic hepatitis group. They concluded that the determination of plasma fibronectin is not important in evaluation of degree of liver fibrosis but it is one of the liver function tests. Whereas, **Grieco et al., (1998)** found significant decrease in plasma fibronectin only in liver cirrhosis group (P<0.001) while in contrast increased levels were associated with acute and chronic hepatitis group.

On the other hand, **Kandemir et al., (2004)** did not agree with our results as regards chronic hepatitis patients. In the present study we also examined the relation between plasma fibronectin and ALT levels as marker of liver inflammation and necrosis. Our data showed no correlation between plasma fibronectin concentrations and ALT levels (P>0.05). These finding was in agreement with **Kandemir et al., (2004)** who found also no correlation between plasma fibronectin concentrations and ALT levels.

Furthermore, we examined the correlation between plasma fibronectin and both of serum albumin concentrations and prothrombin time (PT) as all of them are synthesized in the liver. We found positive correlation between plasma fibronectin and serum albumin (P<0.001). On the other hand, we found negative correlation between plasma fibronectin and (PT) (P<0.001) (i.e., positive correlation with prothrombin concentration). This finding was also reported by **Ricciardi et al., (1987) and Gabrielli et al., (1986).** In contrast **Angelis et al., (1988)** found no correlation between plasma fibronectin and serum albumin in cirrhotic patients.

Other findings in this study, we found 10 cases have a fibronectin level less than 170 µg/ml, 8 of them have a past history of recurrent infection. Moreover, we found 6 patients have fibronectin level < 140 µg/ml and all of them have severe splenomegaly. These findings may indicate that the decrease in plasma fibronectin may result from its increased consumption in the hyperactive reticulo-endothelial system. Since fibronectin acts as opsonin in reticulo-endothelial system, it is not surprising that low levels of this glycoprotein are associated with recurrent infection.

Our previous results indicate that the significant decrease in plasma fibronectin in hepatic bilharzial fibrosis patients is either due to liver function impairment and/or its increased consumption by the hyperactive reticulo-endothelial system.

Our results showed statistically significant increase of serum AFP in hepatic bilharzial fibrosis and as expected in hepatocellular carcinoma groups in comparison with the control group (P<0.001). There was non-significant increase in chronic hepatitis group. So, the increase in AFP was positively related to disease progression with the highest being in hepatocellular carcinoma group (174.9± 92.4 ng/ml, P <0.001). These results were in consistent with **Chu et al., (2004)** and **Arrieta et al., (2007)**. But **Seifi and Bafandeh, (2006)** did not agree with our results as regards chronic hepatitis group only. They found significant difference between HCV positive group in comparison with the control group (P<0.05). On the other hand AFP was also increased in hepatic bilharzial fibrosis mean concentration (41.7 ng/ml). this indicates low specificity of AFP hence, there is a need for additional serum markers that will improve the detection of early hepatocellular carcinoma.

In coclusion serum cystatin C, plasma fibronectin and AFP were closely related to the progression of liver diseases and chronic liver inflammation. This suggests that the above markers may be useful to certain extent in monitoring liver function and follow up the progression of liver diseases. Besides, we need additional markers to improve early diagnosis of hepatocellular carcinoma hence improvement of prognosis.

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