CONTENTS

The Effective Curriculum Design Principles to Reduce the Nauseating Symptom in Training Whirling-kung

Chia-Shen Liao, Jian-Wei Rau*

The Department of Curriculum Design and Human Potentials Development, National Dong-Hwa University, Hualien 97065, Taiwan. rau@mail.ndhu.edu.tw

Abstract: Sufi-whirling is a famous spiritual practice in Sufism and is adopted as a physically active meditation by people intending to promote their physical and mental health in Taiwan, where the term "whirling-kung" is used instead of Sufi-whirling to avoid the religious connotation of Sufi. In this paper the authors intend to inquire the issue of how to design appropriate and effective curriculum for training whirling-kung in order to help learners reduce the symptom of nauseating and conquer the fear of whirling. Through a process of action research, the authors draw up two cruxes of whirling: choiceless awareness and centering. The authors also summarize 10 effective curriculum design principles in whirling-kung training to help the learners reduce the nauseating symptom in whirling-kung training courses: (1) Provide training and practice on "mind-body-scan meditation" before training whirling-kung. (2) Arrange the practice of meditation immediately after whirling, which constitutes a Whirling-Meditation unit (abbr. as WM unit in this paper). (3) Increase the length of WM unit gradually. (4) Arrange several WM units in one session if needed. (5) The break between training sessions should not be too long in order to keep the warmth of training. (6) The number of training sessions should be long enough to have training effects. (7) Adjust every learner's length of WM unit according to the overload training principle. (8) Select appropriate place for training. (9) Select appropriate music for training. (10) Try to arrange the whirling practice at least one hour after the meals.

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1. Introduction

The Sufi poet and mystic Jalalu'ddin Rumi [1] (1207 –1273) began the whirling dance and established the dervish order known as the Mevlevis. Whirling dance is widely called "Sufi whirling" (or Sufi spinning) and is still practiced by the Sufi Dervishes of the Mevlevi order. Nowadays Sufi whirling is worldwide distributed and has become a form of physically active meditation advocated by people intending to promote their physical and mental health.

In Taiwan, the term "whirling-kung" or "spinning meditation" is used instead of Sufi-whirling to avoid the religious connotation of Sufi. In this paper we use the term "whirling-kung". The term "kung" is a short form of the term "kung-fu", denoting the meaning of "skills" in Chinese. Therefore, "whirling-kung" emphasizes that it is a skill that can only be practiced and learned with patience and dedication through appropriately designed curriculum.

For the past few years, more and more people practice whirling-kung for the purpose of rehabilitation from illness in Taiwan. The most famous example is "Taiwan Cancer Friends New Life Association [2]" (TCFNLA). Since 2000, TCFNLA has incorporated whirling-kung training as the core of their curriculum to help cancer patients rehabilitate. Numerous cancer patients participating the TCFNLA's courses have witnessed and reported amazing rehabilitations from cancers after practicing whirling-kung for a period of time [2]. According to TCFNLA's claim:

It (whirling-kung) has both physical and mental benefits. On the physical side, it can improve our metabolism, strengthen the heart function and facilitate the blood circulation. On the mental side, it will enhance our internal awareness, mental detachment, and help with emotional cleansing. As the spinning takes place, the overall effect is to balance of our body and mind and bring our physical and mental well-being into alignment.

Although whirling has been practiced by many people since ancient times, it is still not common in countries other than those in the middle-east area, compared to common activities like walking, running or swimming etc. Without appropriate and sufficient whirling training, most people would get nauseating or vomiting, which in turn cause people to be afraid of whirling or to avoid of learning whirling totally. Under this circumstance, it has become an important challenge in terms of how to help learners go through a series of effective training sessions in order to gradually learn the skill of whirling and reduce the symptom of nauseating caused by whirling.

However, we also found that no relevant article or

literature can be found in terms of how to train whirling effectively and how to reduce the symptom of nauseating caused by whirling. It is also curious enough that no other researchers have explored this issue so far. Based on this background, in this paper the authors intend to inquire the issue of how to design appropriate and effective curriculum for training whirling-kung in order to help learners reduce the symptom of nauseating and conquer the fear of whirling.

2. Research method and process

The authors employed the research method of action research to develop the appropriate curriculum for training whirling-kung gradually. The first author of this paper established two whirling-kung training groups in successive semesters by calling together volunteers from a senior high school in eastern part of Taiwan, shown as the following (Table 1).

The second author has incorporated training whirling-kung as part of his undergraduate and graduate courses in five different classes since 2009 in a National University in eastern part of Taiwan, shown as the following (Table 2).

Both authors went through a similar cycling process of "plan-action-reflection" in typical action research. Based on the reflection of the experience of a previous group or course of training whirling-kung, the authors revise the curriculum design and implement the curriculum again in the successive groups or courses. Both authors share their experience in the process of training whirling-kung in their groups or courses and provide feedbackup to each other. Through this process, we improve our curriculum design gradually and draw up the conclusions as presented below.

3. Research results and discussion

Based on the process of action research as described above, the authors have found the cruxes of whirling in order to avoid nauseating and the effective curriculum design principles to reduce the nauseating symptom in training whirling-kung. Due to the limitations of the paper, we present the research results in this secion without presenting the process of obtaing the research results.

3.1 The fundamental methods of whirling

In this paper, whirling-kung is considered as a kind of physical and mental training and the person who practices whirling-kung is called "whirler". The fundamental method of whirling is defined as: the whirler stands his or her feet in upright position and move the feet continuously so as to revolve the body in clockwise or counter-clockwise directions at certain speed of whirling for a certain period of time. Summarized from the authors' experience of training whirling-kung, there are six possible modes of whirling regarding the way of moving feet, shown as Table 3.

Moreover, based on our preliminary experience, in order to have the effects of practicing whirling-kung, the period of whirling time is at least five minutes and the speed of whirling is at least 30 turns per minute approximately. If the duration of whirling is too short or the speed of whirling is too slow, it might not be considered as practicing whirling-kung. Otherwise, the activity might be just called "walking" instead. However, it is still an issue that awaits future research to resolve in terms of how long or how fast of whirling is sufficient to have the effects of whirling-kung.

During the process of whirling, the whirler could also move his or her feet to different locations by different routes. There is also no limitation in terms of the postures or movements of both hands. However, in this research, the whirler usually whirled continuously at the same location with simple hand postures.

There are two ways of stopping whirling: (1) The whirler slows down the whirling gradually until totally standing still. (2) The whirler stops whirling and stands still instantly and waits for the surrounding revolving view gradually slows down until the view stops completely.

3.2 The cruxes of whirling to avoid nauseating

In the curriculum for training whirling-kung, besides the fundamental methods of whirling, the most important task for the teacher is to guide the learners to grasp the cruxes of whirling in order to gradually reduce the symptom of nauseating caused by whirling gradually until the nauseating symptom completely diminish. Based on the results of this research, there are two cruxes of whirling: "choiceless awareness" and "centering", as explicated below:

3.2.1The first crux of whirling: choiceless awareness

The first crux of whirling is "choiceless awareness", which is a phrase adopted from a famous spiritual leader Jiddu Krishnamurti [3] to emphasize that "our mind becomes absolutely calm and relaxed and we are fully aware of the moment yet our awareness is not focused on any physical or mental image/object [4]". In the context of whirling, "choiceless awareness" means that the whirler should not focus his or her vision on any particular objects in the surrounding and allow the sight to be flowing and blurry. If the whirler focuses his vision on any particular objects, it is more likely to cause nauseating. The whirler just needs to open up his or

her awareness in a choiceless manner and roughly pay attention to his or her own position relative to others and the surrounding objects in order to avoid bumping.

Table 1 The whirling-kung training groups of the first author

Semester	No. of Students	The students' age level	Time spent in training whirling-kung
2010 Fall	30	Senior High School	12 weeks, 2 sessions per week, from 5 min. to 30 min. in each session
2011 Spring	12	Senior High School	8 weeks, 3 sessions per week, from 5 min. to 30 min. in each session
Total	42		

Table 2 The whirling-kung training courses of the second author

Semesters	No. of Students	Course Level	Time spent in training whirling-kung
2009 Spring	30	Undergraduate's course	14 weeks, from 5 min. to 30 min. each week
2009 Fall	29	Undergraduate's course	12 weeks, from 5 min. to 30 min. each week
2010 Spring	17	Master's course	11 weeks, from 5 min. to 30 min. each week
2010 Fall	21	Undergraduate's course	12 weeks, from 5 min. to 30 min. each week
2010 Fall	32	Master's course	11 weeks, from 5 min. to 30 min. each week
Total	129		

Table 3 The six possible modes of whirling in whirling-kung

The axis of whirling / Direction of whirling	Right foot		Body
Clockwise	Use the right foot as the axis and move the left foot forward so as to create clockwise whirling.	Use the left foot as the axis and move the right foot backward so as to create clockwise whirling.	Use the body as the axis and move the left foot backward and the right foot forward at the same time so as to create clockwise whirling.
counter-clockwise	Use the right foot as the axis and move the left foot backward so as to create counter-clockwise whirling.	Use the left foot as the axis and move the right foot forward so as to create counter-clockwise whirling.	Use the body as the axis and move the left foot forward and the right foot backward at the same time so as to create counter-clockwise whirling.

If the whirler could not grasp the crux of "choiceless awareness" while whirling, she or he could also raise one hand in front of the chest and focus on viewing the palm and roughly pay attention to his or her own position relative to others and the surrounding objects in order to avoid bumping. After a certain amount of whirling experience, a whirler might begin to get used to the blurry view while whirling and could try to break away gradually from the reliance on viewing the palm.

3.2.2 The second crux of whirling: centering

The second crux of whirling is "centering", which is a key notion of many meditation techniques [5, 6] to emphasize that meditators focus their attention whole-heartedly on their own feelings, especially the feeling of heart. In the context of whirling, "centering" means that, in the whole process of whirling, the whirler would try to focus his or her attention completely on his or her own physical or mental feelings and stays at the center of stillness within a whirlpool of various sights and body experiences. This is similar to putting oneself in a "typhoon eye" where there is no wind or rain at all. While centering oneself, a whirler might also need to stick to the crux of choiceless awareness at the same time.

In other words, the two cruxes of whirling should be combined together. A whirler should be choicelessly aware of every phenomenon happened in the body, mind and surrounding, and completely

accept all the phenomena without being influenced by them. In this manner, a whirler could stay calm in the center without caring too much about how good or bad one is doing, how good or bad others are doing, etc. This is the key to avoid the nauseating symptom. Even for someone who gets nauseating easily, by grasping both the crux of choiceless awareness and centering, a whirler could gradually conquer the nauseating problem caused by whirling.

Even if the whirler feels nauseating during the process of whirling, he or she still needs to stay calm and feel it without any rejection about the feeling. The nauseating feeling would gradually diminish and vanish eventually. Only when the nauseating feeling stay strong and consistent, the whirler is advised to sit down and meditate on the feeling by using the "body-scanning" technique as explained in the next section until the nauseating feeling diminish totally. This is the key to conquer the nauseating problem caused by whirling.

3.3 The curriculum design principles for training whirling-kung

Besides helping the learners grasp the previous two cruxes of whirling, the authors summarize a few curriculum design principles for training whirling-kung in this section based on the experience in training whirling-kung in our training groups and courses.

3.3.1 Provide training and practice on "mind-body-scan meditation" before training whirling-kung

As explicated above, in any whirling-kung training curriculum, it is the first and most important task to help the learners conquer the nauseating symptom. In order to attain this objective, the teacher had better provide the learners with a certain amount of practice on "mind-body-scan meditation", which is a mindfulness-based meditation [7, 8, 9] and is also known as "vipassana meditation" [10] originally delivered by the Buddha and practiced by many people currently and world-widely. The procedure of "mind-body-scan meditation" is briefly described as follow:

(1) Close the eyes and sis still or lie down in any comfortable position.
(2) Carefully scan the body and mind to find out any physical or mental feeling.
(3) Observe and accept any feeling found without any judgment for a while. Whenever an uncomfortable feeling (including nauseating) is found, feel it and accept it mindfully.
(4) Continuously scan and accept any feeling throughout the body and mind in any direction or sequence.

By this manner, any uncomfortable feeling could be diminishing gradually. The mind-body-scan meditation could be arranged at the beginning stage of the whirling-kung training curriculum at least 10 minutes a time for several times. If the learners acquire this meditation skill, they can apply it to eliminate any nauseating feeling during the process or at the end of practicing whirling-kung. By practicing whirling-kung together with the mind-body-scan meditation again and again, most of the learners would overcome the nauseating symptom gradually.

3.3.2 Arrange the practice of meditation immediately after whirling

In whirling-kung training curriculum, the teacher had better arrange certain amount of time for the practice of meditation immediately after one session of practice whirling. The combination of "whirling + meditation" is named as a "WM unit" in this paper.

Based on the findings from the authors' whirling-kung training courses, in any WM unit, the practice of meditation is extremely important. Not only could it help the learners conquer the nauseating symptom, but also increase the depth of meditation, which is beneficial to the physical and mental health of the learners.

For any WM unit shorter than 20 minutes, the length of time for practicing whirling and meditation is approximately the same. For example, 2 minutes of whirling could be followed by 2 minutes of meditation; 5 minutes of whirling could be followed by 5 minutes of meditation, etc. For any WM unit longer than 20 minutes, 10 minutes of meditation practice is enough for every session of whirling practice. Of course, within a WM unit, the necessary length of meditation practice still depends on the amount of time available and the individual needs. Therefore, the teacher should adjust the length of meditation according to the specific situation.

3.3.3 Increase the length of WM unit gradually

According to the experience of the authors, the teacher had better increase the length of WM unit gradually in designing any whirling-kung training curriculum. In other words, at the early stage of whirling-kung training, the length of WM unit should not be too long. For example, at the beginning, one WM unit might start from 6 minutes (i.e. 3 min. whirling + 3 min. meditation). After several sessions of training, the WM unit could be increased to 8 minutes, 10 minutes, 12 minutes gradually, with an increment of 2 minutes each time. As the learners gain more and more experience in whirling, the increment of WM unit could be enlarged to 8 or 10 minutes Of course, some learners might progress faster than others and the increment of WM unit could be larger than others. In other words, both the

length of WM unit and the increment of WM unit depend on individual learner's situation. In general, the teacher would increase the length of WM unit slowly enough so as to allow the learners adapt to the feeling of whirling gradually.

There are many possible ways of increasing the length of WM unit gradually, shown as the following Table 4 .

In the Table 4, "5W+5M" means 5 minutes of whirling and 5 minutes of meditation, and so on. The authors of this paper mainly adopted the arrangement in Ex.1, starting from 5 minutes whirling and increasing the length of whirling gradually with an increment of 5 minutes each time. For most of the learners, this kind of progress is slow enough. However, for a few learners, the progress might be too fast. In that case, the teacher might adopt the arrangement of training similar to Ex. 2 or Ex.3 in the Table 4.

3.3.4 Arrange several WM units in one session if needed

At the early stage of whirling-kung training curriculum, some learners might not be able to adapt to whirling quickly enough and feel nauseating easily. The teacher might arrange several short WM units in one session and insert some time for discussion, which allows the learners to ask questions or share their experience in whirling, as shown in the following Table 5.

In Ex.3 of the above table, in the first session of training, the teacher arranges three short WM units (with 4, 4, 6 minutes in each unit) and inserts 5 minutes discussion between two WM units. In the second session of training, the teacher arranges three WM units with 20 minutes in each unit and inserts 5 minutes discussion between two WM units. At the beginning of training course, if one WM unit is too long, it might look too intimidating to them. The major purpose of arranging training sessions in this way is to help those learners who get nauseating easily to decrease the fear of long duration of whirling and provide them with more and more confidences in whirling. The insertion of discussion between two WM units could also provide the learners with more time to take a rest and clarify their doubt or anxiety about whirling. For those who do not get nauseating in whirling, of course, it is not necessary to arrange several WM units in one session and the teacher might just arrange the training sessions as shown in Table 4.

3.3.5 The break between training sessions should not be too long in order to keep the warmth of training

In this paper, a period of time of training is called one "session". The duration of one session might be as short as 15 minutes or as long as two hours. The break between two sessions might be as short as 10 minutes or as long as one week. For training whirling-kung, if one session is longer than one hour, it might be too long for the learners, especially for the beginners. Based on our experience in whirling-kung, the most appropriate duration of one session is one hour.

If the break between two sessions is too short, it might be too laborious for the learners and the learners' body might not be able to adapt to the whirling. If the break between two sessions is longer than two days, the learners might lose the warmth of learning. Therefore, the most appropriate break between two training sessions is about one or two days (i.e. one session every two-day).

3.3.6 The number of training sessions should be long enough to have training effects

Most learners could not learn whirling-kung through just a few sessions of training, except for those who naturally do not get nauseating from whirling. In other words, the number of training sessions should be long enough to have training effects. In this paper, a series of training sessions is called a "module". In order to have training effects, the number of training sessions of a module is at least approximately 24. If the number of sessions of a module is too short, some learners might not be able to master the skill of whirling. For example, if a module of training consists of 24 sessions, the module could be arranged as 3 sessions a week for 8 weeks (with one hour per session).

Of course, this is only an approximate and temporary conclusion based on our limited experience so far. The most appropriate number of training sessions per module and the most appropriate interval between two sessions depend on many factors. After all, an excellent curriculum design for training whirling-kung involves various factors related to the learners like the age, the condition of physical and mental health, the attitude and motivation toward whirling, etc. All of these factors await further rigorous research in the future.

3.3.7 Adjust every learner's length of WM unit according to the overload training principle

The overload training principle is a principle that says that "To make progress in bodybuilding training, you need to progressively increase the intensity of your workouts [11]." For example, if one can lift 50kgs easily, it is useless to require him to practice lifting any weight under 50kgs. He needs to practice lifting a little bit heavier than 50kgs. Our bodies are designed to adapt to new conditions or stimuli. Through repeated overload training, our physical tissues or organs could gradually adapt themselves as required in the training [12].

According to the authors' experience, the

learners' whirling capabilities at the beginning of training vary quite a lot. Therefore, the teacher should adjust every learner's length of WM unit at the starting point according to the overload training principle and the learner's capability.

In his second training group, the first author of this paper encountered a learner who was capable of whirling for more than one hour without any nauseating feeling. It was definitely unnecessary to arrange a 15-minute session of whirling practice for her.

In whirling-kung training course, the first session of training is very important for the teacher. Based on the learners' conditions of whirling practice, the teacher could appraise every student's initial whirling capability and arrange ability grouping. In the coming training sessions, the teacher could arrange different lengths of WM units and different increments of WM units for different ability groups.

Moreover, the teacher needs to make a distinction between those who do not get nauseating while whirling and those who get nauseating while whirling but are not afraid of whirling. For the former, the length of WM unit and the increment of WM unit could be longer than the latter at the beginning of training. For the latter and those who are afraid of whirling, the teacher could start with a 4 or 6 minutes' WM unit at the beginning and increment the WM unit by 2 minutes a time. These are some examples of appropriate curriculum designs for whirling-kung training according to overload training principle.

3.3.8 Select appropriate place for training

In whirling-kung training courses, the selection of place for training is also rather important. Based on our experience, it is not appropriate to select the place with narrow space, which might give rise to the feeling of constriction and cause the beginning learners get nauseating easily. When the learners practice whirling in a narrow space, the surrounding objects might look revolving very fast relative to the

Table 4 The possible ways of increasing the length of WM unit

Session # / Ex.	1	2	3	4	5	6	7	8	9	10	11
Ex. 1	5W+5M	5W+5M	10W+10M	10W+10M	15W+15M	15W+15M	20W+20M	20W+20M	25W+20M	25W+20M	…
Ex. 2	5W+5M	5W+5M	7W+7M	9W+9M	11W+11M	15W+15M	20W+20M.	25W+20M	30W+20M	35W+20M	40W+20M
Ex. 3	5W+5M	7W+7M	10W+10M	15W+15M	20W+20M	20W+20M	25W+20M	30W+20M	30W+20M	35W+20M	40W+20M

Table 5 Way of arranging several WM units in one session

Session # / Ex.	1	2	3	4	5	6	7	8
Ex. 3	2W+2M+5D+2W+2M+5D+3W+3M+10D	5W+5M+5D+5W+5M+5D+5W+5M+10D	10W+10M+5D+10W+10M+5D	10W+10M+5D+10W+10M+5D	25W+20M+5D	30W+20M+5D	35W+20M+5D	40W+20M

learners and the learners might be afraid of bumping to other people, objects or wall. All of these feelings and fears might increase the possibilities to cause the learners to get nauseating. Besides, the teacher had better select the place for training with as less noises or interference as possible to decrease the factors of distraction and the possibility of nauseating for the learners.

3.3.9 Select appropriate music for training

During the process of whirling, we usually select appropriate music to accompany with the practice so that the learners could enjoy the music while whirling. Based on the authors' experience in many training courses, the music used should not sound boring or dull. The rhythm of the music should be vivid and brisk. The effects of using different music while whirling are quite different. Since whirling-kung training usually requires a series of training sessions, the teacher could vary the music used in different sessions, especially after several training sessions. At the beginning of training, it is not necessary to change the music every time so that the learners could get used to the music and focus more on the whirling. After 3 or 4 sessions of

training, the teachers could change the music every time to allow the learners obtain different experience with different music and avoid the feeling of boring.

3.3.10 Try to arrange the whirling practice at least one hour after the meals

When the first author of this paper implemented his second training group, at the first session of training, about half of the learners squat down to take a rest quickly. Some of them expressed their feeling of nauseating and vomiting to the teacher after the training session. The authors infer that the students finished their lunch at around 12:10 and started their whirling practice at around 12:40, which is only about half hour after the lunch and was not appropriate for whirling practice.

The second author of this paper always arrange the time for whirling practice at least two hours after the meals and the learners did not have similar reactions. Therefore, we strongly suggest that the teacher had better arrange the whirling practice at least one hour after the meals.

3.4 The effects of whirling-kung training

Based on the experience of the whirling-kung training courses as described above, most of the learners could gradually conquer the fear for whirling and reduce the nauseating symptom. They could master the skill of whirling eventually and enjoyed the whirling because it allows them to feel relaxed and calm. Besides providing the effects of exercise or losing weight, whirling-kung could also help the learners reduce the nauseating problem in daily lives or in situations that would easily cause motion sickness.

However, a few learners still could not overcome the nauseating symptom. Future research might be necessary to explore other effective techniques or curriculum design for this kind of learners.

Corresponding Author:
Jian-Wei Rau, Ph.D.
The Department of Curriculum Design and Human Potentials Development, National Dong-Hwa University, Hualien 97065, Taiwan
E-mail: rau@mail.ndhu.edu.tw

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A Model for Predicting Intention to Use E-learning based on Epistemological Beliefs

Mohammad Hassan Seif, Mohammad Reza Sarmadi, Isa Ebrahimzadeh, Hossein Zare

Payame Noor University, PO BOX 19395-3697, Tehran, IRAN. hassanseif2012@gmail.com

Abstracts: This paper investigates the impact of epistemological beliefs on intention to use e-learning emphasizing on the mediator role of motivational beliefs, goal orientation, self-efficacy, ease of use, attitude and perceived usefulness. A sample of 562 students from universities providing virtual education selected for further analysis. Data analysis using path analysis confirmed all of the hypotheses investigated at this paper.
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Keywords: epistemological beliefs, self-efficacy, Goal Orientation, motivational beliefs, ease of use, usefulness

Introduction:

Development and e-learning providers in different countries need to understand how these are elements of e-learning learners understand and react to it, and how this strategy will promote learning (Koohang and Durant, 2003) plus interest of learners and to identify factors influencing their opinions and beliefs about e-learning in education is to help managers and practitioners in their own mechanisms to ultimately attract more students with the learning environment (Grandon et al, 2005)

The overall objective of this study is to investigate the impact of epistemological beliefs on intention to use e-learning courses and virtual universities in Iran among the students.

Three categories of variables exist in this study, the predictor, the criterion variable and the mediating variables. Criterion variable in this study is the behavior of e-learning usage. Predictor variables include epistemological beliefs; intermediate variables included six variables, computer self-efficacy and perceived ease of use and perceived usefulness, attitude, self-regulatory learning and learning goal orientation.

Research Hypotheses:

Hypothesis 1: epistemological beliefs have a positive direct effect on goal orientation, motivational beliefs and self efficacy.

Hypothesis 2: Goal Orientation has a positive direct impact on motivational beliefs, self-efficacy, perceived ease of use, perceived usefulness and attitude towards e-learning.

Hypothesis 3: motivational beliefs have the most direct is positive impact on perceived ease, sense of usefulness and intention to use e-learning

Hypothesis 4: The self-efficacy has a direct positive impact on, perceived usefulness, attitude and intention to use e-learning and perceived usefulness.

Hypothesis 5: Perception of ease of use has a direct positive effect on, perceived usefulness, attitude and intention to use e-learning.

Hypothesis 6: perceived usefulness has a direct positive impact on attitude toward e-learning and intention to use e-learning

Hypothesis 7: Attitudes has a direct effect positive to the intended use of e-learning

Hypothesis 8: epistemological beliefs has an indirect positive impact on intention to use e-learning through the mediator role of goal orientation, motivational beliefs, self-efficacy beliefs, ease of use, perceived usefulness and attitude

Hypothesis 9: Goal orientation has an indirect positive impact on intention to use e-learning through the mediator role of motivational beliefs, self-efficacy beliefs, ease of use, perceived usefulness and attitude

Hypothesis 10: motivational beliefs have an indirect positive impact on intention to use e-learning through the mediator role of ease11: self-efficacy beliefs has an indirect positive impact on intention to use e-learning through the mediator role of ease of use, perceived usefulness and attitude.

Hypothesis 12: perceived usefulness and perceived ease through the mediation of attitude have the positive indirect effect on intention to use e-learning

Hypothesis 13: Perceived usefulness of e-learning using mediated role of attitude has indirect effect on intention to use e-learning.

In this paper the results of six first hypotheses are presented.

Literature Review:

Rozell and Gardner (2000) to investigate the cognitive, motivational processes associated with the performance of computer-driven model is path analysis, this purpose, 600 students in undergraduate management information systems were selected, and the results indicate that computer self-efficacy has a

direct and significant effect on student academic performance.

In the study of Neber and Schommer – Aikins (2002) the role of cognitive variables, motivational, epistemological, and the environment is emphasized. Number of samples of 133 elementary and secondary school students are. Variables include research in science and knowledge, beliefs, motivation, goal orientation, epistemological beliefs and intentions, it could be included. Path analysis showed that in science research on goal orientation, epistemological beliefs and self-regulation of the strategy is effective, while epistemological beliefs may influence the efficacy.

In the Liaw's (2001) paper, perceived usefulness of the web has a significant impact on the intended use of the Web. Perceived enjoyment of the Web on the Web and to use the perceived usefulness of the web has a significant effect.

Jung (2003) in his doctoral thesis entitled "Evaluation of facilitating learning in adults with information technology and university education» has stated that the teaching - learning facilitated by information technology, the unique issues that are different from typical lesson creates. These issues indicate that further evaluation is needed of factors affecting learning.

Figure 4-1shows the * **P < 0.05** ** **P < 0.01** fitted path analysis model to use e-learning

In the Sen's (2005) research, at four different times during the fall semester 2004 data collected through questionnaires. Results and significant positive effect on computer self-efficacy on perceived usefulness and perceived ease of use was apparent in every four times.

Ravindvan et al (2005) predicted the involvement of teachers with cognitive variables and epistemological beliefs and goal orientation. The results showed that the positive relationship between epistemological beliefs and goal orientation exists and these two variables are significant predictors of teachers' cognitive engagement.

Research Methodology

The study of factors affecting intention to use e-learning using a causal model in the virtual university students will explore.

Based on the nature and method of this research can be placed in groups descriptive correlation research. In this study the relationship between predictor variables of epistemological beliefs, computer playfulness and intermediate variables of self- efficacy, learning goal orientation, self-regulatory beliefs, perceived usefulness and perceived ease of use together with the criterion variable of intention to use e-learning will be investigated.

Statistical population and Research Progress:

In this research, students of virtual universities who are familiar with e-learning selected as population of the study. From the operator universities of e-learning in Iran, the University of Tehran, Iran University of Science and Technology, Mashhad University and Shiraz University cooperated with the researchers. The present study used random stratified sampling and 562 questionnaires distributed and 477 questionnaires returned for further statistical analysis.

Results

Hypothesis 1: epistemological beliefs have a positive direct effect on goal orientation, motivational beliefs and self efficacy.

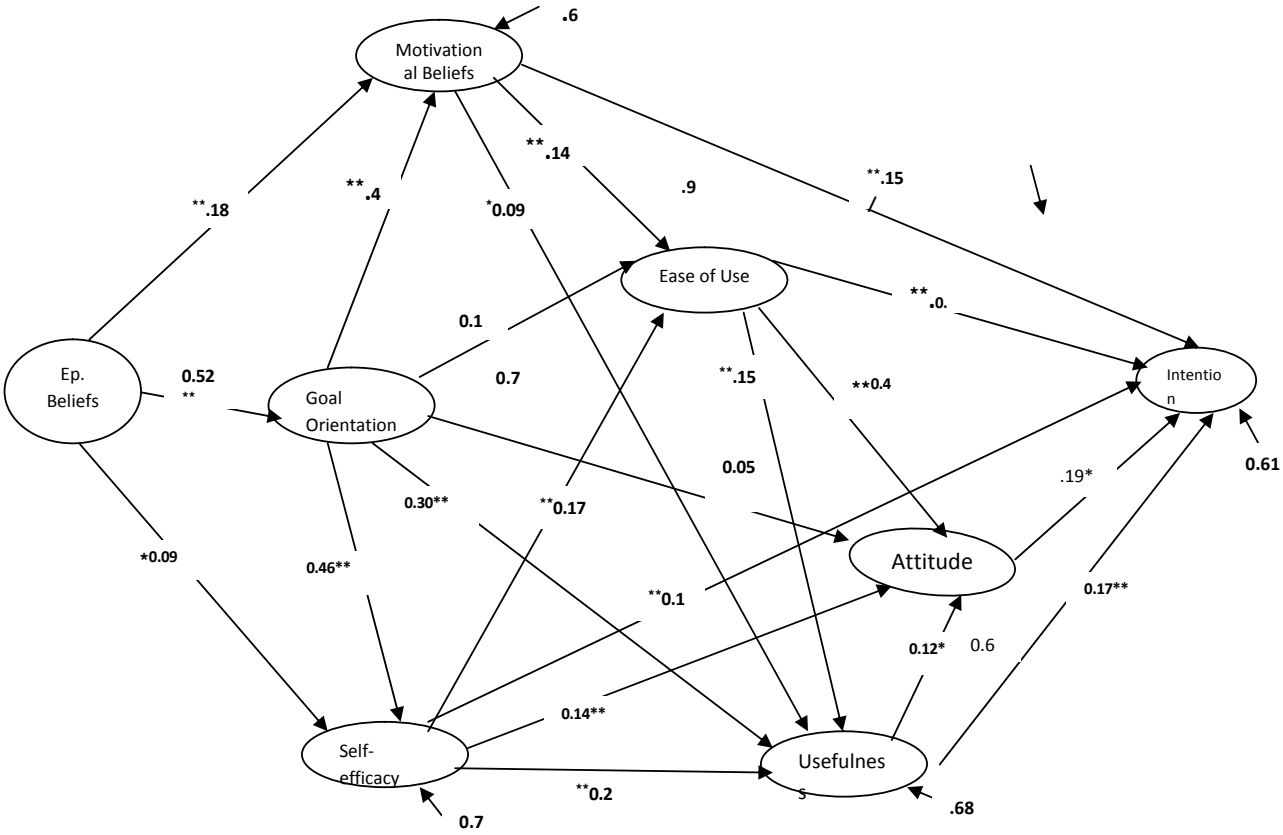
Based on the results the direct effect of epistemological beliefs on goal orientation (0.52) considering (t=22.13) at level 0.01 is significant. Direct effects of epistemological beliefs on motivational beliefs (0.18) considering (t=4.03) at level 0.01 is significant. Direct effects of epistemological beliefs on self-efficacy (.09) and considering (t=2.03) at level 0.05 is significant. The first hypothesis is confirmed in this study.

Hypothesis 2: Goal Orientation has a positive direct impact on motivational beliefs, self-efficacy, perceived ease of use, perceived usefulness and attitude towards e-learning.

According to the results direct effect of orientation on motivational beliefs (0.45) considering (t=10.18) at level 0.01 is significant. Direct effects of goal orientation on self-efficacy (0.46) considering (t=9.93) at level 0.01is significant. Direct effect of orientation on the understanding of ease (0.10) considering (t=1.74) is significant. Direct effect of orientation on the understanding of the usefulness (0.30) considering (t=6.05) at level 0.01 is significant. Finally, direct effect of attitude toward e-learning on goal orientation (0.05) and considering t=1.01) is significant.

Hypothesis 3: motivational beliefs have the most direct is positive impact on perceived ease, sense of usefulness and intention to use e-learning based on the results, the direct effect motivational beliefs on perceived ease (0.14) considering (t=2.62) at level 0.01 is significant. Direct effect of the motivational beliefs on perception of the usefulness (0.09) considering (t=1.99) at level 0.05 is significant. Motivational beliefs' direct effects on intention to use e-learning (0.15) considering (t=3.96) at level 0.01is significant. The third hypothesis is confirmed by the study.

* Figure 4-1 the fitted path analysis model to use e-learning



Hypothesis 4: The self-efficacy has a direct positive impact on, perceived usefulness, attitude and intention to use e-learning and perceived usefulness. Direct effect of self-efficacy on perceived ease (0.17) considering (t=3.40) at level 0.01 is significant. Direct effect of self-efficacy on the perception of the usefulness (0.22) considering (t=4.86) at level 0.01 is significant. Direct effect of self-efficacy on attitude toward e-learning (0.14) considering (t=3.02) at level 0.01 is significant. Finally, the direct effect of self-efficacy on intention to use e-learning (0.18) considering (t=4.36) at level 0.01 is significant, so the fourth hypothesis is confirmed in this study.

Hypothesis 5: Perception of ease of use has a direct positive effect on, perceived usefulness, attitude and intention to use e-learning.

Results showed direct effect of perceived ease of use on perceived usefulness (0.15) considering (t=3.74) at level 0.01 is significant. Direct effect of ease of use on attitude toward using e-learning (.44) and considering (t=10.86) at level 0.01 is significant. Direct effect on perceived ease of use on intention to use to e-learning (0.22) considering (t=4.19) at level 0.01 is significant. The fifth research hypothesis is also confirmed.

Hypothesis 6: perceived usefulness has a direct positive impact on attitude toward e-learning and intention to use e-learning

Results showed that the direct effect of perceived usefulness on attitude toward e-learning (0.12) considering (t=2.57) at level 0.05 is significant. The direct effect of perceived usefulness on intention to use e-learning (0.17) and considering (t=4.11) at level 0.01 is significant. The sixth research hypothesis is confirmed.

Conclusions:

Findings of the study are a confirmation of models of technology acceptance, theory of planned behavior and theory of reasoned action in different

approaches. As we can see the model presented above may be a brief introduction to the above mentioned theories. In the study 13 hypotheses proposed but the current paper discussed on the 6 hypotheses. However the results of the study confirmed the direct effect of epistemological beliefs on goal orientation, self-efficacy beliefs and attitude toward e-learning. This shows that the construct " epistemological beliefs" is predictor of the three mentioned and theories of planned behavior, Technology acceptance model, and reasoned action theory typically lack such ideologically constructs. It should be noted that although some developments has been occurred on the above models but, there is a need also to consider variables of ideological beliefs while investigating the above models. Researchers of the study suggest studies that focus philosophically and ideologically on the 3 above mentioned models so, the investigation of "philosophy of use" as a predictor variable in future studies is recommended. Also the finding of the study suggests that when designing the e-learning environment it is a good idea to focus on epistemological beliefs. The authors suggest that the most effective way is that the designers and users have a "shared perception of value" in the design, and in this regard it is vital to notice to the ideological beliefs such as epistemological beliefs and philosophy of use.

The other findings of the study are to some extent the confirmation of the predictor role of the variables included in the study in predicting intention to use. Although the predictor role of some variables hasn't been confirmed in some studies but, the researchers discuss that these differences may be due to demographic differences and sample conditions of the other studies.

This study has been conducted in an Eastern culture, so the researchers advise more consideration when using in other cultures and further studies need to focus on cultural variables. So, this may be the other suggestion of the current study.

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Serum Interleukin-6 (IL-6), Vascular Endothelial Growth Factor (VEGF), and VEGF/Platelets Ratio as Markers for Hepatocelluar Carcinoma

Ehab F. Moustafa[1], Ghada M. Galal[2], Sahar M. Hassany[1], Mohamed Z. Abd Elrahman[3], and Madleen Adel A. Abdou[3]

[1]Department of Tropical Medicine and Gastroenterology, Faculty of Medicine Assiut University
[2]Department of Tropical Medicine and Gastroenterology, Faculty of Medicine Sohag University
[3]Clinical Pathology Department, Faculty of Medicine, Assiut University
ehabmostafa_99@yahoo.com

Abstract: Background: Primary liver cancer is the fifth most common cancer worldwide and the third most common cause of cancer mortality. **Aim of the work**: To evaluate the usefulness of serum IL-6, serum VEGF, and VEGF/Platelets ratio in hepatocellular carcinoma (HCC) diagnosis. **Patients and methods:** Fifty-eight cirrhotic patients with hepatocellular carcinoma were included in the study (51 males and 7 females) and 18 liver cirrhosis patients without HCC (15 males and 3 females) were recruited as a control group. All patients were subjected to full medical history, clinical examination, laboratory investigations complete blood count, liver function tests, AFP, serum IL-6, serum VEGF and calculation of VEGF/platelets ratio. **Results**: Patients had significantly higher values of AFP (*P*=0.0001), IL-6 (*P*=0.004), VEGF (*P* =0.001) and VEGF/Platelets ratio (*P* =0.005) than cirrhotic patients without HCC (control group). Sensitivity and specificity of serum IL-6, VEGF and VEGF/Platelets in detecting HCC, was found to be 34.5 % & 94.4%, 43.1 % & 88.9% and 41.4 % & 88.9% respectively. Sensitivity and specificity of serum IL-6, serum VEGF and VEGF/platelets ratio for detection of portal vein thrombosis were 65.5% & 83.3%, 63,8% & 77,8%, and 58,6% & 72,2% respectively. There was significant positive correlation between VEGF and AFP (r=0.794, *P* =0.0001), VEGF/Plat and AFP (r=0.760, *P* =0.0001) and IL-6 and AFP (r=0.804, *P* = 0.0001). **Conclusion:** Serum IL-6, serum VEGF, and VEGF/platelets ratio are significantly higher in HCC patients than liver cirrhosis patients without HCC. The clinical utility of these biomarkers in HCC diagnosis is still doubtful because their sensitivity is not more than that of AFP. They may have a good role in detection of portal vein thrombosis (tumor invasion).

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Key words: Hepatocellular carcinoma, diagnosis, vascular endothelial growth factor, IL-6, alpha feto protein.

Abbreviations: hepatocellular carcinoma (HCC), vascular endothelial growth factor (VEGF), Plt (platelets), AFP (alpha feto protein).

1. Introduction

Primary liver cancer is the fifth most common cancer worldwide and the third most common cause of cancer mortality [(1)]. Hepatocellular carcinoma (HCC) accounts for between 85% and 90% of primary liver cancers [(2)]. Its incidence is increasing worldwide ranging between 3% and 9% annually [(3)]. In Egypt, HCC was reported to account for about 4.7% of chronic liver disease (CLD) patients [(4)]. More than 80% of cases of HCC occur in a background of cirrhosis and most frequently involve the right lobe [(2, 5)]. Major causes of cirrhosis are HBV, HCV, and alcohol. Investigations in Egypt during the last decade have shown the increasing importance of HCV infection in the etiology of liver cancer, estimated to account for 40–50% of cases, and the declining influence of HBV and HBV/HCV infection (25% and 15%, respectively) [(6,7)].

Recently, the survival of patients with HCC after diagnosis has improved (8), which is attributed to advances in diagnostic techniques and to the application of various curative treatment options (surgical resection, liver transplantation, and percutaneous ablation). The major diagnostic modalities for HCC include serum markers, various imaging modalities and histological analysis. The overall sensitivity and accuracy of US-guided biopsy generally exceeds 85% [(9)]. There are virtually no false-positive findings. The negative predictive value of biopsy remains low. Therefore, in patients with negative biopsy findings, HCC cannot be definitely ruled out.

Pathologically, patients with chronic liver disease, particularly those associated with a high degree of hepatocyte regeneration, can express AFP in the absence of cancer. Also, AFP is elevated in

hepatocarcinogenesis, embryonic carcinomas [10] and in gastric [11] and lung cancer [12]. Some patients with cirrhosis and/or hepatic inflammation can have an elevated AFP, even without the presence of a tumor. The test had a sensitivity of 39% - 65%, a specificity of 76% - 94%, and a positive predictive value of 9% - 50% for the presence of HCC in previously published studies [13].

Tumor angiogenesis is essential for tumor growth, invasion and metastasis [14]. Tumor angiogenesis is mediated by a number of angiogenic factors and vascular endothelial growth factor (VEGF) is one of these factors. It was demonstrated that there is strong VEGF expression in various solid tumor types. In HCC, VEGF expression in tumor tissue has been found to be correlated with aggressive behavior, and poor prognosis [15]. By measuring circulating VEGF concentrations with the enzyme-linked immunosorbent assay (ELISA), the expression of VEGF in patients with various malignancies has been made possible [16].

VEGF content of platelets is higher in cancer patients than in healthy subjects [17]. It is known that platelets concentrate plasma proteins such as VEGF and later transport them into their granules [15]. So, authors have suggested that VEGF secreted from tumor cells could be stored and transported by platelets in the blood stream, and that this reservoir of VEGF might have a role in tumor angiogenesis and invasion [17,18]. Accordingly, VEGF load in platelets may predict tumor angiogenic activity better than serum VEGF. **George *et al.*** [19] used serum VEGF per platelet count to correct variation of serum VEGF levels in patients with different platelet counts. Serum VEGF per platelet count correlated with advancing stage of colorectal cancer, suggesting its role as a standard measure of circulating VEGF. There is still little data regarding the prognostic significance of serum VEGF per platelet count in patients with HCC. **Kim *et al.*** [20] reported that serum VEGF per platelet count was higher in patients with HCC than those with liver cirrhosis and it was an independent prognostic factor with the presence of portal vein thrombosis in their study. They concluded that serum VEGF per platelet count could be a feasible prognostic indicator during the follow-up of patients with HCC.

Interleukin-6 (IL-6) is a multifunctional cytokine largely responsible for the hepatic response to infections or systemic inflammation. Serum IL-6 levels are elevated in patients with chronic liver inflammation including alcoholic hepatitis [21], hepatitis B [22], HCV infections [23] and steatohepatitis [24]. Furthermore, serum IL-6 levels are reportedly higher in patients with HCC than in those without [25]. In chronic hepatitis, IL-6, produced mainly by activated Kupffer cells, intensifies local inflammatory responses and induces compensatory hepatocyte proliferation, facilitating malignant transformation of hepatocytes [26]. Also, IL-6 induces the hepatic acute phase response by modulating the transcription of several liver specific genes during inflammation [27]. **Malaguarnera *et al.*,** found that there was a significant positive correlation between IL-6 and the size of the tumor [27]. Moreover, Giannitrapani et al., indicated that IL-6 could be more sensitive marker in identifying HCCs than AFP [28].

Aim of the work:

To evaluate the usefulness of VEGF, VEGF/Platelets, interleukin-6 (IL-6) in HCC diagnosis

2. Patients and Methods:

Patients:

Fifty-eight patients with hepatocellular carcinoma (HCC on top of L.C) were included in the study (51 males and 7 females, mean age 59.6± 8.3 years, range 27 – 85 years). They were admitted to Tropical Medicine and Gastroenterology Department, Assiut University Hospital, Egypt from January 2008 to January 2010. The diagnosis of HCC was based mainly on the typical findings of triphasic abdominal computed tomography. Eighteen patients with liver cirrhosis without HCC (15 males and 3 females, mean age 60.2±9.7 years, range 40– 75 years) were also admitted to the department and served as a control group (Table 1). Tumor characteristics such as size, number, and portal vein patency were assessed by real time abdominal ultrasonography (Table 2).

Specimen collection and analysis:

For patients and control subjects, blood was obtained by venipuncture without a tourniquet. Complete blood count was done on Coulter Hmx, USA. Prothrombin time and concentration were estimated according to standard procedure using Sysmex CA500 coaglometer, Germany. Liver function was measured by Hitachi 911, Roche, Germany autoanalyser. Serological tests for hepatitis B surface antigen (HBsAg) and anti-HCV were carried out by Micro particle Enzyme Immunoassay (MEIA) technology using Abbot AXSYM System, USA autoanalyser.

Serum alpha foetoprotein was measured by ELISA DS-EIA-AFP from Diagnostic system Ltd, Germany. Serum vascular endothelial growth factor was measured by ELISA (according to the manufacturer instruction from Biotechnology, USA). Serum IL-6 was measured by ELISA from Orgenium kit Finland, catalogue no.ILO6001).

Statistical analysis:

Data entry and analyses were performed using a statistical software package (SPSS, Version 10.0, Inc., Chicago, IL). Student's t- test, ANOVA test, Chi-square test, Fisher exact probability test, correlation coefficient (r) and spearman's rank correlation (r) were used when appropriate. P<0.05 was considered statistically significant.

Ethical approval:

The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki. An ethical approval was obtained from our local Ethics Committee (Medical Ethics Committee, Faculty of Medicine, Assiut University). Patients were enrolled after written informed consent was obtained.

3. Results:

The base line clinical and laboratory data of patients (HCC on top of liver cirrhosis) and controls (liver cirrhosis) are demonstrated in Table 1. According to Child-Pugh classification, 21 (36.2%) patients were Child A cirrhosis, 20 (34.5%) were Child B and 17 (29.3%) were Child C. Cirrhotic patients of the control group were 6 (33.3%) Child B and 12 (66.7%) Child C.

In HCC group 33 patients were HCV Ab positive, 19 patients were HBsAg positive and 6 patients were both HCV Ab and HBsAg positive, while in control group (cirrhosis without HCC) 10 patients were HCV Ab positive and 8 patients were HBsAg positive.

Abdominal ultrasound data of patients and controls revealed that thirty- seven patients (63.8%) had single hepatic focal lesion and 21 (36.2%) had multiple hepatic focal lesions varying in size between 1 to 11 cm. Portal vein was patent in 51 (87.9%) patients with focal lesions and was thrombosed in 7 (12.1%). All cirrhotic patients without focal lesions (controls) had patent portal vein (Table 2).

Table 3 shows that HCC patients had significantly higher values of AFP (401.5±665.4 vs 31.2±104.6; *P*=0.0001), IL-6 (138.1 ±257.6 vs 31.2±104.6; *P* =0.004), VEGF (398.9±67.2.5 vs 52.3±150.6; *P*=0.001) and VEGF/Platelets ratio (3.4±6.1 vs 0.7±2.2; *P* =0.005) than cirrhotic patients without hepatic focal lesions (control group).

Studying serum markers of HCC in patients with single and multiple focal lesions revealed that no significant difference in all the studied markers between both groups (Table 4).

Diagnostic value of IL-6, VEGF and VEGF/Platelets

By using the mean levels of IL-6, VEGF and VEGF/Platelets in patients with liver cirrhosis without HCC (23.1 pg/ml, 52.3 pg/ml and 0.7) as cut-off levels, the sensitivity and specificity of these 3 markers in detecting HCC, were found to be 34.5 % with 94.4% specificity, 43.1 % with 88.9% specificity and 41.4 % with. 88.9% specificity respectively (Table 5).

To evaluate the diagnostic value of these markers for detection of portal vein thrombosis, mean levels of IL-6, VEGF and VEGF/Platelets in HCC patients with absence of portal vein thrombosis was used as cut-off level, serum VEGF was found to have a sensitivity of 63.8% and a specificity of 77.8% in detecting portal vein thrombosis, serum IL-6 was found to have a sensitivity of 65.5% and a specificity of 83.3% in detecting portal vein thrombosis and VEGF/Platelets ratio was found to have a sensitivity of 58.6% and a specificity of 72.2% in detecting portal vein thrombosis (Tables 6-8).

Results of correlation studies:

Correlation studies of the tested variables revealed that there was significant positive correlation between VEGF and AFP (r=0.794, *P* =0.0001; Fig 1), VEGF/Plat and AFP (r=0.760, *P* =0.0001; Fig 2) and IL-6 and AFP (r=0.804, *P* = 0.0001; Fig 3)

Table 1: Base line clinical and laboratory data of patients (HCC on top off liver cirrhosis) and controls (liver cirrhosis)

	Cases (n=58)	Control (n=18)
Age(ys) Mean Range	 59.6 (27 – 85)	 60.2 (40 – 75)
Sex Male/Female	 51/7	 15/3
Prothrombin Time(sec)	16.3	19.0
Prothrombin concentration(٪)	63.3	48.4
platelets(x10^9/l)	163.0	126.4
ALP(IU/l)	169.3	121.6
Positive HCV Ab	33 (56.9%)	10 (55.6%)
Positive HBsAg	19 (32.8%)	8 (44.4%)
Positive HCV Ab & HBsAg	6 (10.3%)	0
Child classification: Child A Child B Child C	 21 (36.2%) 20 (34.5%) 17 (29.3%)	 0 6 (3.3%) 12 (66.7%)

Table 2. Sonographic data of patients (LC+HCC) and controls (LC)

Variable	Patients (LC+HCC) n=58	Controls (LC) n=18
Size of focal lesion (cm)		
Range	1-11	----
Mean±SD	4.2±2.1	----
N of focal lesions		
Single	37 (63.8%)	---
Multiple	21 (36.2%)	---
Portal vein		
Patent	51 (87.9%)	18 (100%)
Thrombosed	7 (12.1%)	0 (0%)
Ascites		
Yes	31 (53.4%)	18 (100%)
No	27 (46.6%)	0 (0%)

Data are expressed as mean±SD or as number, (%).

Table 3. Platelets count, Alkaline phsphatase (ALP), α feto protein (AFP), serum IL-6, serum vascular endothelial growth factor (VEGF) nd VEGF/platelets ratio in patients (HCC) versus control (liver cirrhosis without HCC).

Variable	Patients (LC+HCC) n= 58		Controls (LC) n= 18		P value
	Range	Mean±SD	Range	Mean±SD	
AFP (ng/dl)	1-2502	401.5±665.4	2-450	31.2±104.6	0.0001
IL-6 (pg/ml)	1-1100	138.1±257.6	1-355	23.1±83	0.004
VEGF(pg/ml)	4-2300	398.9±672.5	5-650	52.3±150.6	0.001
VEGF/ Platelets	0.03-21.4	3.4±6.1	0.01-9.6	0.7-2.2	0.005

Table 4. Serum markers of HCC in patients with single vs multiple focal lesions.

	n. of focal l (single)		n. of focal l (multiple)		P value
	Mean	SD	Mean	SD	
AFP (ng/dl)	340.89	611.943	508.25	754.453	0.362
IL6 (pg/ml)	138.54	269.82	137.4	240.98	0.987
VEGF(pg/ml)	419.15	734.47	363.13	562.03	0.763
VEGF/plat	3.93	6.77	2.54	4.66	0.361

Table 5. Sensitivity and specificity of serum IL-6, VEGF and VEGF/Platelets in detecting HCC.

	Cut off value	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
IL6(pg/ml)	23.1	34.5	94.4	95.2	30.9
VEGF(pg/ml)	52.3	43.1	88.9	92.6	32.7
VEGF/plat	0.7	41.4	88.9	92.3	32.0

Table 6. Performance of VEGF at cut off point for detection PV thrombosis

	Cut off VEGF value (pg/ml)	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
PV thrombosis	19.5	63.8	7.8	90.2	740.0

Table 7. Performance of IL-6 at cut off point for detection PV thrombosis

	Cut off IL6 value (pg/m)	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Portal V TH	5.6	65.5	83.3	92.7	42.9

Table 8. Performance of VEGF/Plat at cut off point for detection PV thrombosis

	Cut off VEGF/plat value (pg/ml)	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
PV thrombosis	0.162	58.6	72.2	87.2	35.1

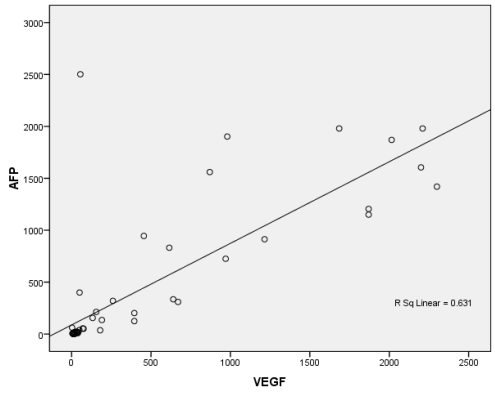

Fig 1. Correlation between AFP and VEGF in HCC patients

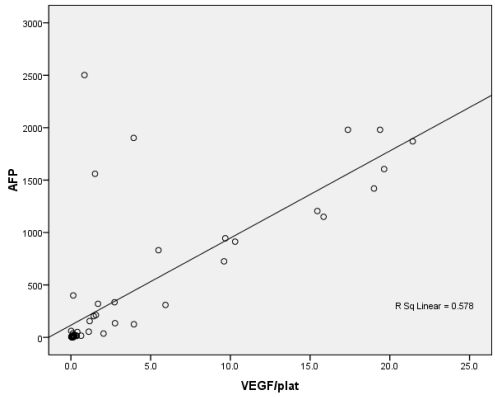

Fig 2. Correlation between AFP and VEGF/Plat in HCC patients

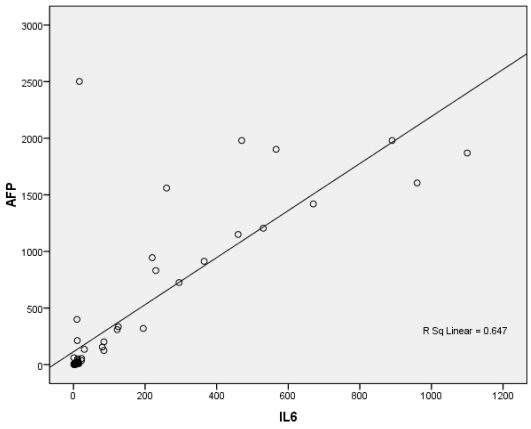

Fig 3. Correlation between AFP and IL-6 in HCC patients

4. Discussion

Surveillance of cirrhotic patients for early diagnosis of HCC has been recommended by all practice guidelines for optimal HCC management [29-31]. The AASLD practice guidelines on the management of HCC were published in 2005 in order to provide an evidence-based approach to screening of HCC, in addition to diagnosis, staging and management of this tumor [30].

The most important tumor marker for HCC is alpha fetoprotein (AFP). The common method for screening high risk patient using AFP marker can detect more early tumors and prolong the survival of patients [32]. The sensitivity of AFP assays has increased. In HCC, the reported range of AFP is 10 to more than 100,000 µg/L. Approximately 40% of patients with HCC have AFP levels greater than 1,000 µg/L [33].

Serum AFP is the most commonly used marker for this neoplasm, but its real clinical usefulness is unclear, a systematic review and critical analysis [34] of the use of this marker in HCC detection yielded sensitivity and specificity rates ranging from 41% to 65% and 80 to 94%, respectively. AFP levels greater than 400 µg/L, however, are generally considered diagnostic of HCC, especially when a liver mass is visualized at ultrasound (US) or computed tomography (CT) [35].

Our results showed that HCC patients have significantly higher values of AFP, serum IL-6, serum VEGF, and VEGF/Platelets ratio than cirrhotic patients without HCC (control group).

Our findings showed significant positive correlations between VEGF and AFP, VEGF/Plat and AFP and IL-6 and AFP.

Many studies showed that serum VEGF has a prognostic value in various cancers [36-38]. But the issue of whether serum VEGF level can reflect tumor VEGF expression has become a major obstacle in its clinical application. Indirect evidence has suggested that the tumor is a major source of serum VEGF in cancer patients. A significant decrease in serum VEGF after tumor resection has been documented [37,38]. Previous study [39] reported that serum VEGF levels in mesenteric venous blood draining from the tumors were several-fold higher compared with peripheral blood in colorectal cancer patients, this remark suggested the secretion of VEGF from the tumors into the circulation. On the other hand, **Salgado *et al.*** [40] found no significant increase in

serum or plasma VEGF levels in the vein draining the tumors in patients with various carcinomas.

Hepatocellular carcinoma (HCC) is a highly vascular tumor characterized by Neo vascularization and a high propensity for venous invasion. A strong VEGF expression was observed in the tissue of HCC and associated with tumor progression and metastasis [13]. **Poon *et al.*** [41], reported that serum VEGF level showed significant elevation in patients with HCC, and high serum VEGF levels were associated with the absence of tumor capsule, the presence of venous invasion and microsatellite nodules, and advanced TNM stage. **Li *et al.*** [42] also reported that serum VEGF was a predictor of invasion and metastasis of HCC. Other previous studies suggested that platelets aggregate at metastatic sites, due to factors released from metastatic cells and vascular invasion, resulting in microthrombosis, tumor adhesion, and may release VEGF to the circulation [43, 44].

Jinno *et al.* [45] concluded that P-VEGF (plasma VEGF) was a promising tumor marker for remote metastasis of HCC and its specificity, sensitivity and overall accuracy were high enough to be clinically useful.

Moreover, **Li *et al.*** [46] found that pre-TACE (transarterial chemoembolization) plasma VEGF was found to be markedly elevated in the majority of patients with HCC and the increase was closely related to a more advanced stage of diseases. This finding suggested that HCC cells were an important source of P-VEGF. But the lower VEGF levels in HCC patients overlapped considerably with those in normal controls, thus limiting the application of VEGF as a tumor marker in early detection of HCC. Also, they found that high plasma VEGF levels were associated with the presence of extrahepatic metastasis and portal vein involvement. Because vascular invasion by tumor cells is indispensable for remote metastasis, portal vein involvement and A-V shunting can reflect the ability of tumor cells to invade blood vessels. They suggested that plasma VEGF could be used as a tumor marker in detecting vascular invasive phenotypes of HCC. They concluded that, Plasma VEGF level is an independent predictive factor of tumor progression, especially vascular invasion.

On the other hand they found no correlation between plasma VEGF and serum AFP, suggesting that they had different mechanisms of production, and P-VEGF might be an independent predictive factor.

Kim *et al.* [19] suggested that serum VEGF per platelet count predicts tumor aggressiveness and treatment outcome. In the current study, serum VEGF per platelet count was higher in patients with HCC than in those with liver cirrhosis. The presence of HCC could contribute to this difference, because both groups had liver cirrhosis and similar characteristics such as liver function and viral markers. This may suggest the possible role of serum VEGF per platelet count as an indicator of the development of HCC in patients with liver cirrhosis during follow-up. In the multivariate analysis for factors predicting survival, high serum VEGF per platelet count (>1.4 pg/106) and portal vein thrombosis were independent prognostic indicators for overall survival. This suggests that serum VEGF per platelet count could be a better prognostic factor than serum VEGF itself.

It was noticed that serum IL-6 level was elevated in patients with primary liver cancer [47] and its levels were significantly higher in HCC patients than in healthy controls [48, 49]. High serum IL-6 may promote HCC development in hepatitis B patients [50]. Therefore, IL-6 could be considered a HCC biomarker and a high risk factor for HCC. Several studies revealed that increased IL-6 is positively correlated with the occurrence and progression of primary liver cancer. Karin group showed that obesity can promote liver inflammation and augment cancer risk via the increased expression of IL-6/STAT3 and TNF/STAT3, where STAT3 mean Signal Transducer and Activator of Transcription 3, an acute phase response factor [51]. Also, they found that diethylnitrosamine (DEN) can cause HCC in 100% of male mice but in lower than 30% of female mice. Other studies concluded that DEN increases serum IL-6 much more remarkably in male mice. Blockade of IL-6 result in absence of this difference in both sexes, so this indicates that IL-6 is an important risk factor of HCC [52]. All the above, suggest that inflammation and IL-6 may be correlated with the development of HCC.

There is strong evidence about the role of the cytokine IL-6 in the process of liver damage and carcinogenesis. Liver cirrhosis is associated with increased hepatic expression of several cytokines, including IL-6 [53]. IL-6 was also shown to induce the expression of the mitogenic, motogenic, morphogenic and pro-neoangiogenic hepatocyte growth factor which are expressed at high levels in HCC [54]. Also, the expression of IL-6 into non-metastatic HCC cells makes them highly metastatic (55). Beside this IL-6 may also decrease HCC cell apoptosis. In one study in a mouse model, IL-6 proved able to reduce Fas-induced apoptosis [56]. It has been postulated that IL-6 may directly stimulate hepatic DNA synthesis, in mice showing a lack in DNA synthesis following hepatectomy [57]. Also, it was reported that IL-6 is considered a cause of natural killer cell dysfunctions, resulting in tumor escape from immune surveillance [58].

Several reports indicate a potential role for IL-6 as a tumor marker for HCC [27, 28, 49].

The sensitivity and specificity of serum IL-6, VEGF and VEGF/Platelets in detecting HCC, was found to be 34.5 % with 94.4% specificity, 43.1 % with 88.9% specificity and 41.4 % with. 88.9% specificity. **Li *et al.*** [46], Found that sensitivity and specificity of P-VEGF in detecting HCC were 73.3% and 62.6%.

Our results showed that serum VEGF had a sensitivity of 63.8% and a specificity of 77.8% in detecting portal vein thrombosis. Serum IL-6 had a sensitivity of 65.5% and a specificity of 83.3% in detecting portal vein thrombosis and VEGF/Platelets ratio was found to have a sensitivity of 58.6% and a specificity of 72.2% in detecting portal vein thrombosis. These also agree with **Li *et al.*** [46], where they found that sensitivity and specificity of P-VEGF in detecting portal vein thrombosis of positive tumors were 62.5 % and 64.9%.

5. Conclusions:

In conclusion, serum IL-6, serum VEGF, and VEGF/platelets ratio are significantly higher in HCC patient than liver cirrhosis patients without HCC. The clinical benefit of using these biomarkers in HCC diagnosis is still doubtful because their sensitivity is not more than that of AFP. They may have a good role in detection of portal vein thrombosis (tumor invasion), with sensitivity and specificity of 65.5% and 83.3%, 63, 8% and 77,8%, and 58,6% and 72,2% respectively.

Correspondence author
Ehab F. Moustafa
Department of Tropical Medicine and Gastroenterology, Faculty of Medicine Assiut University
ehabmostafa_99@yahoo.com

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Magnibursatus diplodii n. sp. (Derogenidae: Halipeginae) from white sea bream, *Diplodus sargus*, Off Sirt, Libya

Elsayed M. Bayoumy[*1,2] and Gasem M. Abu-Taweel[2]

[1]Hydrobiology Department, National Research Centre, Dokki 12622, Giza, Egypt
[2]Biology Dept., College of Education, Dammam Univ., P. O. Box 2375, Dammam - 31451, Saudi Arabia
[*]bayoumy2004@yahoo.com

Abstract: Examination of the 35 specimens of White Sea bream, *Diplodus sargus* Linne 1758 (Sparidae)were caught from the Sirt Coast, Libya and revealed the presence of one new halpigian parasite, *Magnibursatus diplodii* n. sp. with incidence 25.7% (9 out of 35 fish examined). The main characters of the obtained parasite are ; the. Body is small and slender and at the same time, the forebody is shorter than the anterior one. Oral sucker is subterminal, and ventral sucker cup-shaped, strongly muscular, substantially larger than oral one, protuberant. Testes are two in number ,, oval in shaped and separated from each other. Seminal vesicle very elongate, coiled. Pars prostatica short, poorly developed. Ovary spherical to oval in shaped, and separated from posterior testis. Vitellarium comprises 2compact, entire, contiguous masses, situated side by side posterior to ovary. The morphological characters and measurements of the present parasite were discussed with the previously related species. Moreover, Sirt coast is considered a new geographical area for halpegian parasites.
[Elsayed M. Bayoumy and Gasem M. Abu-Taweel. ***Magnibursatus diplodii* n. sp. (Derogenidae: Halipeginae) from white sea bream, *Diplodus sargus*, Off Sirt, Libya.** *Life Science Journal*. 2012;9(2):939-945]. (ISSN:1097-8135). http://www.lifesciencesite.com. 139

Key words: Parasite**,** Digenea, Derogenidae, *Magnibursatus diplodii*, *Diplodus sargus*.

1. Introduction

The perciform family Sparidae comprises more than 100 species worldwide. Sparids are demersal fishes living in coastal waters and occupying a variety of trophic niches (Bargelloni *et al*. 2005). Although the White Sea bream *Diplodus sargus* Linne, 1758, is a common and commercial sparid in the Eastern Atlantic Ocean and Mediterranean Sea (Whitehead *et al.* 1984), the digenean fauna is known mainly from the northern shore of the Mediterranean Sea (Bartoli 1987 a, b**;** Bartoli and Gibson 1989**;** Bartoli and Bray 1996**;** Bartoli *et al*. 1989a, b**,** 2005**;** Sasal *et al*. 1999; Ternengo *et al*. 2005**;** Pérez -del Olmo *et al*. 2006, 2007, 2008**&** Kostadinova and Gibson, 2009**)**. However, on the southern Mediterranean little work was done (Gargouri and Maamouri, 2008 & Gargouri *et al*. 2011).

Records of the derogenids subfamily Halipeginae Poche, 1926 are scarce and tend to restricted to small number of host groups. As far as we are aware, the halpegian genus *Magnibursatus* Naidenova (1969) consists of 7 nominal species, most of them being parasites of sparid fishes from Black – Sea and northern shore of the Mediterranean Sea fishes (Kostadinova *et al*. 2003 &2004; Kostadinova & Gibson, 2009). Consequently, the number of species is greater than previously believed, indicating some recent radiation, where additional material from *Diplodus sargus* may help reveal whether the morphometric variation reflects host or population differences (Kostadinova & Gibson 2009).

Thus, the present article aims to study the prevalence and light microscopical description of new halpigian species of the genus *Magnibursatus* Naidenova (1969), from *Diplodus sargus* Linne 1758, Sirt Coast, Libya.

2. Materials and methods

A total of 35 host fishes, *Diplodus sargus* Linne 1758, (Sparidae), were collected alive from fishermen in Sirt Coast, located in the middle of the Libyan coast between Tripoli and Benghazi (31°:12.19 N and 16°:35.18 W). After capture, fish were maintained alive in aquaria and anaesthetized and killed just before autopsy for parasites. After death, gills and the digestive tract were removed and each of its anatomical parts of the later isolated and opened. Digenean specimens were collected under a dissecting microscope and studied while still alive and later as permanent preparations. Individuals were fixed in Bouin's fluid between slide and coverslip without pressure, stained in acetic carmine and mounted in Canada balsam. Only ovigerous specimens were studied using a differential interference microscopy. Illustrations were made using a drawing tube. Measurements are given as the range in micrometers, with the mean in parentheses. All measurements are in micrometers. The term 'forebody' refers to the distance between the anterior extremity of the body and the anterior margin of the ventral sucker. The term 'hindbody' refers to the

distance between the posterior margin of the ventral sucker and the posterior extremity of the body.

3. Results

Family: Derogenidae Nicoll, 1910
Subfamily: Halipeginae Poche, 1926
Magnibursatus Naidenova, 1969
Magnibursatus diplodii sp. n.
Host: *Diplodus sargus* (L.) – white seabream (Perciforms: Sparidae).
Locality: Sirt Gulf, Libya.
Sites: Gills and oesophagus.
Prevalence: 9 of 35 fish 25.7% (9 out of 35 fish examined).
Intensity: 1-3
Etymology: *M. diplodii* is named after the specific name of the host, *Diplodus sargus*.
Description: (Figs. 1-3; Table 1).

[Based on 20 whole-mounted adult specimens.] Body small, slender. The total body length measured 803-850 (827), widest at level of ventral sucker. Forebody relatively shorter than the anterior one; It was 295-326 (319) in length, its maximum width at lateral aspect were 78-94(89) and was 108-125 (119) at ventral aspect. Hindbody relatively long; 357-375 (361), its maximum width at lateral aspect were 83-95(91) and was 135-148 (142) at ventral aspect. Worms usually take up lateral position to make approximately right-angle when mounted (Fig.1a&2b). Tegument unarmed. Pre-oral lobe distinct [8-14 (12)]. Oral sucker subterminal, subglobular [41- 53 ×52 -64 (46×58)]. Ventral sucker cup-shaped, strongly muscular, substantially larger than oral sucker, protuberant [131-152×136-161(146×150)]. Prepharynx was absent. Pharynx subglobular; with dimensions [17-21×22–33(19×27)]. Oesophagus short. Intestinal bifurcation just posterior to pharynx. 'Drüsenmagen' present. Caecae with thick epithelial lining, end blindly fairly close to posterior end of body.

Testes 2, oval, smooth, oblique to tandem, separated from each other; anterior testis somewhat sinistral, [56-71×49-62 (65×50)], well-separated from ventral sucker by [16–23 (19) (AT/BL = 2.3%)]; posterior testis [61-73×49-62 (69×54)], at [186-198(191)] from posterior end of body (PT/BL = 23%). Seminal vesicle very elongate, coiled. Pars prostatica short, poorly developed. Hermaphroditic duct is short. Sinus-sac large, broadly oval, comparable in size to oral sucker, in anterior half of forebody, it measures [75-89×45-63(81×50)] and its posterior extremity separated by [74-85(81)] from ventral sucker. Its posterior 2/3 with multi-layered muscular wall, male and female ducts unite within its proximal thin-walled portion. Genital atrium is shallow. Permanent sinus-organ not observed. Genital pore is median, just posterior to level of pharynx or more anterior.

Ovary is spherical or transversely oval, entire, median, posterior to and separated from posterior testis by 23-31 (27). It is slightly smaller than testes and it measures [49-58×39-51 (54×46)]. Oviduct is with thick-walled. Laurer's canal thick-walled, not surrounded by gland-cells, terminates as Juel's organ. Proximal part of Juel's organ contains spermatozoa. Mehlis' gland strongly developed, delimited by membrane. Proximal part of uterus heavily convoluted, with some eggs, but nearly filled by spermatozoa, forming uterine seminal receptacle (Fig. 1&2), coils from level of ovary almost at posterior end of body, separated from the end of the body(Post-ovarian region) 113-124 (119), overlaps vitelline and Mehlis' gland dorsally. Uterine coils pass over dorsal faces of ovary and most of testes; large uterine loops present between anterior testis and ventral sucker; numerous uterine loops in forebody; uterus opens into posterior wall of sinus sac, forming muscular metraterm considerably shorter than sinus-sac (Fig. 3). Vitellarium comprises 2 compact, entire, contiguous masses, situated side by side posterior to ovary and measures 36-47×32-42 (42×38). Vitelline reservoir is absent. Vitelline duct short joins oviduct just prior to Mehlis' gland. Eggs numerous, operculate, with numerous fine, terminal threads filaments; threads very obvious in fully developed eggs from fore-body, but difficult to see in eggs from hind-body; It measures 10-15×5-9(13×7).

Excretory vesicle Y-shaped, with very short wide stem which bifurcates just posterior to Vitelline glands; arms wide, run forward in dorso-lateral field, re-unite dorsally at level of posterior pharynx. Excretory pore is terminal.

4. Discussion

The trematode under discussion is a parasite commonly referred to as "a derogenid". In the most recent revision of the family Derogenidae by Kostadinova & Gibson (2009), three species, *M. barretti, M. bartolii*, and *Magnibursatus* species were included in the genus *Magnibursatus* from the gills and oesophagus of *D. sargus*, Spain.

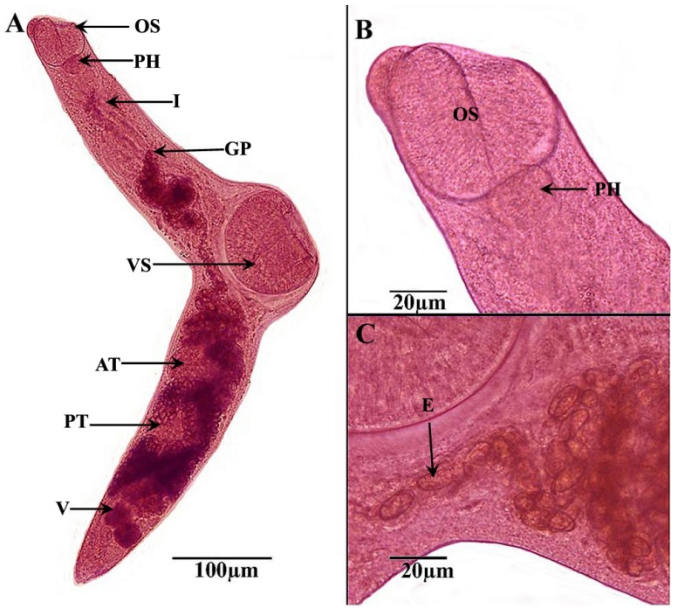

Fig. 1. Light microscopic photomicrographs of *Magnibursatus diplodii* sp. n. (A) Whole fluke (lateral view). Note: Fluke take up lateral position to make approximately right-angle. (B) Anterior end of the specimen showing the ventral sucker and the subglobular eosophagus. (C) The ventral sucker area showing operculate filamentous eggs. *Abbreviations*: AT, anterior testes; E, egg; GP, genital pore; I, intestine; OS, oral sucker; PH, pharynx; PT, posterior testes; V, vetelarium; VS, ventral sucker.

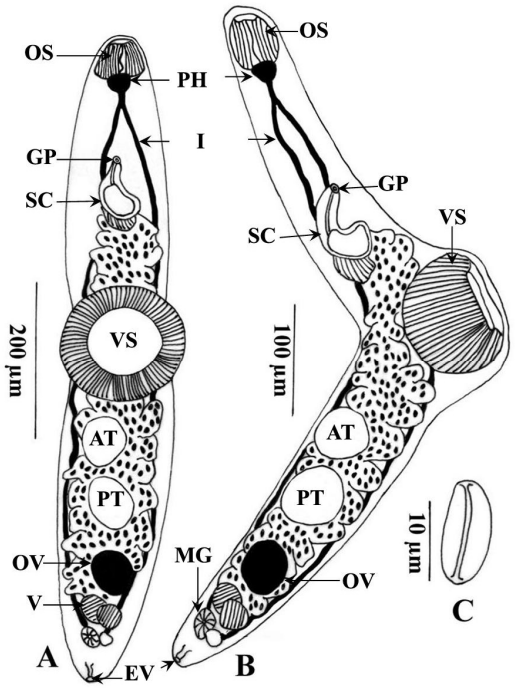

Fig. 2. Schematic drawing of *Magnibursatus diplodii* sp. n. **A)** Ventral view of flattened adult fluke. **B)** Lateral view of adult specimen, Note: Worm take up lateral position to make approximately right-angle. **C)** Egg. *Abbreviations*: AT, anterior testes; EV, excretory vesicle; GP, genital pore; I, intestine; MG, Mehlis' gland; OS, oral sucker; OV, ovary; PH, pharynx; PT, posterior testes; SC, sinus sac; V, vetelarium; VS, ventral sucker.

Table (1): Comparison between the previously described related species of *Magnibursatus* with the present material *Magnibursatus diplodii* (all measurements are in µm).

Species	***M. barretti***	***M. bartolii***	***M. bartolii***	***Magnibursatus* sp.**
Host	*Diplodus sargus*	*Diplodus sargus*	*Boops boops*	*Diplodus sargus*
Locality	Off Burriana (Spain)	Off Santa Pola (Spain)	NE Atlantic coast (Spain)	Off Burriana (Spain)
Source	Kostadinova & Gibson (2009)	Kostadinova & Gibson (2009)	Kostadinova *et al*. (2003)	Kostadinova & Gibson (2009)
Site	oesophagus	Gills & oesophagus	oesophagus	oesophagus
Measurements	**Range**	**Range**		
Body length	520–605		1.32 – 1.78	697
Forebody maximum width (ventral aspect)	130–155	–	363	–
Forebody maximum width (lateral aspect)	117	189–233	250–304	139
Hindbody maximum width (ventral aspect)	59–86	–	334	–
Hindbody maximum width (lateral aspect)	73	138–139	146–279	97
Pre-oral lobe length	8–11	13–15	13–29	11
Oral sucker	42–63 × 38–71	86–105 × 76–96	100–146 × 100–154	74 × 63
Pharynx	25–29 × 25–31	32–34 × 40–42	42–79 × 46–67	32 × 25
Ventral sucker	115–149 ×115–143	185–225 × 185–225	259–313 × 325	166 × 166
Sinus-sac	71–76 ×19–42	162–174 × 76–94	275× 179	130 × 65
Anterior testis	42–48 × 31–48	86–90 × 78–86	88–175 × 154	76 × 53
Posterior testis	36–52 × 32–50	96–99 × 82–97	67–175 × 163	82 × 55
Ovary	27–32 × 29–36	57–76 × 76–84	50–129 × 125	53 × 46
Vitelline masses	24–28 × 19–23	51–56 × 42–46	46–121 × 38–113	39 × 28
Eggs	13–19 × 7–9 (16 × 8)	23–25 × 11–12 (25 ± 0.7 × 11 ± 0.3)	18–26 × 9–14 (24 × 12)	–
Distances				
Forebody length	166–178	327–548	525–734	246
Hindbody length				
Posterior extremity of sinus sac to ventral sucker				
Anterior testis to ventral sucker	13–42	31–153	92–179	23
Posterior testis to ovary				
Post-testicular region	145–180	220–405	284–396	185
Post-ovarian region	109–136	143–248	154–234	153
Ratios				
FO/BL (%)	29–32	35–36	36–42	36- 41
AT/BL (%)	2–7	3–10	6–11	3
PT/BL (%)	27–30	24–27	21–25	6-11
OV/BL (%)	21–23	15–16	11–15	11-14
Sucker-width ratio	1:2.01–3.03	1:2.34–2.43	1:2.11	1:2.63
Sinus-sac length /forebody length ratio			1: 2.5-4.8 (3.3)	
Ventral sucker to ovary			134-403 (252)	
Posterior testis to ovary			0-33 (3)	

Table 1. *Continued*

Species	***M. minutus***	***M. blennii***	***M. diplodii***
Host	*Neogobius eurycephalus*	Three diff. fish species	*Diplodus sargus*
Locality	Black Sea	off Corsica (France)	off Sirt coast (Libya)
Source	Kostadinova *et al*. (2003)	Kostadinova *et al*. (2004)	Present study (n = 13)
Site	Alimentary canal	Oesophagus & Ant. Intestine	Gills & oesophagus
Measurements	**Range**		**Rang (Mean)**
Body length	571–826	675-1.406 (1.036)	803 -850 (827)
Forebody maximum width (ventral aspect)	150–154		108 - 125 (119)
Forebody maximum width (lateral aspect)	142–192		78 -94(89)
Hindbody maximum width (ventral aspect)	150		135 -148 (142)
Hindbody maximum width (lateral aspect)	133–158		83 -95 (91)
Pre-oral lobe length	13–25		8-14 (12)
Oral sucker	63–75 × 46–88	88-154 × 86-165 (120 × 112)	41- 53 ×52 -64 (46×58)
Pharynx	25–42 × 25–42	32-64 × 35-80 (44 × 59)	17 -21 ×22 –33 ((19×27)
Ventral sucker	104–150 × 138	106-176 (134)	131-152 ×136-161 (146×150)
Sinus-sac	113 × 75	95-186 × 63-103 (134 × 85)	75-89 ×45-63 (81×50)
Anterior testis	54–92 × 71	58-147 × 77-205 (109 × 134)	56-71 ×49-62 (65×50)
Posterior testis	71–83 × 92	59-176 × 92-208 (114 × 138)	61-73 ×49-62 (69×54)
Ovary	50–63 × 63	44-128 × 63-160 (76 × 105)	49-58 ×39-51 (54×46)
Species	M. minutus	M. blennii	M. diplodii
Vitelline masses	33–63 × 26–63		36-47×32-42 (42×38)
Eggs	22–30 × 11–15 (25 × 13)	23-29 × 11-14 (26 × 12.5) (n = 55)	10-15×5-9(13×7) (n=50)
Distances			
Forebody length	188–313	285-591 (440)	295 - 326 (319)
Hindbody length		275-724 (473)	357 – 375 (361)
Posterior extremity of sinus sac to ventral sucker		63-250 (152)	74 -85 (81)
Anterior testis to ventral sucker	4–21	16-96 (52)	16 - 23 (19)
Posterior testis to ovary			23 - 31 (27)
Post-testicular region	125–234	139-348 (227)	186 -198 (191)
Post-ovarian region	75–154	96-240 (151)	113- 124 (119)
Ratios			
FO/BL (%)	27–40	37.4-47.5 (42.6) %	38.6
AT/BL (%)	0.5–4.8	1.6-9.2 (5.0) %	2.3
PT/BL (%)	22–30	18.6-26.4 (21.6) %	23
OV/BL (%)	13–19		14.0-14.6 (14.4)
Sucker-width ratio	2.11	1:0.93-1.22 (1.12)	1:2.52-2.62 (1:2.58)
Sinus-sac length /forebody length ratio			1:3.9
Ventral sucker to ovary			2.7:1
Posterior testis to ovary			1.28:1

FO/BL, forebody mean length as a proportion of body mean length; **PT/BL**, post-testicular field mean length as a proportion of body mean length; **AT/BL**, distance from ventral sucker to anterior testis as a proportion of body mean length; and **OV/BL**, post-ovarian field mean length as a proportion of body mean length.

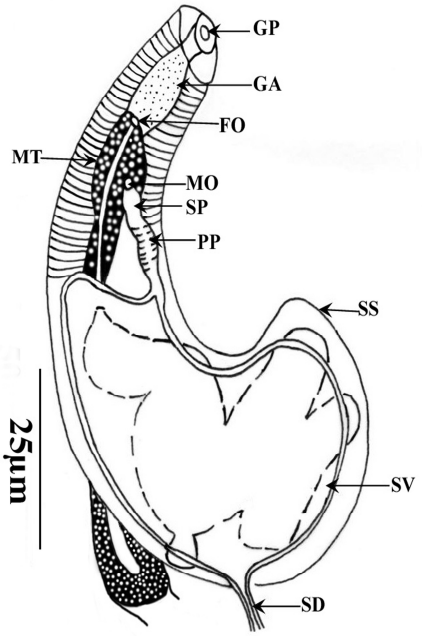

Fig. 3. Schematic drawing showing details of the terminal genitalia (Dorsal view of the sinus-sac). *Abbreviations*: GA, genital atrium; GP, genital pore; HD, hermaphroditic duct; FO, female genital opening; MO, male genital opening MT, metraterm; PP, pars prostatica; SD, spermiduct; SP, sphincter; SS, sinus-sac; SV, seminal vesicle.

As proposed by Gibson & Bary (1979) and Kostadinova *et al*. (2009), the parasite under discussion possessed the characteristic features of the genus *Magnibursatus* (Halipeginae). It was identified as, *M. diplodii* n. sp. due to; the possession of a large muscular ventral sucker which is substantially larger than the oral sucker; the position and space occupied by the sinus-sac in the forebody; the distribution of the uterine coils in the forebody; the relative length of the forebody; the position of the gonads; and having an anterior testis that well separated from the posterior one and from the ventral sucker by uterine coils.

Comparing the present material with the previously closed related species (table 1), It show that a relatively differences in measurements. After comparison of Kostadinova *et al*. (2003 & 2004) and Kostadinova & Gibson (2009)'s materials; and the present specimen, it was observed that most measurements of the present fluke has great deference in almost measurements; especially that of suckers, and testis and ovary (table 1).

M. diplodii n. sp. differs from *M. bartolii* Kostadinova *et al*. (2003), *M. blennii* Kostadinova *et al*. (2004), *M. caudofilamentosa* (Gibson and Køie, 1991) and *M. skrjabini*, Vlasenko (1931) in its slender body with markedly smaller dimensions (size of body and all organs), relatively short forebody and notably larger sucker-width ratio. The new species appears most similar to *M. minutus* Kostadinova, *et al*. (2003) and a form with similar body dimensions that described from a gobiid host in the Black Sea (Kostadinova *et al*., 2003). Moreover, The new species can be further distinguished from *M. minutus* by: (i) its distinctly smaller oral sucker (41-53×52-64(46×58) vs.63-75×46-88, ventral sucker (sucker-width ratio 1:2.52-2.62(1:2.58) vs. 1:1.86–1.94); (ii) more posterior located ovary (OV/BL 14.0-14.6 (14.4) vs. 13–19%); and (iii) much smaller eggs (10-15×5-9(13×7) vs. 22–30×11–15(25×13). The above differences and the considerable geographical and host separation justify, in our opinion, the distinct status of *M. diplodii* n. sp.

However, the present specimens measured are somewhat smaller (within the lower range for body size of *M. bartolii*) and this results in most metrical features varying within or below the lower limits reported for *M. bartolii* in Boops boops from the NE Atlantic by Kostadinova *et al*. (2003) (Table 1).

From the above we can concluded that the present derogenid; *M. diplodii*; is a new species and Sirt Coast is a new geographical area. Moreover, the results of the current and previously obtained can conclude that the restriction of some digeneans to only one host species relates to the transmission

modality of the infective stages of parasites and with the diet of the host (Bartoli, 1987b).

***Corresponding author**
Dr. Elsayed M. Bayoumy
Hydrobiology Department,
National Research Centre,
Dokki 12622, Giza, Egypt
E-mail**:** bayoumy2004@yahoo.com

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X-ray diffraction and IR spectroscopy for nano-sized ITO doped with some metal oxides

A.M. Youssef[1], H.A. Abbas[1,*], F.F. Hammad[1], A.M.A. Hassan[2] and Z.M. Hanafi[1]

[1]National Research Centre, Inorganic Chemistry Department, Dokki, Egypt.
[2]Al-Azhar University, Faculty of Science, Chemistry Department, Nasr city,Egypt.
hu_abbas2005@yahoo.com

Abstract: Nanocrystalline indium tin oxide (ITO) doped with 2, 4 and 6 mole % of CuO, Cr_2O_3, and ZrO_2 powder have been synthesized by pechini method. The crystalline structure of all the prepared samples was identified using XRD and IR spectroscopy. The morphology and average grain size of the prepared powder were determined using TEM and XRD. The effect of different dopant, different concentration, dopant cation valence and ionic radius on the crystalline structure, lattice parameter, crystallite size and strain were investigated. All samples have single cubic bixbyite phase structure except ITO samples doped with Zr. They have cubic bixbyite structure as predominant phase and traces of rhombohedral phase. Pure ITO sample has higher lattice parameter value than those of ITO samples doped with ZrO_2 and lower lattice parameter value than those of ITO samples doped with CuO and Cr_2O_3.
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Key words: Nano-sized doped ITO, Structural properties, IR, TEM

1. Introduction

ITO has been widely used in the microelectronic applications, including transparent heating elements for aircraft and car windows, solar cells, heat reflecting mirrors for glass windows, gas sensors, photovoltaic devices, in chemistry as a photocatalyst, biological systems and in a variety of electro-optical devices, such as liquid-crystal flat panel displays [1-8].

Generally tin-doped indium oxide (ITO) is a preferred transparent conductive oxide (TCO) material for these applications because of its favorable electrical and optical properties. One disadvantage of the use of ITO is its cost. It is not the most economical choice for mass production of devices utilizing TCO films [9].

However, some critical factors such as chemical and thermal instability and lower surface energy limits the wider application of ITO films [10, 11], and the optical-electrical properties of ITO films should be further improved.

Recently, indium tin oxides with improved properties have been intensely investigated, including the application of new deposition techniques such as ion-beam-assisted deposition [12], pulsed laser deposition [13] and the use of ultra high density ITO targets [14].

This paper is to complete our previous work where the effects of various concentration of SnO_2 on the structural, electrical properties and the sensitivity to the CO_2 gas of In_2O_3 were studied [15]. The present work aims also to study systematically the effect of co-doping of In_2O_3 with Sn^{4+} and different concentrations of various dopants such as Cu^{2+}, Cr^{3+}, and Zr^{4+} on the crystal structure, lattice parameter, crystallite size and strain in order to obtain the optimum condition required to prepare nano-sized powders that could be used in the gas sensing applications.

2. Experimental

Nano-sized powders of ITO co-doped with CuO, Cr_2O_3 and ZrO_2 were prepared by using the citrate technique base on pechini method [16] which is the most popular method used for preparation of metal oxides. In this method ethylene glycol and citric acid are used as gel former and complexing agent respectively. The starting materials were Indium (99%, Aldrich), $SnCl_4.5H_2O$ (99%, Aldrich), $ZrO(NO_3)_2.2H_2O$ (99.9%, Alfa), $Cr(NO_3)_3.9H_2O$ (98% QUALIKEMS Fine Chemicals), $Cu(NO_3)_3.3H_2O$ (99%, S d fine-chem limited), $C_6H_8O_7$ (99.5%, Pharmaceutical Chemicals) and $C_2H_4(OH)_2$ (99%, ChemPur). Citric acid (CA) was added to chelate metal cations at the CA: Me^{x+} molar ratio of 2:1. After 15 min of reaction, ethylene glycol (EG) was added into solution at a CA: EG molar ratio of 80:20. The colourless solution thus obtained, was then heated up to 150 °C, and kept under stirring to promote the esterification and polymerization reactions. After nitrous oxides and water elimination, a clear resin was obtained. The polymeric resin was charred at 250 °C to remove organic substances. The brown powder thus produced was ball milled and calcined for 18 h at 500 °C to obtain the nano-sized ceramic powder. Chemical analysis was performed on the previously prepared samples to adjust the stoichiometry. The crystalline structure of all the

prepared powder samples were identified using X-ray diffractograms provided with computer controlled X-ray diffractometer "formally made by The PHILIPS® MPD X'PERT diffractometer". The X-ray tube used was a Copper-tube operating at 40 Kv and 30 mA. X-ray diffractometer with a CuK_α radiation (λ= 1.5418 ˚A) the lattice parameter, the average crystallite size and the micro-strain were calculated using Rietveld software MAUD 2.074[17]. TEM pictures of the samples were obtained using a JEDL model 1230. FTIR spectra of the samples in the form of KBr pallets were recorded on (Japan FTIR-6100) at a spectral resolution 2 cm^{-1}.

3. Results and discussion

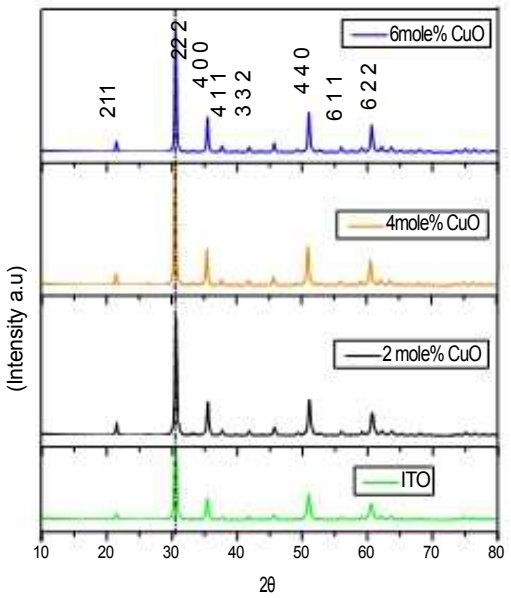

Fig.1: XRD patterns of undoped ITO and that doped with different concentrations of CuO.

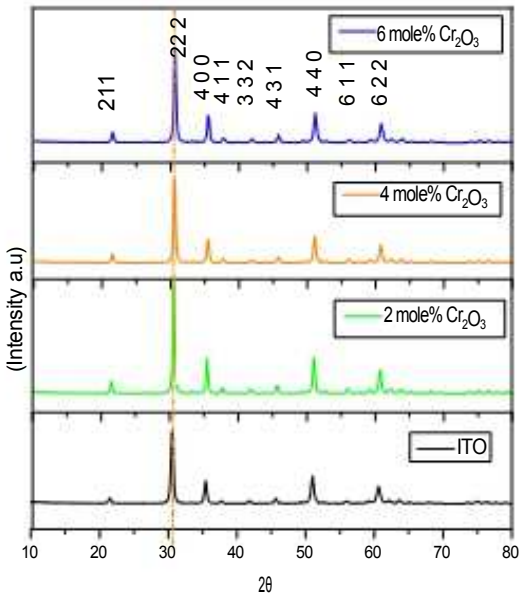

Fig.2: XRD patterns of undoped ITO and that doped with different concentrations of Cr_2O_3.

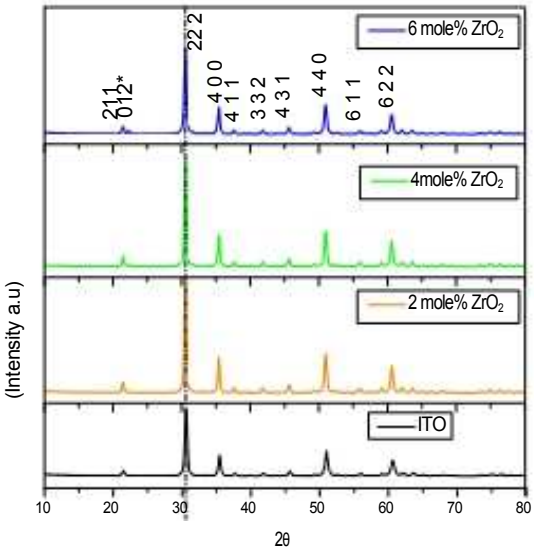

Fig.3: XRD patterns of undoped ITO and that doped with different concentrations of ZrO_2.

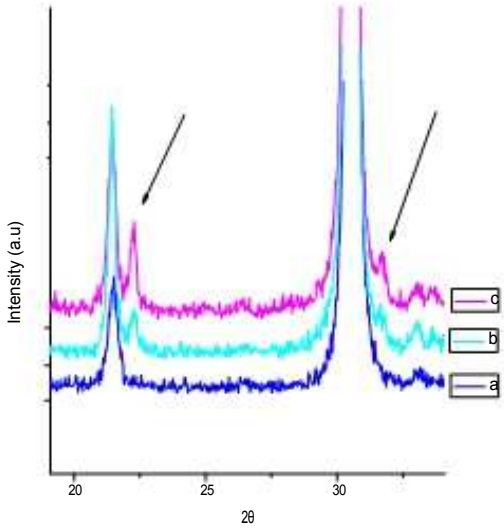

Fig.4: XRD pattern of a) undoped ITO b) ITO: 4Zr, and c) ITO: 6Zr.

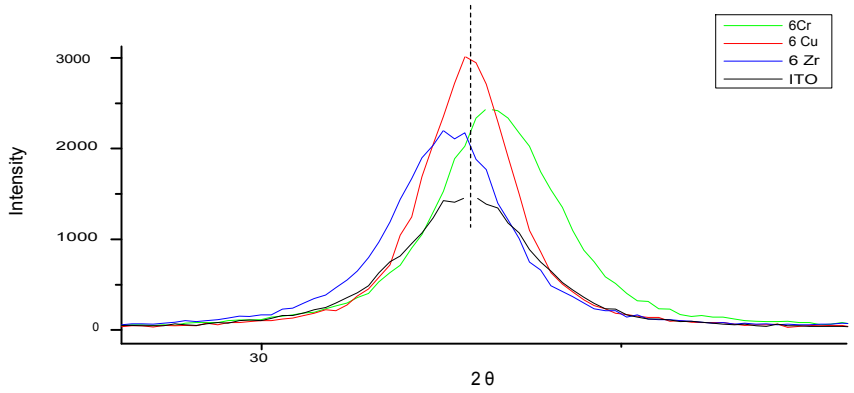

Fig.5: The XRD peak shift for undoped ITO, ITO: 6Cu, ITO: 6Cr, ITO: 6Zr.

Figs. 1-3 Shows the x-ray diffraction patterns of undoped ITO and that doped with different concentrations of CuO, Cr_2O_3 and ZrO_2 at room temperature. It is clear that there is no phases for tin oxide and no other impurity phases were observed. The undoped ITO has single cubic bixbyite structure.

For ITO:Cu system Fig.1, it is clear that all samples have single cubic bixbyite structure [18] and there are no phases were detected for CuO. The XRD peak intensity for all the ITO: Cu samples are higher than that of the undoped ITO. The XRD peaks are shifted almost to higher theta value and the peak broadening (FWHM) decreases as the CuO concentration increases.

The peak positions for ITO:Cr of a cubic bixbyite structure [18] are shown in Fig.2. All diffraction lines from the Cr-doped ITO could be indexed assuming the same bixbyite structure as pure ITO with no detectable impurity phase as Cr, CrO, Cr_2O_3 or CrO_2 up to 6 mol% Cr doping.

Fig.3 shows that for ITO:Zr system, there is mixture of two phases, where the cubic [18] bixbyite is the predominant phase and as the concentration of ZrO_2 increases, traces of the rhombohedral phase [19] starts to grow. There are no phases corresponding to ZrO_2 were detected. It is clear that the intensity and peak broadening for all samples increases with increasing of ZrO_2 concentration as shown in Fig.4.

Zhang et al [20] deposited ITO:Zr thin films on glass substrates by co-sputtering with an ITO target and a zirconium target. The averaged metal atomic ratio of ITO:Zr thin films was In:Sn:Zr= 9:1:0.2. All samples are crystallized in the cubic bixbyite structure of indium oxide.

There is slight shift of the peaks towards higher theta value as the concentration of Cr_2O_3 and CuO increases as compared to that of the ITO. The peaks are slightly shifted towards lower theta value than that of ITO for ITO:Zr system as shown in Fig.5.

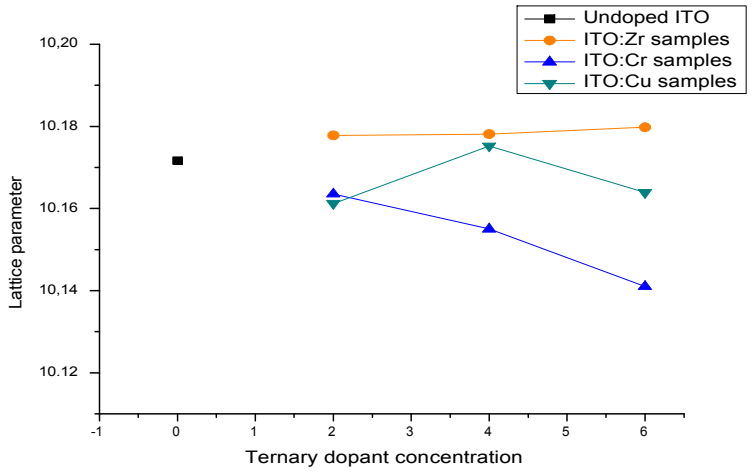

Fig.6: The lattice parameter of all the prepared samples as a function of ternary dopant concentration

Fig.6: shows the lattice parameter of all the prepared samples as a function of ternary dopant concentration. The lattice parameter values slightly increase for ITO:Zr system as ZrO_2 concentration increases and they are higher than that of the undoped ITO. For ITO:Cu system the lattice parameter increases till 4 mole % of CuO and above this concentration it decreases. This might be due to that In^{3+} ion is fully substituted by Sn^{4+}/Cu^{2+} and the addition of CuO oxide leads to decrease of the lattice parameter due to segregation of CuO along the grain boundaries. In future the concentration of CuO along the grain boundaries will be checked using EDX. When the Cr_2O_3 concentration increases the lattice parameter decreases. The ITO:Cr system exhibits the largest decrease of the cell parameter versus x with respect to ITO, in agreement with the much smaller average ionic radius of the couple Sn^{4+}/Cr^{3+} (0.65pm [21]), compared to In^{3+} (0.8pm [21]).

For ITO:Zr and ITO:Cu systems, both the size and the oxidation state have to be considered. Although, the average ionic radius of Sn^{4+}/Cu^{2+} couple (0.71pm [21]) is close to that of Sn^{4+}/Zr^{4+} couple (0.705pm [21]), the lattice parameter of ITO:Zr system is larger than ITO:Cu system. This may be discussed considering the electric neutrality requirements. Doping of In_2O_3 or ITO will be accomplished by introducing oxygen vacancies for electrical neutrality [22-24]. For ITO:Zr system one oxygen vacancy will be created for substitution of two In^{3+} ions by one Sn^{4+} ion and one Zr^{4+} ion. No oxygen vacancies will be created for substitution of two In^{3+} ions by Sn^{4+} ion and Cu^{2+} ion.

There is linear dependence of the a lattice parameter on dopant concentration in case of ITO:Zr and ITO:Cr systems. This result confirms the homogeneity of the substitution procedure of In^{3+} by Sn^{4+} and M ions within the studied range of composition where M=Zr^{4+}, Cu^{2+}.

Bizo et al [25] studied the substitution of In^{3+} by Sn^{4+}/M^{2+} couple in $In_{2-2x}Sn_xM_xO_3$ solid solutions where M=Ni, Mg, Zn, Cu and Ca. All the solid solutions have cubic bixbyite structure and the Ni solid solution has the lowest lattice parameter value. The highest lattice parameter was observed for Ca solid solution.

Kim et al [26] reported the structural characteristics of Cr-doped indium tin oxide (ITO) films grown on SiO_2/Si substrates by pulsed laser deposition. From XRD measurements, only films of cubic bixbyite structure were grown up to 20 mol% Cr doping. The lattice parameter decreases as the concentration of Cr_2O_3 increases.

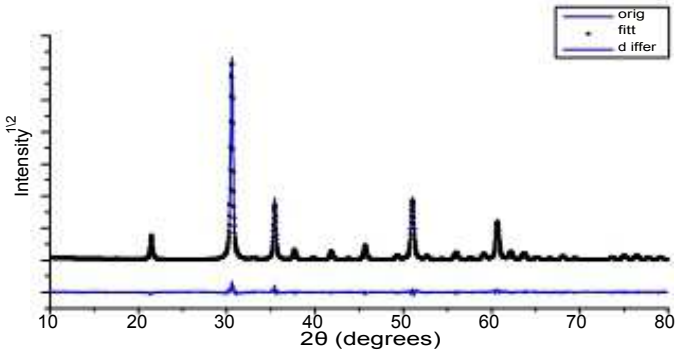

Fig.7: The results of individual profile fitting of ITO: 2Cr, Rietveld refinements (program MAUD) on powder diffraction patterns of products obtained after calcination of sample at 500 ˚C.

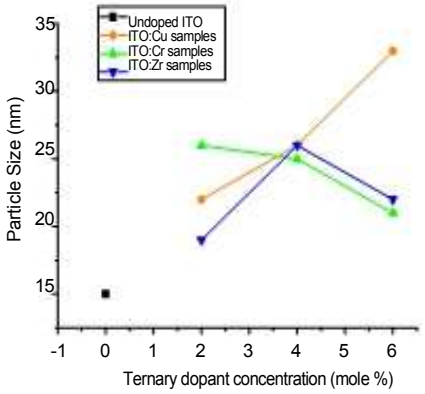

Fig.8: Particle size value as a function of concentration.

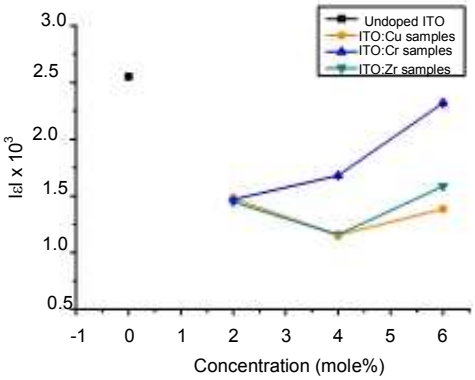

Fig.9: micro-strain (ε) value as a function of concentration.

Rietveld software method was employed to estimate the particle sizes and the strain of all the prepared powder samples [17]. Fig.7shows the results of individual profile fitting of ITO: 2Cu XRD powder pattern using Rietveld refinements (program MAUD).

It is clear that the effect of particle size as a function of concentration is not a systematic relation. The cubic grain size increases and then decreases as a function of dopant concentration for ITO:Zr. The cubic grain size increases in case ITO:Cu while it decreases in case ITO:Cr as a function of dopant concentration as shown in Fig.8. The cubic grain size for all the prepared samples is in the 19-33nm range.

Discussing the effect of dopant concentration on micro-strain $|\varepsilon|$ it is clear that the micro-strain value decreases and then increases in case of ITO:Cu and ITO:Zr systems while it increases in case of ITO:Cr system as the dopant concentration increases as shown in Fig.9. ITO:Cr system has higher strain values than that of ITO:Cu and ITO:Zr systems. This might be due to difference in average ionic radii of Sn^{4+}/Cr^{3+} couple and In^{3+} ion is higher than the difference in ionic radii of In^{3+} and Sn^{4+}/Cu^{2+} or Sn^{4+}/Zr^{4+} couples.

In conclusion, the dominant factor in line broadening may be due to the presence of strain resulted from addition of different concentrations besides the change in lattice parameter values which resulted from created defects.

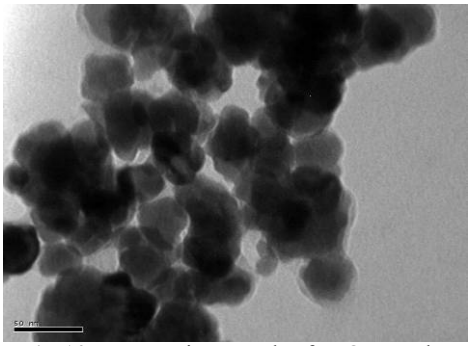

Fig.10: TEM micrograph of ITO sample

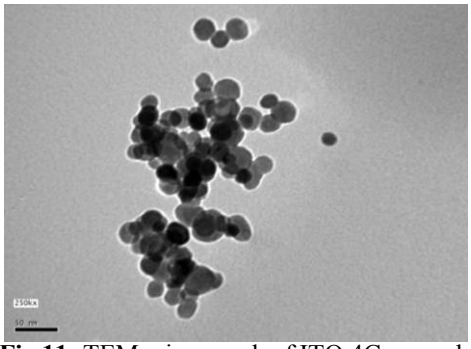

Fig.11: TEM micrograph of ITO:4Cu sample

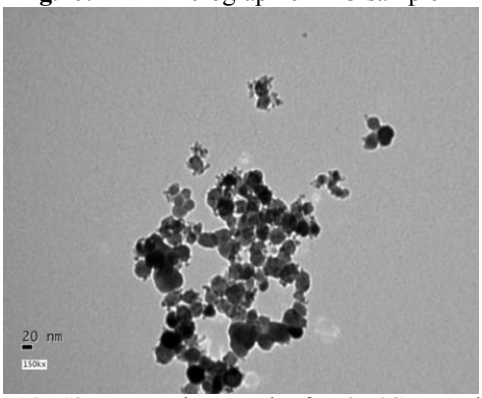

Fig.12: TEM micrograph of ITO: 4Cr sample

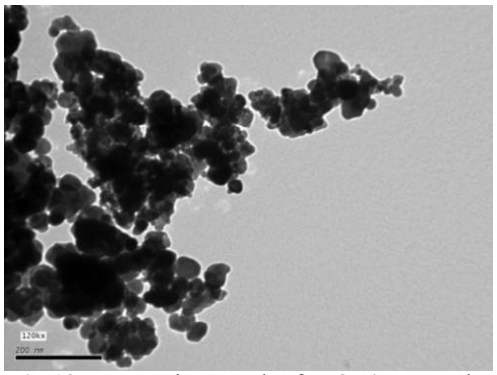

Fig.13: TEM micrograph of ITO: 4Zr sample

Figs. 10-13 show TEM image of ITO, ITO:4Cu, ITO:4Cr and ITO:4Zr nanopowder. It is shown that the particles of the samples are spherical in shape with 19-33 nm size range. The particle size obtained from TEM is nearly in the same range of that calculated form XRD pat-terns.

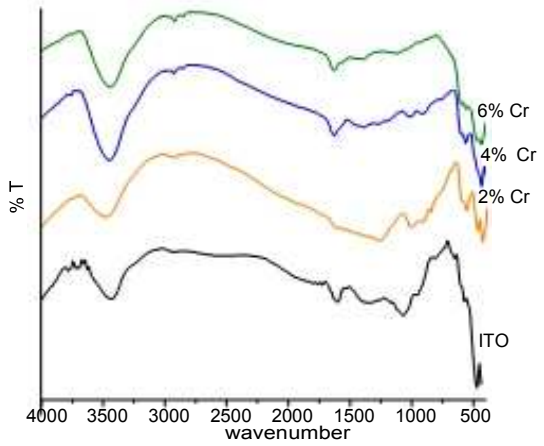

Fig.14: IR spectra of the undoped ITO and that doped with different concentrations of Cr_2O_3 all samples.

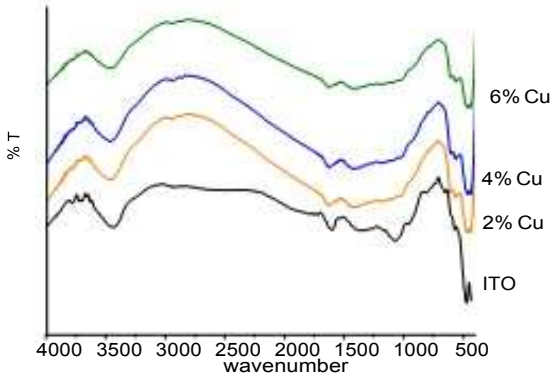

Fig.15: IR spectra of the undoped ITO and that doped with different concentrations of CuO all samples.

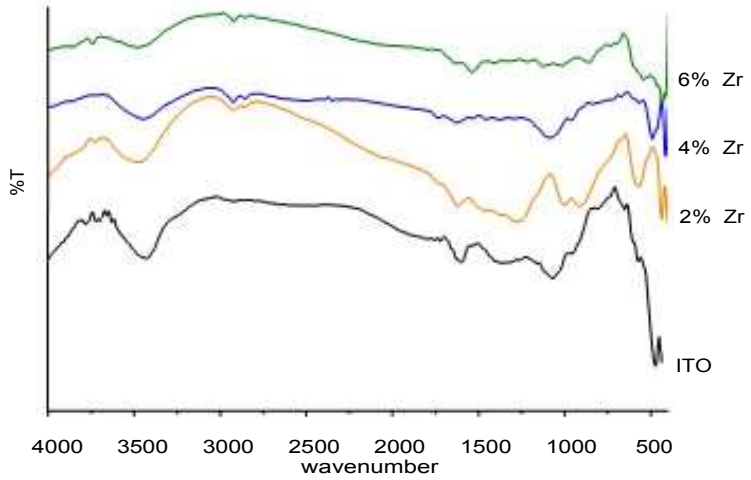

Fig.16: IR spectra of the undoped ITO and that doped with different concentrations of ZrO_2 all samples.

Figs. (14-16) show the IR spectra of undoped ITO, ITO:Cr, ITO:Cu and ITO:Zr systems in the range (400-4000 cm^{-1}). The bands present in the frequency range 400-800 cm^{-1} may be due to the stretching and bending vibrations predicted by a factor group analysis and lattice vibration [27]. To discuss the band appearing in this range on the above principle, some information on the crystal lattice of In_2O_3 must be given. In_2O_3 has the C-type cubic lattice and belongs to the space group T^{7h} (la_3)[28]. There are two coordination, the In(1) polyhedron is essentially regular and has a single metal bond oxygen distance d(1). The In(2) polyhedron is quite distorted with only 2-fold system, there are three pairs of metal-oxygen distances which vary considerable in length. The In-O bond lenghts are (6) $In_{(1)}$-$O_{2.18}$ **Å**, (2)$In_{(2)}$-$O_{2.13}$ **Å**, (2) $In_{(2)}$-$O_{2.19}$**Å**, (2) $In_{(2)}$-$O_{2.23}$ **Å**.

The large difference in the $In_{(2)}$-O bond lengths is attributed to an unequal distribution of the repulsive forces among the oxygen atoms that form the polyhedron around $In_{(2)}$[28].

For all the prepared samples, The band at about 520 cm^{-1} belong to γ-OH displacement [29] and the peak at about 560 may be attributed to Sn-O, Sn-OH and\or free carriers [30-31]. The bands at about 1080 and 1170 cm^{-1} may be assigned as δ(OH) of bridging and terminal hydroxyl group of In(OH), respectively[32] and/or may be due to M-O valence oscillation band. The peaks at 1385-1450 and about 1620 cm^{-1} belong to the absorbed water in the precursors [30]. The peaks observed at 1385-1450 cm^{-1} were assigned as other mode of vibration of δ(OH) of OH group bonded to In^{3+} and Sn^{4+}, respectively [32]. The two peaks at about 3150 and 3400 cm^{-1} were attributed to the OH group **[30].** Different authors [33-35] attributed the shift and/or broadening of Infra-red absorption band to the change of lattice parameter, lattice imperfections, the

variation of M-O bonding strength and/or change of free electron present.

The bands corresponding to addition of Cr or Zr oxides affect only on the band positions. This may be attributed to the occurrence of the defects created by the addition to the In_2O_3 crystal lattice [36-37]. For ITO:Cu, there is no shift in the band position above 4 mole% CuO as the concentration of CuO increases. This behaviour is previously discussed in the XRD part. The results obtained from the IR spectra are confirmed by that obtained from the XRD part.

4. Conclusion

Nano-sized indium tin oxide with different amounts of dopants (CuO, Cr_2O_3 and ZrO_2) was prepared by the citrate technique and studied with particular intention to dopant concentration, dopant cation nature, valency and radius**.** XRD patterns of ITO:Cu and ITO:Cr systems have cubic with bixbyite-type structure. ITO samples doped with Zr have cubic bixbyite structure as predominant phase and traces of rhombohedral phase starts to grow as the concentration of Zr increases. There is slight shift of the peaks positions as compared to the ITO as the dopant content increases for all the prepared systems. These shifts results from the difference between ionic radii of In^{3+} and Sn^{4+}/M couple where M=Cu^{2+}, Cr^{3+}or Zr^{4+} and this is reflected on the lattice parameter values for all the investigated systems. Particle size calculated from the XRD patterns and TEM measurement showed that all the prepared oxide samples are in the nano range (19-33nm). Results obtained from XRD analysis confirm that obtained from IR studies in the fact that the addition of dopants shifts the peak position either in XRD patterns and IR spectra for ITO:Cr and ITO:Zr and also confirmed that there is no shift in the peak position in case of ITO:Cu system for the addition of 6mole % of CuO .

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Perspectives on the relationship between invisibility, richness, plant size, seed production, seed bank and community productivity of invasive *Argemone ochroleuca* Sweet in Taif, Saudi Arabia

Sanaa, A. I. Moussa

Botany Department, Faculty of Science, Cairo University, Giza, Egypt
Present Address: Girl's Faculty of Science, Biology Department, King Abdulaziz University, Jeddah, KSA
sanaa_moussa1958@yahoo.com

Abstract: *Argemone ochroleuca* Sweet is an invasive desert weed species; a worldwide medicinal plant with economic potentialities. It is recently introduced ,in Saudi Arabia especially in Taif Governorate. The later is one of the largest areas, in the Southeastern of K.S.A. characteristic by its high diversity in local climatic and topographic conditions. Dryness is a characteristic climatic feature of the area since rainfall is less than 10 inches and a maximum temperature of 37^0C are reached in September. Temperature decreases the dryness in the angle of rain and evaporation, thus it affects the vegetation of the area . The large widespread of the species due to its high propagation ability encourages the studying of the inter-relationship between its invisibility and some of the biological characteristic features such as species richness of its communities, average plant size, seed production ability and *Argemone* stored seeds (number of seeds contained.$m^{-2}$of surface soil). Some of the chemical characters of the soil such as the organic matter content as well as the variations in topographic features of the different habitats could be main reasons for the variations in species productivity in the different habitats. Nine localities representing three different habitats; sand plains, dams& wadies were selected. *Argemone ochroleuca* is highly reproducible invasive desert weed; it produces a huge average number of 189 capsules, with a maximal number (258) at Al-Shafa and a minimal (162) at Jabajeb. An average number of 453 seeds per capsule- with a higher value (473) at Al-Shafa- was counted. An overall average number of 85, 850 seeds per individual were obtained. The immense number of seeds evinces the high propagation of the species. *A. ochroleuca* tends to inhabit the less fertile soil and reduces the native plant diversity in Taif. *A.* seeds stored in superficial soil (seed bank; SB) attained an average value of 7, 736. m^{-2}; the highest (13,600) at Jabajeb. Species richness; SR, was negatively correlated with invisibility (INV). The later was positively correlated with both plant size (PS) and seed production (SP), while negatively correlated with SR & SB. PS was positively correlated with INV and SR. A negative correlation was also between SR and all other variables. PS was negatively correlated with either SR or SB.

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Keywords: Reproduction, Organic Matter, Community variables.

1. Introduction

A. mexicana is a principal weed of beans and maize in Tanzania, cereals in Australia and India, cotton in Nicaragua, potatoes in India, tobacco in Argentina and Puerto Rico, and wheat in Pakistan (Holm *et al.,* 1977). It grows as a common weed in many crop fields such as, sugarcane, potato, tea, tomato and bean (*Phaseolus vulgaris*). It reduces the wheat grain yield (Rawson& Bath, 1980) as its seed is an undesirable contaminant in grain sold for seed or stock food, in turn; a high level of control is required. *A. mexicana* has an inhibitory effect on germination and seedling growth of vegetables (Hazarika & Sannigrahi, 2001) and weed residues may affect Bambara groundnut (*Vigna subterranea*) and sorghum (*Sorghum bicolor*) growth and development because of the inhibitory effects of allelochemicals present (Karikari *et al*. 2000). Grazing animals generally avoid this weed but can be poisoned if it is consumed in hay or chaff. The value of wool is decreased when contaminated by the prickly fruits of *A. mexicana* (Parsons & Cuthbertson, 1992). Harvesting by hand of low-growing field crops can be a painful experience in the presence of *A. mexicana*. Ownbey (2007) differentiates *A. ochroleuca* from *A. mexicana* on the basis of differences in flower bud shape and petal colour. Seedlings are readily controlled by light tillage. Long cultivated fallow or vigorous perennial pastures will control large infestations (Parsons and Cuthbertson, 1992). *A. mexicana* is an invasive weed of different aged soil dumps (Kumar *et al.,* 2011) and there is an increament in vegetation diversity as man precedes towards the upper aged soil dumps. Herbicides which control *A. mexicana* include 2, 4-D, 2, 4-DB, dicamba, diuron, fluroxypyr, hexazinone, isoproturon, karbutilate, MCPA, metribuzin, oxadiazon, picloram and terbutryn. A biological control programme of *A. mexicana* and of closely related *A. ochroleuca* has been initiated in Australia. This native of Mexico is naturalized in most warm countries of the world in sub-humid as well as semiarid regions.

Invasive species are one of the most significant threats to native species diversity, and identifying the factors that make places more or less invasible

has been one of the most important issues in the study of invasions (Wilcove *et al.,* 1998; Pimentel *et al.,* 2000). From a theoretical perspective, the reasons some communities are more invasible than others is a question intrigue ecologists (Cadotte *et al.,* 2009) because it underlines fundamental concepts in community ecology: species coexistence and assembly (Tilman, 2004). Serpentine systems attributed this behavior to the often extreme environment within these communities. Spatial heterogeneity, spatial scale (local or regional) and productivity are critical elements in understanding the invasibility of communities. Harrison& Cornell (2008) studied richness at either local or regional scales. Starzomski *et al.,* (2008) found that local richness did not depend on regional richness during any time of community assembly. Elton (1958) first proposed that a high richness of native species armors sites against invasion by making reasons less available to newly arriving species. Many studies supported this idea by detecting negative relationships between native and exotic diversity at small spatial scale – the scale of interaction between individuals - (Brown& Peet, 2003). Later authors revealed that competition from resident species has strong and significant effects on both establishment and performance of invaders. Native and exotic diversity could positively correlated only on larger spatial scale and furthermore the most diverse regions are often the most invaded, particularly for plant communities (Harrison *et al.,* 2006). Davis *et al.,* (2007) demonstrated that the relationship between native and exotic diversity flipped from negative to positive at scales at which spatial heterogeneity in the environment came into play.

The history of introduction of *A. mexicana,* now occupying large tracts of deteriorated rangelands in Asir region, KSA is not traceable. *A. ochroleuca* was most widespread in Taif area (Shorbaji & Abidin, 1999). The two species are growing in almost all types of soil and at different climatic conditions. In addition, all stages of growth can be observed in the same area at the same time of the year. Studies showed that noxious weeds also decrease wildlife forage quality (Medina, 1998) essentially needed by livestock. Therefore, determining *Argemone*'s rangeland distribution and its invasion ecology are essential for planning control measures of this weed. Recently, Moussa *et al.,* (2012) studied the vegetation in the different habitats in Taif, KSA and explained that the selected localities are of moderately diversed native 35 species belonging to 25 different families; the largest of which is Compositae. They added that *Argemone* has the highest importance value.

2. Materials and Methods:

During the year 2008/09 vegetation study of *Argemone ochroleuca* was undertaken in nine selected localities in Taif Governorate, KSA (Moussa *et al.* 2012) that lies in the desert and semi-desert region of the world. These localities represent different habitats that varied from: sand plains (as in Al-Shafa; 25 km Southwest of Taif, Jabajeb; Northwest of Taif& Al-Arafah; 35 km North of Taif), dams (as in Gadeer; 7 km South of Taif& Ekrima; 6 km Northwest of Taif) and wadies (such as in Thumalah & Wadi S'ab; 25& 5 km Southwest of Taif, respectively, Saysid and Jaleel; 14& 28 km Northwest of Taif, successively). Vegetation was studied in order to cover all vegetation variations in all directions around Taif and the importance value of the species - that represents the invisibility - was calculated. Richness as a measure of the species diversity (Pielou, 1975; Magurran, 1988) as well as the average *Argemone* plant size was estimated for the different localities. *Argemone* productivity tests (average number of flower buds, flowers& fruits per individual plant as well as the average number of seeds per fruit) were calculated. Finally, the average number of seeds produced per individual was estimated at all selected localities. Soil samples (five replications, 0.25 m^2 each) from the underneath of the plants were superficially collected, intermixed, air-dried then re-divided for homogeneity. Percentage of organic matter content (Walkley& Black, 1934) as well as organic carbon content (Page *et al.,* 1982), was estimated. Seed trapping activities determines the quality of invasive species arriving at each locality. So, seed bank (the number of *Argemone* seeds stored.m^{-2} soil) was evaluated using five replications of 5 g soil samples. Separation took place by the floating method using 40 % $CaCl_2$ and examination took place using an electric binocular (X 20). Because *Argemone sp.* has a tendency to multiply rapidly thereby choking the land to compete with other range plant species and reduce the land value, the variation between community parameters (invisibility; INV, species richness; SR, plant size; PS, seed production; SP, *Argemone* seed bank; SB& soil organic matter content as a measure for community productivity; OM& OC) in relation to the different localities was assessed using One-Way analysis of variance (ANOVA). Species richness as a measure of species diversity was calculated as the average number of species recorded in each locality. Relationships between community variables and each other were tested using simple linear correlation coefficient (*r*). The statistical package SPSS version 10.0 for windows was used for different statistical analyses..

3. Results and Discussion:

Argemone ochroleuca is an invasive worldwide medicinal plant with economic potentialities. *A. mexicana* is adapted to a wide range of habitats, including humid and semi-arid areas and a wide range of soil types. It occurs as a weed of arable land, pastures and in waste places, roadsides and fence rows. In East Africa it is

reported in grasslands and savannas (Lyons& Schwartz, 2001). It is known from sea level to elevations of 2900 m in Tanzania (Holm *et al*., 1977). Taif is the largest city in KSA, distinguishing by a strategic site. It lies between East and Southwest of KSA. It is also characterized by its mountainous topography and mild climate. The first documentation of the genus in Saudi Arabia was given by Migahid (1974). Hussein *et al.* (1983) found *A. mexicana* in different investigated desert areas in KSA. Currently, the two species were already identified in the same country (Collenete, 1985; Chaudhary & Al-Jowaid, 1999). *A. mexicana* now occupies large tracts in deteriorated rangelands in Asir region. *A. ochroleuca* is most widespread in Taif Governorate (Shorbaji & Abidin, 1999). Both species are distributed in the western and southern parts of the Kingdom, the former being unintentionally introduced from the New World. In the present study, nine locations in Taif representing different habitats; sand plains (open areas), dams and wadies were selected to detect hazardous role of *Argemone* as an invasive weed. Recently, vegetation strategies were studied at the same localities in Taif, KSA (Moussa *et al.,* 2012).

Fig. 1. *Argemone Mexicana*

Table (1): Productivity tests of *Argemone ochroleuca* plants at the different localities.

Locality	Av. no. of flower buds / indiv.	Av. no. of flower / indiv.	Av. no. of fruits / indiv.	Av. no. of seeds / fruit	Av. no. of seeds / indiv.
Al-Shafa	21	12	258	472.7	122,160
Jabajeb	18	12	162	435.5	70,515
Al-Arafah	20	10	223	460.7	102,558
Gadeer	20	16	163	463.3	75,438
Ekrima	20	16	215	440.1	94,708
Thumalah	19	12	170	466.9	79,185
Wadi S'ab	22	11	164	433.0	71,188
Saysid	16	16	167	447.0	74,644
Jaleel	18	14	179	458.6	82,257
Mean value	19.3	13.2	189	453.1	85,850

A. ochroleuca is a highly reproducible invasive desert weed (Table 1). By the end of 2009, estimates of the species productivity revealed that the number of flower buds ranged from a lowest value of 16 (in Saysid locality) to a highest of 22 (in Wadi S'ab locality) per individual plant. Meantime, the number of produced flowers ranged from 10 (as a lowest value in Al-Arafah locality) to 16 (a highest value, simultaneously monitored in Gadeer, Ekrima& Saysid localities). The species produces huge numbers of fruits that ranged from a lowest of 162 (at Jabajeb) to a highest of 258 per individual (at Al-Shafa). The later locality was also distinguished by giving a maximized number of 473 seeds per fruit whereas a minimal number of 433 seeds was given at Wadi S'ab locality. So an overall maximal number of 122,160 seeds per individual *Argemone* plant were rained at Al-Shafa locality while a minimal overall number of 70,515 seeds were detected at Jabajeb. It was observed that seeds produced in large quantities tend to fall near the parent plant producing dense stands. The plant is known to break off at the base and be windblown for long distances helping to disperse seeds. The immense numbers of produced seeds evinces the high propagation of the species. Seed production of *Argemone* varies throughout the world. Mauritius reports the greatest seed production with an average of 60 to 90 capsules per plant with 300 to 400 seeds in each capsule (Holm *et al.,* 1977). They added that most seeds fall around the base of the parent plant where they form a carpet of seedlings. Dispersal occurs in surface water and in mud adhering to farm machinery and the feet of man and livestock. Seeds are readily eaten by a number of bird species in Puerto Rico as indicated by the presence of many seeds of the species in birds' stomachs (Barnés, 1946). In Ethiopia, most seeds do not normally germinate the year after shedding. Instead they enter the seed bank and seedlings establish, even in well-maintained field, probably for many years (Karlsson *et al.*, 2003). Number of seeds buried in superficial soil layer (seed bank of the species) attained a mean value of 7, 7 36. m^{-2} that ranged from a minimal value of 3,000 at Ekrima (dam topographic habitat) to a maximal value of 13,600 at Jabajeb (an open sand plain habitat). An intermediate mean value of 7, 275 seeds was recorded in wadies of Thumalah, S'ab, Saysid and Jaleel. On raining, water is stored behind the dams that help in dispersing most of seeds on flooding, thus attaining the lowest seed bank values. The intermediate values found in wadi habitats might be a result of water runoff that carries seeds from the surrounding higher terraces and settles them down the wadies. The possession of maximized seed bank value by the sand plain topographic habitat ensures the intensive receive of seeds arriving by different dispersal means. Plant size (PS) varied at the studied localities; the largest of which (5.8 m^3) was detected at Al-Shafa, while the smallest (3.7 m^3) was distinctive at Gadeer. An average value (4.2 m^3) was recorded.

Along with spatial scale, site productivity likely affected the invisibility of communities and thus the relationship between native and exotic diversity, (Davis *et al.,* 2007) especially at small scales, where competitive exclusion potentially varied with site productivity. Authors continued that productive sites had a common positive relationship between native and exotic diversity, whereas unproductive sites had a common negative relationship. Generally, in Taif, *Argemone* tends to inhabit the less fertile (less productive) soils, having low organic matter and organic content (Table 2). Former parameter varied from a least value of 0.55 (at Al-Shafa) to a highest of 3.75 % (at Jabajeb) while the second one ranged between 0.24 (also at Jabajeb) and 1.67 % (at Al-Shafa). This comes in concordance with Parsons& Cuthbertson (1992) who mentioned that the species tends to grow best in soils of low fertility. They added that in Australia, it is peculiarly adapted to colonize derelict areas low in phosphorus. *A. mexicana* is better suited to grow at sites deficient in nitrogen whereas the closely related *A. ochroleuca* does better where phosphorus is limiting (Ramakrishnan & Gupta, 1972). Moussa *et al.* (2012) reported that slight alkalinities as well as complete lacking of carbonates are characteristic features for the studied localities. They added that high EC is expressed at Jaleel and owed that trend to the possession of higher contents of Ca^{+2}, Cl^- & SO_4^{+2}. They continued that highest Mg^{+2}content is detected at Ekrima, while Na^+ is exceedingly measured at Thumalah. Neither species appear to have obvious restriction to particular agronomic or environmental situations (Karlsson *et al*., 2003). In southern India it occurs up to an altitude of 800 m a.s.l. and when growing in undisturbed land, it can produce fresh weights of 6-9 t/ha but, in cultivated land, it is generally not an aggressive competitor (Holm *et al*., 1977).

Argemone invisibility (INV); expressed as the importance value; varied with the variation in localities. The highest invasibility (180) was detected at Al-Arafah, followed by 150 at Al-Shafa, while the lowest value (97) was recorded at Wadi S'ab. The remaining localities attained intermediate values. Species richness showed negative correlation (Tables 2& 3) with the invisibility; as the former decreases with increasing the later and vice versa. To-date little is known about the impact of *Argemone* on biodiversity. Kumar and Rohatgi (1999) postulated that the species decreases biodiversity in India.

Community productivity; expressed as organic matter and organic carbon content (Table 2) was moderately detected (3.75& 1.67 %, respectively) at Jabajeb where the least species richness (2.6) was recognized. Hodgson *et al.* (2002) found a positive relationship between diversity and productivity and

a negative diversity – invisibility, productivity – invisibility relationship among bacterial colonies.

One Way ANOVA test showed significant differences (F-ratio = 0.00; sign. =6.51, 22.09, 5.38& 34.99) between invisibility (INV), species richness (SR), plant size (PS) & seed bank (SB) values of the nine localities (Table 2). The highest value of invisibility (180±26.07) was found at Al-Arafah locality, while that of species richness (9.07±2.12) was attained at Gadeer. Al-Shafa was distinguished by possessing the largest plant size (5.72±0.76) as well as the highest seed productivity (122,160±11,258). Higher organic matter& organic carbon (3.75; 1.67%, respectively) were detected at Jabajeb.

The calculation of correlation coefficient (*r*) between the different community variables (Table 3) indicated that invasibility (INV), plant size (PS) & seed productivity (SP) had the highest number of correlations with high significant positive correlations between each other. Correlation between INV, SR, PS, SP& SB and each other (Table 3) indicated that INV positively correlated with both plant size (PS) & seed production (SP). Plant size (PS) positively correlated with invasibility (INV) & seed productivity (SP). The later (SP) positively correlated with invisibility (INV) & plant size (PS).On the other side, there was a negative correlation between INV, SR& SB. A negative correlation was also obtained between SR& all other variables and also between SP, SR& SB. Plant size (PS) also negatively correlated with seed bank (SB).

Table (2): Means of *Argemone* variables (±S.D) at the nine studied localities ; INV, Invasibility ; SR , Species Richness ; PS , Plant Size ; SP , Seed Productivity ; SB , Seed Bank ; OM, organic matter content; OC, organic carbon content; SD , Standard Deviation.

Argemone variables	Locations									Total ± SD	F-ratio	Sig.
	1	2	3	4	5	6	7	8	9			
INV (Invasibility)	150 ±32.37	120 ±17.13	180 ±26.07	101 ±15.64	138 ±17.16	110 ±22.23	97 ±36.63	109 ±20.64	120 ±10.61	125.0 ± 33.13	6.51	0.00**
SR (Species Richness)	3.07 ±0.96	2.60 ±2.31	4.07 ±1.28	9.07 ±2.12	7.13 ±2.23	7.73 ±1.87	6.20 ±2.51	7.20 ±2.08	7.93 ±1.39	6.11 ± 2.86	22.09	0.00**
PS (m^3) (Plant Size)	5.72 ±0.76	3.88 ±0.68	4.18 ±0.28	3.66 ±0.54	3.95 ±0.49	4.63 ±0.75	3.70 ±0.73	4.10 ±0.64	4.25 ±0.41	4.23 ± 0.82	5.38	0.00**
SP (seed/indiv.) (Seed Productivity)	122,160 ±11,258	70,516 ±19,935	102,558 ±13,540	75,439 ±11,585	94,708 ±17,184	79,185 ±17,228	71,189 ±14,887	74,644 ±17,105	82,257 ±36,720	85,851 ±41,108.	0.87	0.55 n.s
SB (seed.m^{-2}) (Seed Bank)	7,100 ±1,387	13,600 ±2,219	6,700 ±908	10,120 ±756	3,000 ±353	7,200 ±1,095	8,600 ±1,025	5,700 ±570	7,600 ±418	7,736 ± 2,978	34.99	0.00**
OM (%)	0.55	3.75	2.41	2.00	0.78	0.73	3.44	1.56	0.94	1.79		
OC (%)	0.24	1.67	1.07	0.89	0.35	0.33	1.53	0.70	0.42	0.80		

Numbers from 1-9 are the studied localities (1,Al-Shafa ; 2, Jabajeb ; 3, Al-Arafah ; 4, Gadeer ; 5, Ekrima ; 6, Thumalah ; 7, Wadi S'ab ; 8, Saysid ; 9, Jaleel; (**), significant at 1%.

Table (3): Pearson's - product moment correlation coefficient (*r*) between the estimated community variables. For variable abbreviations& units, see Table (2).

Variables					
INV.		-0.590	0.420	0.801**	-0.324
SR	-0.590		-0.404	-0.479	-0.274
PS	0.420	-0.404		0.778*	-0.225
SP	0.801**	-0.479	0.778*		-0.447
SB	-0.324	-0.274	-0.225	-0.447	
	INV	SR	PS	SP	SB

** Correlation is significant at the 0.01 level (2-tailed), * correlation is significant at the 0.05 level.

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Ultrastructural Changes Occur In Mice Lungs after Cessation to Exposure of Incense Smoke

Sammar Omar Rabah.[1] and Sahar Ragab El Hadad [1,2]

[1]Biology Department, Faculty of Science, King Abdulaziz University, Jeddah, KSA
[2]Genetic Engineering Research and Bioinformatics Center, VACSERA, Egypt
saharelhadad@hotmail.com

Abstract: Background: Incense woods are special kind of trees called Agarwood, which characterized by good smelling odors and many medical benefits. Incense smoke is heavily used in Saudi Arabia although comprehensive studies of its effects on health are limited. The present study demonstrated lung ultrastructure changes of mice after exposure and cessation to Incense smoke. Eighty mice are divided equally into four groups, three groups are exposed to different concentrations of Incense smoke (2, 4 and 6 gm) for three months, while the fourth group is control one. At the end of each month, lungs of five animals from each group are gathered, while the last five animals from each group are kept for another 60 days without exposure to the Incense smoke to allow for recovery.
Results: Transmission electron microscope investigations of all exposed groups showed hypertrophy and hyperplasia in Clara Cells and some an enlargement of the macrophage to the point that it fills a large part of the alveolar lumen. Scanning electron microscope marks presence of mucus materials attached to the epithelial bronchioles. After prevention of exposure to the Incense smoke for 60 days, necrosis and degeneration in some cells of epithelial bronchioles, fibrosis of peri-bronchial, thickening in alveolar walls and aggregation of lymphoid cells were demonstrated. **Conclusion:** Based on the above findings and other related studies (not published), we conclude that exposure to Incense smoke causes harmful effects due to sever changes in pulmonary ultrastructure, such effects do not disappear even when Incense smoke inhalation was stopped. Therefore, we recommend that Incense smoke should use only in open places to reduce its harms.
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Key words: Incense smoke, lungs, ultrastructure of lungs, Agarwood

1. Introduction

Incense woods are special kind of trees called *Aquilaria agallocha* and *Aquilaria malaccensis*. These trees produce wood called Agarwood, which is characterized by good smelling odors [1]. This wood has variable benefits as producing Arabian Incense or as traditional medicine against many diseases [2]. Many countries used to use this kind of Incense wood in their temples whereas there are no good air circulation such as Bangladesh, Bhutan, India, Indonesia and most countries of South East Asia [3]. Previous study was conducted to examine the environmental situation and its impact on the health of workers employed in factories producing Incense in India. This study has recorded the presence of some symptoms such as pain in the chest and stomach, runny nose, cough, and quiver in hands, and this consider as the most common disease of workers factories producing Incense [4].

Burning of Incense smoke produced very small particles similar to those particles produced from the burning of tobacco [5, 6]. Many previous studies reported that Incense smoke containing particular matters, carcinogenic material, and polyaromatic hydrocarbons (PAHs) [7]. Smoke produced from Incense wood burning consider one of the most important factors of air pollution, which has a negative impact directly on infants and children [8, 9], caused increases the risk of some lung diseases such as asthma [10]. Daily use of incense smoke at Saudi population consider as risk factor for chronic obstructive lung disease [11]. In addition to, some studies conducted on both the cellular and physiological changes showed direct influence of respiratory organs especially lung as a result of persistent exposure to incense smoke [12, 13]. Furthermore, many histological studies discussed and recorded severity changes in lung tissues of rats after continuous exposure to Incense smoke. These changes ranged from, moderate inflammation, and lymphocytes infiltration [14], to severe changes including lung carcinoma [15].

In the present study, we investigated the ultrastructure changes due to continuous exposure to Incense smoke on mice lungs and its harmful effect. In addition, we planned to study if the observed ultrastructure changes enhanced by stopping exposed the same mice groups to Incense smoke for recovery. This research derives its importance from the fact that Incense is heavily use in Saudi Arabia in the absence of comprehensive studies of its effects on health.

2. Material and Methods

Mice:

Six to eight-week-old female BALB/c mice were raised and maintained throughout experimentation in the Center of King Fahed for Medical Research at King Abdel Aziz University, Jeddah, KSA, and maintained under standard laboratory conditions including diet and temperature of 22^{O}C (+/- 2) with continuous supply of water. Its body weights were almost 25-30 g.

Incense smoke used in the experiment:

Incense smoke was used in this study was bought from Jeddah local market, and it was burned using cubes of artificial coal.

Mice exposed to Incense smoke:

Eighty female BALB/c mice were divided into four groups. Group of twenty mice Group I was comprises of twenty mice and kept in open air along the experimental period. The remaining 60 mice were divided equally into three groups (II, III, IV), and were exposed daily (except Friday) for three months to different concentrations of Incense smoke (2, 4 and 6gm) respectively. A box was used to expose mice to different concentration of Incense smoke individually and to make them inhaled it.

Ultra structure studies:

Transmission electron microscopy:

Tissues samples were fixated in 2.5 % glutaraldehyde and 2% paraformaldehyde in 0.2 M sodium cacodylate buffer at pH 7 2. The lung slices were chopped into blocks, washed in 1 M sodium cacodylate buffer and post-fixed in 2 % osmium tetroxide for 30 minutes. After blocks specimens were washed by second buffer, they were dehydrated in a series of 70, 96 and 100 % ethanol solutions. After dehydration in graded alcohol solutions, samples were cleared in 2 changes of propylene oxide for 8 minutes and embedded in pure resin for 30 minutes. All samples sections were cut on an ultra microtome and the 1 micron thickness of the transverse sections of lungs were stained with 1% touledine blue stain at 95^{o}C for transmission ultra microscope investigation [22].

Scanning Electron Microscope:

Tissue samples were fixed in 3% Gluteraldehyde and 2% osmium tetroxide. Then they were dehydrated in graded series of ethyl alcohol, then immersed in acetone and infiltrated in a mixture of acetone and hexamethyldisilazana (1:1.v:v) The dried samples were fixed on the specimen stubs and were coated with a gold stain [22].

3. Results

Mice that are not exposed to Incense smoke (Group I)

Transmission electron microscope sections of group I showed lining epithelium composed of two types of cells; the first one is non-ciliated cells which are long cells characterized by large number of variable sized mitochondria and the cell membranes were mostly closely in contact. The second is ciliated cells, which are short cells with large vesicular nucleus and few cell organelles having cilia and embedded in between the non-ciliated cells (Fig. 1a). In addition, lung sections of control group showed the septal capillaries containing red blood cells and the other type is the alveolar cells which is characterized by large in size, bulging in the alveolar lumen having microvilli. The cells contained vesicular nucleus and the cytoplasm included moderate number of variable sized mitochondria as well as a few lamellar bodies (Fig. 1b). Scanning electron micrograph of the surface epithelium of the bronchiole showed that non ciliated cells have small microvilli while the ciliated cells have long cilia (Fig. 2a). Also, the surface of alveolar lumen of control group is showing smooth elongated cells (type I), and large cell with numerous variable size microvilli (type II) and the presence of red blood cell attached to the wall of the alveoli (Fig. 2b).

Ultrastructure changes on mice lungs after exposure to different concentrations of Incense smoke

Manifestations in mice lungs after daily exposing to 2 gm of Incense smoke have been illustrated by both transmission and scanning electron microscope. Transmission electron microscope sections illustrated that most epithelial cells were Clara cells (non-ciliated cells). The Clara cells were greatly hypertrophied and contained large number of variable shape and size mitochondria. Numerous electron dense granules as well as numerous dilated smooth endoplasmic reticulums were present at the periphery of Clara cells. Other lining cells were ciliated and appeared embedded in between the hypertrophied Clara cells. Fibroblast cell were present under the bronchiolar basement membrane and the bronchiolar capillaries (Fig. 3a). In addition, macrophage were containing numerous elongated cytoplasmic processes and the septal capillaries contain RBCs were lining by endothelial cells (Fig. 3b, 3c). Furthermore, alveolar cells types II have surfactant granules, large macrophage and numerous crystals beside other cell organelles embedded in mucin matrix (Fig. 3c). Scanning electron micrograph of lung sections of group II that continuously exposed to 2gm of Incense smoke for 90 days showed presence of mucinous secretion attached to the epithelial lining in mice lung sections. Swollen and separation from the neighboring cells

were demonstrated in non-ciliated cells while the cilia of the ciliated cells adhered to each other and non-erected (Fig. 4).

Exposing mice daily to 4gm of Incense smoke for 90 days (Group III) illustrated the same changes of group II in addition, the presence of basal cell and ciliated cells, which embedded in a numerous hypertrophied Clara cells (Fig. 5a). Also, alveolar lumen showed presence of large elongated, large number of variable sized mitochondria, some phagosomes, membranous vacuoles and rough endoplasmic reticulum. The cells have large cytoplasmic processes and alveolar capillary contains RBCs (Fig. 5b). Lung alveoli showed some macrophage cells characterized by presence of large number of cytoplasmic processes and the cytoplasm contains numerous light electron dense needle like crystals (Fig. 5c).

Moreover, mice exposed to 6 gm of Incense smoke for 90 days (Group IV) showed numerous changes of the lung bronchiole as rupturing of some Clara cells associated with releasing the cytoplasmic contents in the lumen which having electron dense granules. Other Clara cells were very rich with secretory endoplasmic reticulum, mitochondria that found mainly related to the cell membrane (Fig. 6a). Also some peri-bronchial fibrosis was manifested by the presence of fibroblast cells and large amount of collagenous fiber bundles (Fig. 6b). In addition, the hypertrophied Clara cells filling the alveolar lumen was observed in some sections. The mitochondria of the Clara cells were swollen and the cytoplasm contains a numerous membranous vacuoles having electron dense material (Fig. 6c).

Ultrastructure changes in Mice lungs left for recovery for 60 days

Mice lung sections, which daily exposed to 2 gm of Incense smoke for 90 days and left for 60 days for recovery showed numerous ciliated cells that adhered to the basement membrane in micrograph of the terminal bronchiole that have shrunken nucleus with condensation of the nuclear chromatin. The cytoplasm contains large number of variable sized vesicle. The non-ciliated cells were large and contain large number of variable sized and shaped mitochondria. Some cells appear to have three nuclei one of them is large and vesicular, while the other is compact banana shape and the third appears spherical contains nucleolus but the nuclear sap appears more electron dense than normal. The lumen contains mucinous secretions. The collagenous connective tissue forming the lamina propria of the bronchiole was observed under the basement membrane (Fig. 7a, 7b). By using Scanning electron micrographs of the surface of bronchioles showing the non ciliated cells have microvilli and some of them appeared finger like separate from the other cell and some of them adhered to each other (Fig. 8a). Otherwise, surface of bronchioles show some non ciliated cells appeared as tongue shaped while other ciliated cells have large number of non-erected cilia. Several spherical bodies adhered to the surface of both ciliated and non ciliated cells on the surface of the bronchioles (Fig. 8b). Micrograph of the alveolar surface of the lung showing a wide lumen and the lining cells were flat and branched with thickening of the alveolar septa (Fig. 8c).

Further studies was carried out on lung sections belonging to group III that left for 60 days for recovery were reported that the lining epithelium of terminal bronchioles were varies greatly in height while the non-ciliated cells or Clara cells were long and contain vesicular nucleus. The cytoplasm contains large number of mitochondria, which varies greatly in size and shape. In addition, the same group is showing the presence of some electron dense secretory granules at the periphery of the cells. Ciliated cells seemed to be short and contain numerous vesicles and characterized by the presence of peri-nuclear spaces with dentations of the nucleus. The sub-epithelial layer formed by collagenous connective tissue (Fig. 9). Scanning electron micrograph of the alveoli shows compression of the lumens and the septa show thickness. The alveolar cells formed by long and flat pneumocytes having variable size and long microvilli (Fig. 10).

Ultrastructure study of bronchial epithelium belonging to mice that daily exposed to 6 gm of Incense smoke and left for 60 days showed degenerative changes represented by shrinkage and condensation of the nuclear chromatin in the nuclei of some ciliated cells as well as non-ciliated ones. The lumen contains mucin secretion, secretory granules and deciliated cilia (Fig. 11a). Furthermore, the bronchial epithelium belonging to the same group is showing some Clara cells in state of necrobiosis associated with fragmentation of the nuclear chromatin and the cytoplasm contains large number of minute vesicles. Other cells appear electron dense either in the nucleus or in the cytoplasm with presence of numerous light electron granules seemed to be mucin granules. The other cells either ciliated or non-ciliated showing the presence of large number of vesicles varies in size (fixation artifact) all nuclei having pei-nuclear spaces (Fig. 11b). Also the lamina propria formed by a thick layer of collagenous connective tissue in the epithelial lining showing both the non-ciliated and ciliated cells in state of necrobiosis which manifested by presence of large inter-cellular vesicular spaces and presence of numerous SE vesicles in the cytoplasm as well as

mucinous granules. The ciliated cells showed compact and more electrons dense with deciliation of the cilia (Fig. 11c), and the septal wall belonging to group IV showing bundles of collagen fibers in the septal wall (Fig. 11d). In addition, high magnification of the previous photo belonging to group IV showing thickening of the basement membrane with light electron dense deposit in some areas appears fibirillar. The pneumocyte type II, Clara cells have greatly swollen mitochondria, which occupy all the cells and some of them is destructed and change to vesicles (Fig. 11e). Otherwise, scanning electron micrograph belonging to this group showed great thickening of the septal wall of some alveoli with dilatation of the lumen and compression of others (Fig. 12a). Also, number of ciliated cells is observed as they increase in relation to non-ciliated cells. Furthermore, the presence of wide spaces between the lining cells, with presence of some spherical bodies adhered to the surface of the non-ciliated cells was observed (Fig. 12b). High magnification of the bronchiole belonging to the same group are showing the ciliated cells long non erected cilia and the non ciliated cells have variable sized and shaped microvilli with presence of spherical bodies attached to the surface, while, wide werea of peri-bronchial collagen fibers in the lung lobule is reported in some sections (Fig. 12c).

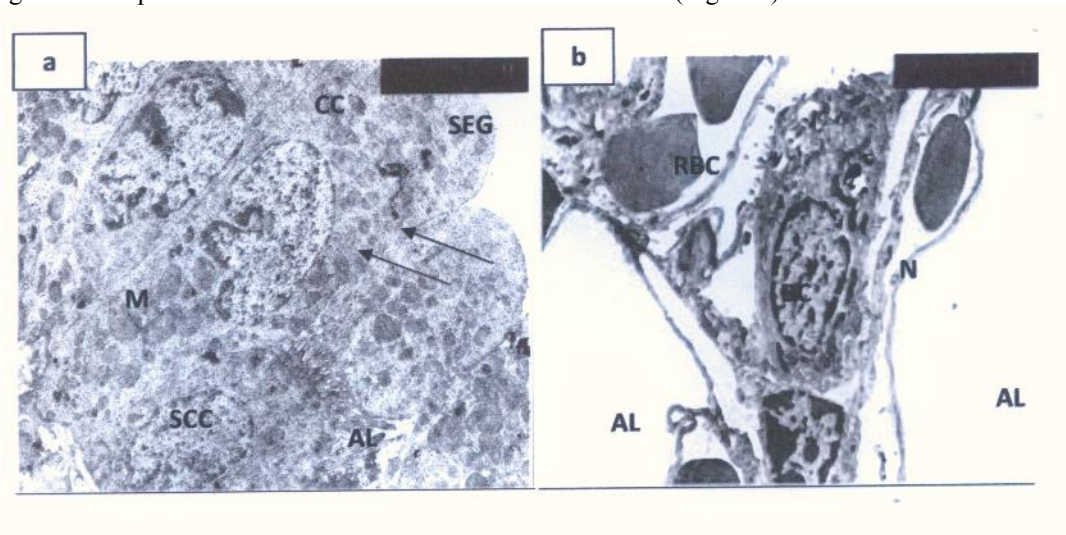

Fig. 1. Transmission electron micrographs of the terminal bronchiole of lung belonging to control group
(a) The lining epithelium formed by two types of cells non-ciliated cells (CC). Large number of variable sized mitochondria (M). The cell membranes ( ) are mostly closely in contact with each others. Ciliated cells are short cells with large vesicular nucleus (SEG) and few cell organelles having cilia and embedded in between the non-ciliated cells (SCC). Mag. 8800X.
(b) The septal capillaries containing red blood cells (RBC) and alveolar lumen cells (AL), the nucleus of the cell (N) is vesicular and the cytoplasm contain both moderate number of variable sized mitochondria (M) and few lamellar bodies (LB). Mag. 8800X.

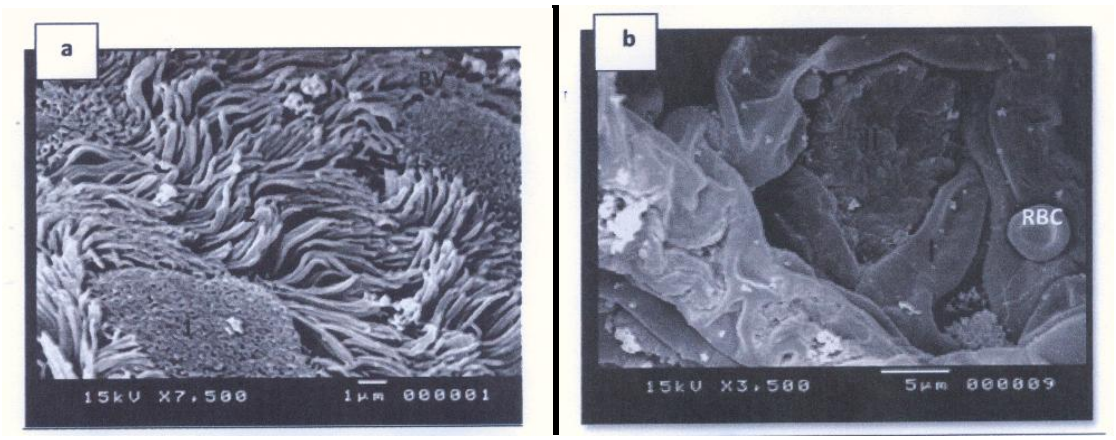

Fig. 2. Scanning electron micrographs of the surface epithelium of the bronchiole belong to control group.
(a) Non ciliated cells contain small microvilli (I) and ciliated cells have a long cilia (II).
(b) Smooth elongated cells (type I), and large cell with numerous variable size microvilli (type II). Note presence of red blood cell (RBC) attached to the wall of the alveoli and Alveolar wall and the alveolar lumen (AL).

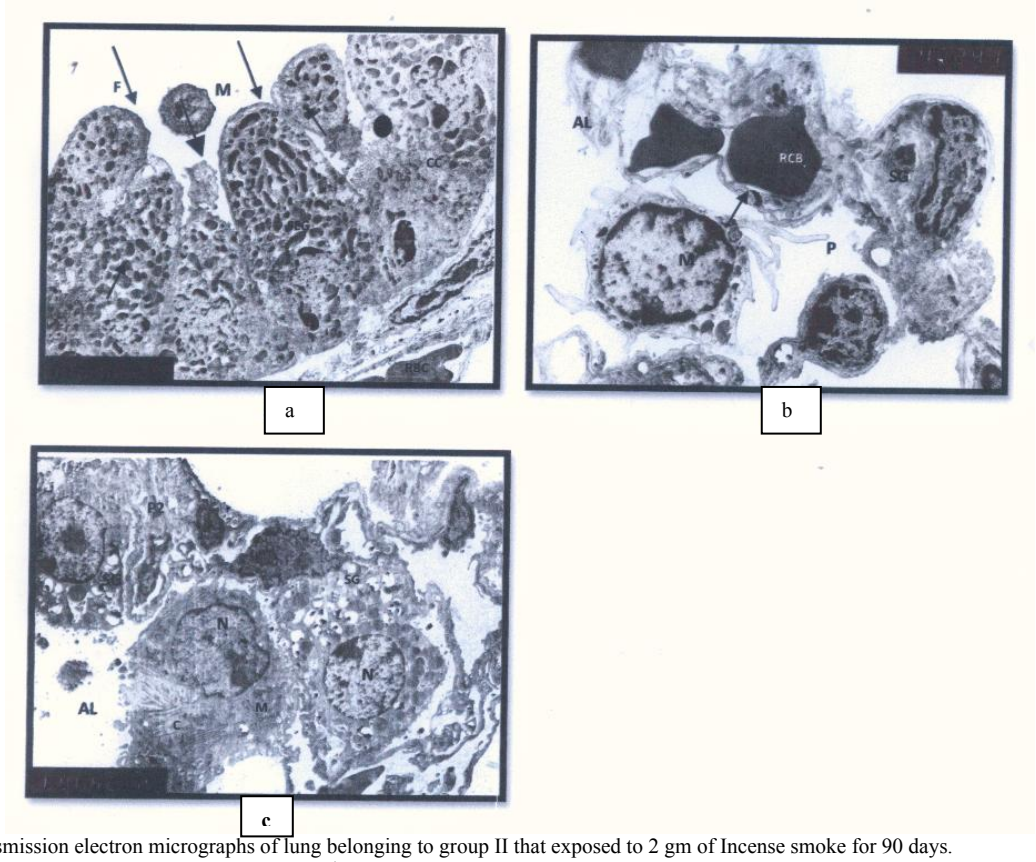

Fig.3: Transmission electron micrographs of lung belonging to group II that exposed to 2 gm of Incense smoke for 90 days.
(a) Clara cells (CC) are greatly hypertrophied (←) containing large number of variable shape and size mitochondria (M) with presence of numerous electron dense granules (SEG). Ciliated lining cells (↘) are appeared embedded in between the hypertrophied Clara cells. Fibroblast cells (F) observed under the bronchiolar basement membrane and the bronchiolar capillaries. Mag. 6000X.
(b) Macrophage (M) contain numerous elongated processes. The septal capillaries contain RBCs and lined by endothelial cells (P). Alveolar cell type II with surfactant granules (SG) is present. Mag. 8800X.
(c) Alveolar cells type II which contain numerous surfactant granules (SG) and the lumen of the alveoli (AL) contain large macrophage (M). Numerous light electron dense crystals (C) beside the other cell organelles embedded in mucin matrix demonstrated in alveolar cells (P2). Mag. 8800X.

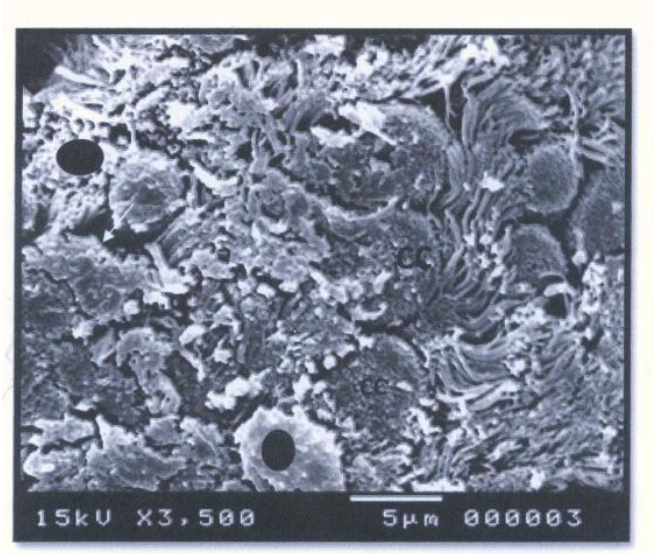

Fig. 4: Scanning electron micrographs of the surface epithelium of the bronchiole belong to group II showed the presence of mucinous secretion (←) attached to the epithelial lining, the non-ciliated cells (CC) swelled and separated from the neighboring cells while the cilia of the ciliated cells (●) are observed. Mag.6000 X.

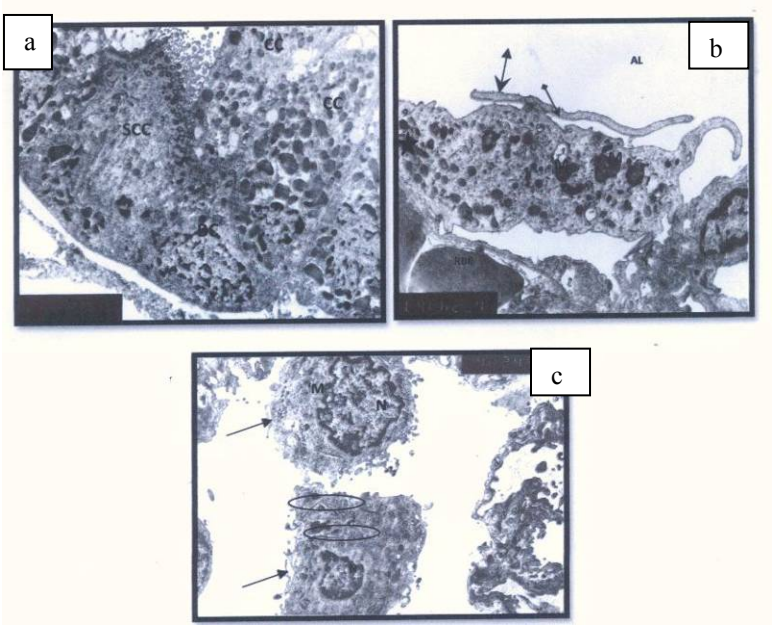

Fig. 5 Transmission electron micrographs of the lung bronchiole belonging to mice that exposed daily to 4gm of Incense smoke (Group III).
(a) Basal cell (BC) and ciliated cells (CC) embedded in a numerous hypertrophied Clara cells (SCC). Mag. 8800X.
(b) Large elongated macrophage (★) in the alveolar lumen which contains a large number of electron dense variable sized mitochondria (M) and some phagosomes (↙↗).
The macrophage has large cytoplasmic processes (↙↗). The alveolar capillary contains RBCs. Mag. 15000X.
(c) Macrophage cells (M) characterized by presence of large number of cytoplasmic processes (↗) and the cytoplasm contains numerous needle like crystals (⬭). Mag. 8800X.

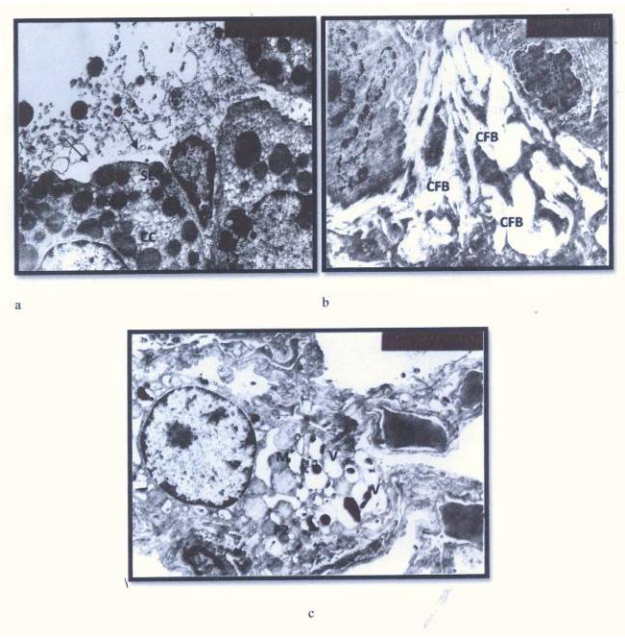

Fig. 6: Transmission electron micrographs of the lung bronchiole belonging to mice that exposed to 6 gm of Incense smoke (Group IV).
(a) Clara cells (CC) rupture and release the cytoplasmic contents in the lumen, which have electron dense granules (↘). Secretory endoplasmic granules (SEG), mitochondria and electron dense granules found mainly related to the cell membrane of Clara cells. Mag. 15000X.
(b) The presence of fibroblast cells and large amount of collagenous fiber bundles (CFB) Mag. 8800X.
(c) The hypertrophied Clara cells (P2) contain the swollen mitochondria (M), and the cytoplasm contain a numerous membranous vacuoles (V) have electron dense material. Mag. 11000X.

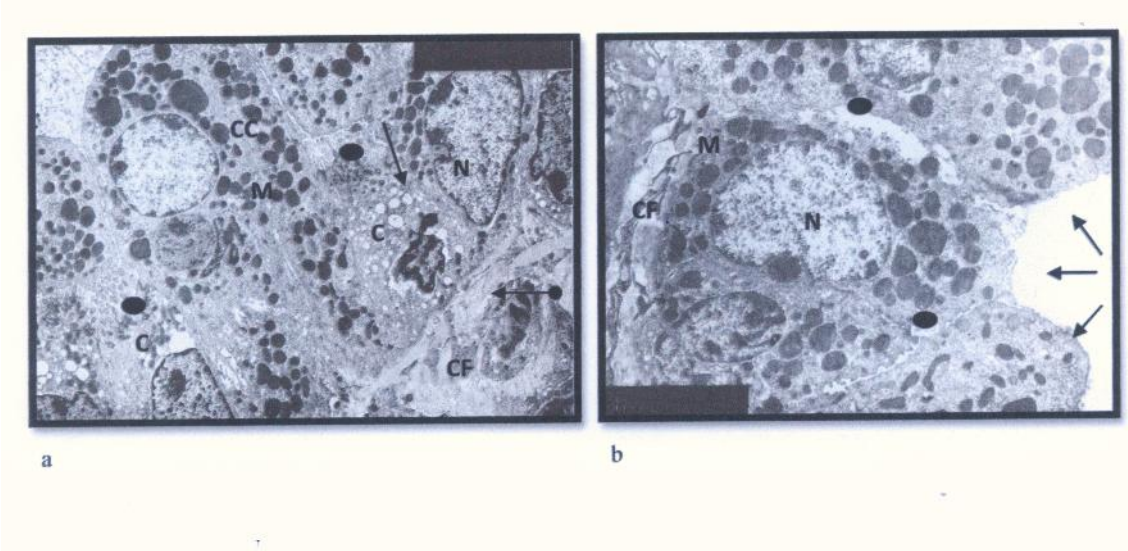

Fig7: Transmission electron micrographs of the lung bronchiole belonging to group II after prevention from exposure to Incense smoke for 60 days.
(a) Ciliated cells (C) are numerous, short closely adhered to the Basement membrane (←●) and have shrunken nucleus (N). The cytoplasm contains large number of variable sized vesicle (↖). The cilia embedded in between the non-ciliated cell not reaching the surface (●). The non-ciliated cells (CC) are large and containing large mitochondria (M). The lumen contains mucinous secretions, and collagenous connective tissue (CF) observed under the basement membrane. Mag. 6000X.
(b) Different lengths of non-ciliated cell (↖) and presence of both intercellular spaces (●), vesicular nucleus (N) and large numbers of mitochondria (M). Sub-epithelial collagenous connective tissue is found (CF) Mag. 8800X.

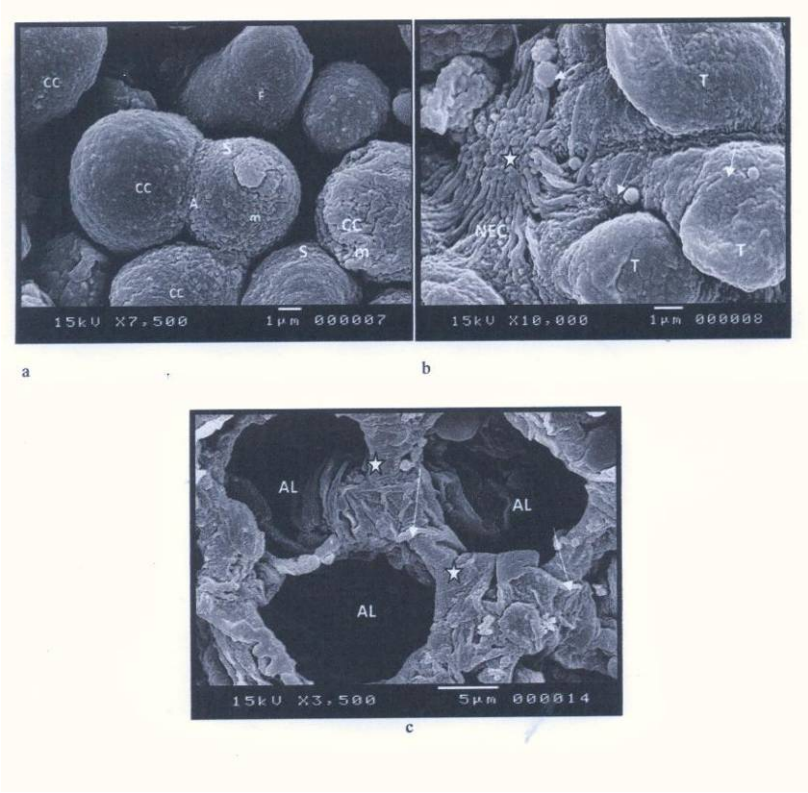

Fig. 8: Scanning electron micrographs of the surface of bronchioles belonging to group II that left for recovery for 60 days.
(a) Non-ciliated cells have microvilli (CC). Some non ciliated cells appear finger like (F) separated from each other cells (S) and some of them adhered together.
(b) Tongue shaped non-ciliated cells (T) while ciliated cells have large number of non-erected cilia (NEC). Several spherical bodies (←) adhered to the surface of cells
(C) Presence of wide lumen (AL). The lining cells are flat (★), also thickening of the alveolar septa (←) is observed

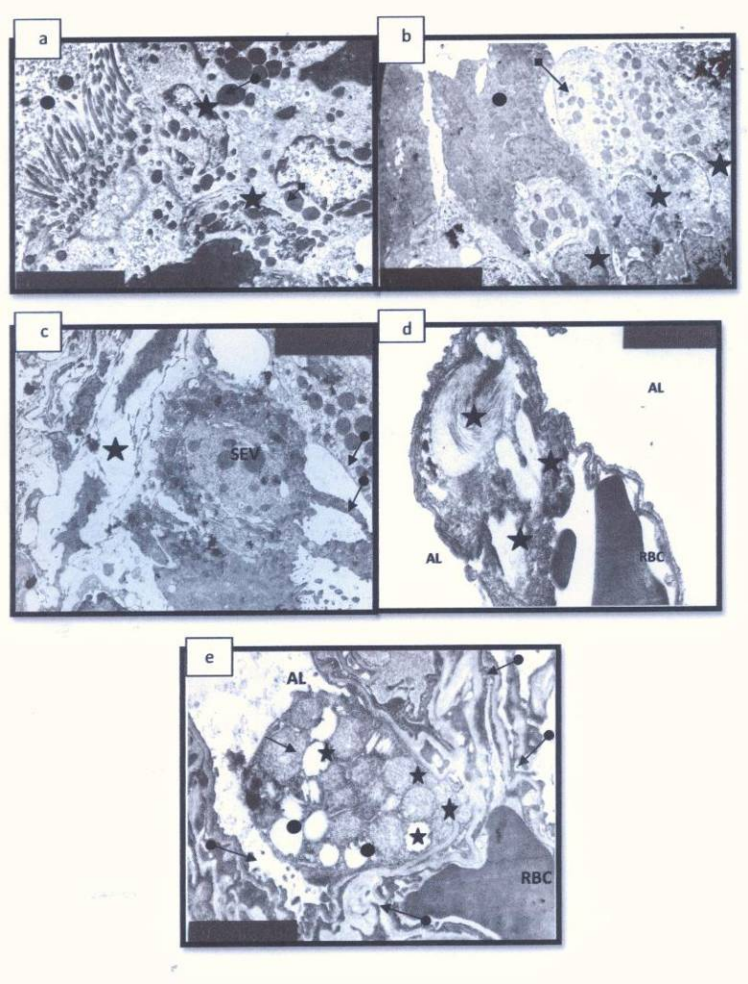

Fig. 9:- Transmission electron micrographs of bronchial epithelium belonging to group IV and left for 60 days for recovery.
(a) Degenerative changes of the nuclear chromatin ( ) in the nuclei of both ciliated cells and non-ciliated ones. Some nuclei appear elongated (★) and other having dentations (●). The lumen contains mucin secretion, secretory granules and deciliated cilia Mag. 11000X.
(b) Fragmentation of the nuclear chromatin of Clara cells (●). The presence of numerous light electron granules ( ) seems to be mucin granules in the cytoplasm. The other cells either ciliated or non-ciliated having intercellular vesicular spaces (★) Mag. 6000 X.
(c) The thick layer of lamina propria characterized with by thick layer of collagenous connective tissue (★). Clara cells epithelial lining showed presence of large inter-cellular vesicular spaces ( ) and presence of numerous vesicles in the cytoplasm (SEV) Mag. 8800X.
(d) Bundles of collagen fibers in the septal wall (★) Mag. 6000 X.
(e) High magnification of the previous photo (D) showed thickening of the Basement membrane (●) with light electron dense deposit in some areas appears fibirillar ( ). The pneumocyte type II, Clara cells have greatly swollen mitochondria ( ) which occupy all the cells and some of them is destructed and change to vesicles (★) Mag. 8000X.

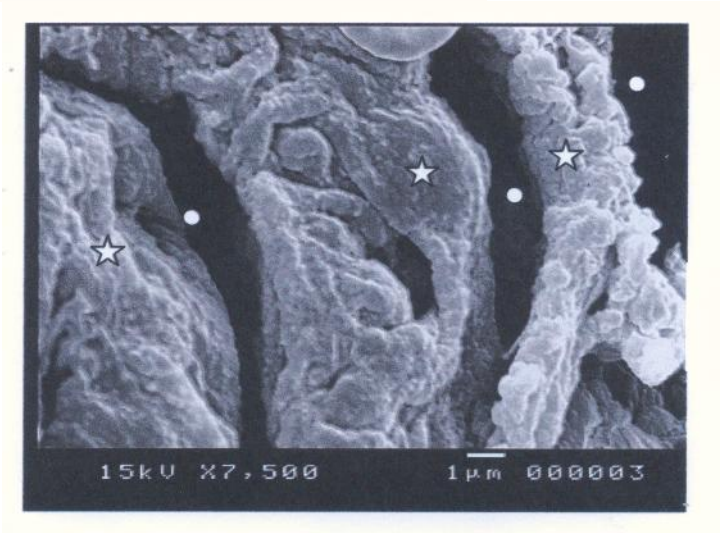

Fig. 10: Transmission electron micrograph of the terminal bronchioles belonging to group III and left for 60 days for recovery showed the non-ciliated cells (SCC) contain vesicular nucleus (N). The cytoplasm contains large number of mitochondria (M) and some electron dense secretory granules (SG) at the periphery of the cells. The sub-epithelial layer formed by collagenous connective tissue (★) Mag. 6000X.

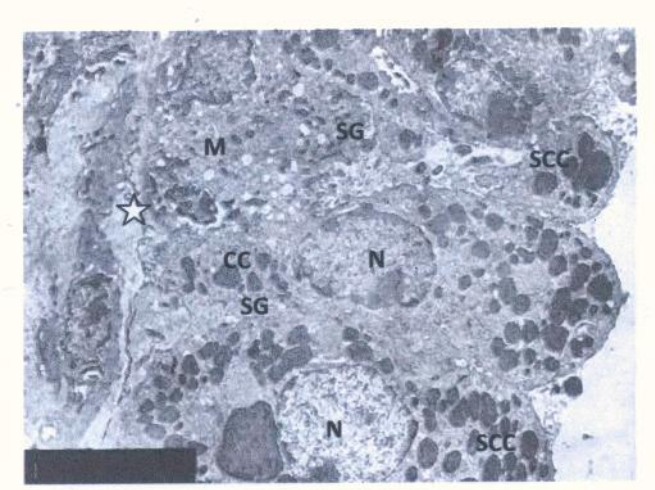

Fig. 11: Scanning electron micrograph of the alveoli belonging to group III and isolated for 60 days for recovery showed compression of the lumen and thickened septa (●). The alveolar cells formed by long and flat pneumocytes that had variable size (★) and long microvilli.

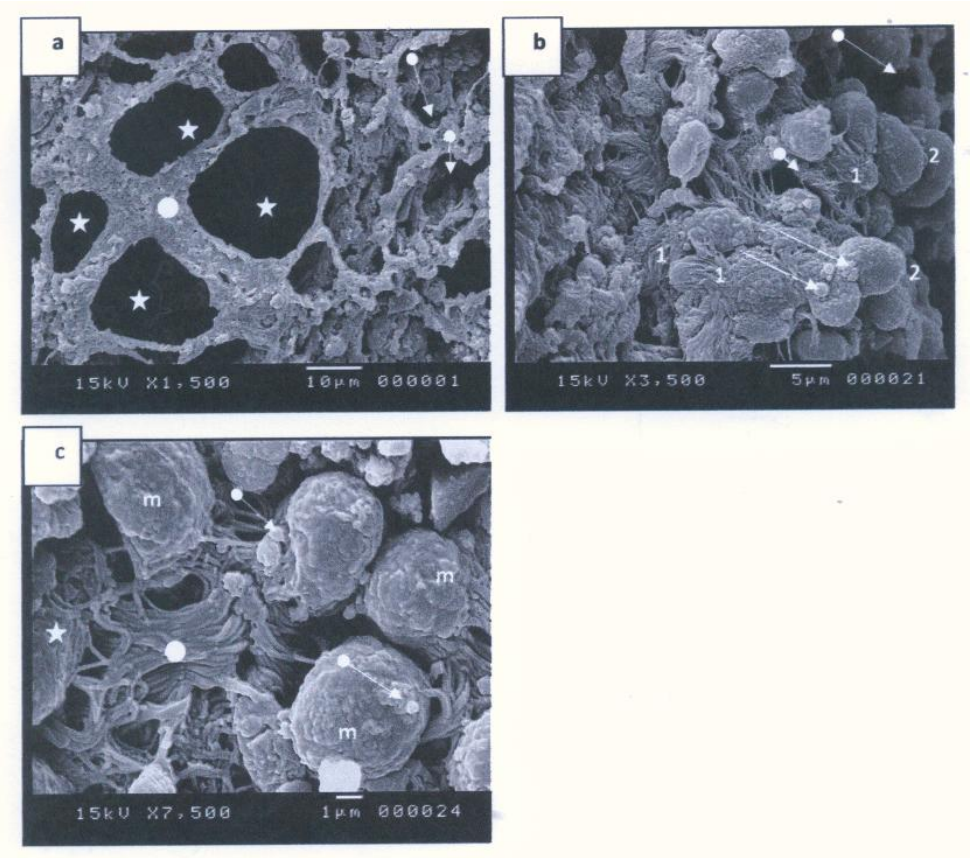

Fig. 12: Scanning electron micrograph belonging to group IV after left for 60 days as a recovery time.
(a) Increasing in thickens of the alveoli septal wall (●) with dilatation of the lumen (★) and compression of others (●→).
(b) Increasing the numbers of ciliated cells (1) in relation to non-ciliated cells (2), and presence of wide spaces (→) between the lining cells. Some spherical bodies (↘) is reported.
(c) High magnification of the bronchiole section showing the variable size and shape of microvilli (m) of non-ciliated cell. The ciliated cells long and have non erected cilia (★) with presence of spherical bodies (→) attached to the surface of the cells. In addition, wide area of peri-bronchial collagen fibers (●) in the lung lobule is recorded in the same section.

4. Discussion

Many previous studies had been reported the harmful effect of the Incense smoke on population especially children and infants [8, 9, 11]. The present ultrastructure findings of current search demonstrated sever changes in mice lungs exposed to different concentration of Incense smoke and continue with the same changes, even after cessation of exposure to Incense smoke. The results of transmission electron microscope of mice lungs exposed daily to 2, 4, and 6 gm of Incense smoke for 90 days recorded the presence of Clara cell hypertrophy due to excessive growth and an increase in the size of these cells associated with an increase in the number hyperplasia. Also, appearance of different size and shape of mitochondria were reported after exposure to either 2 or 4 gm of Incense smoke while giant vesicular macrophage observed after exposure to 6 gm of the Incense smoke whereas they fill out a large part of alveoli cavity. The alveoli cavity

characterized by the presence of numerous elongated processes, and some crystals in form of needle shape. These crystals may perform from metal deposition, or as a remaining due to an interaction of cell organelles, and fibrosis in the tissue around the bronchi (Peri-bronchial fibrosis) was observed. The present results were confirmed with the findings of (Alarifi *et al*., 2004a) study that noticed blockage of alveoli cavity due to presence of large size of the vesicular macrophages in mice lungs sections daily exposed to 4 gm of Incense smoke for 14 weeks, but disagree as they didn't mention any changes occur in the epithelia bronchi layer as hyperplasia or hypertrophy. White collagen fibers have been recorded in liposome walls. Also, they reported an increase in vesicular cell size (P2) which cause cell hypertrophy, associated with mitochondrial overgrown that contains a large amount of Alservictant [13, 16-18]. This difference in results may be due to different concentrations of Incense smoke, which experimental animals were exposed, or perhaps due to the duration and the time of the exposure to Incense smoke.

Ultrastructure study have been carried on mice lungs after cessation for 60 days to exposure of different concentration of Incense smoke for 90 days have reported the continuation of histological changes with the increase in intensity where there is no improvement in lung tissue. Many findings were observed in lung tissues, as necrosis of some epithelial bronchi cells and accumulation and aggregation of lymphoid cells in different locations. Also, lung tissues were recorded changes that occur due to degeneration such as the existence of smooth endoplasmic vesicles in epithelial cells of the bronchioles, the contraction and the concentration in the chromatin nuclei of some epithelial cells. In addition, we noted swelling in cells vesicular P2 that contain swollen and destructive mitochondria These results confirmed with previous studies who demonstrated the ultrastructure of rats lungs exposed to Incense smoke for 14 weeks and without giving the animals a rest period. They have been noticed cells necrosis and accumulation of lymphoid cells, and the cells contain swollen vesicular P2 and mitochondria [14, 19-21].

Current study reported severe changes in mice lungs after cessation for 60 days exposure to different concentration of Incense smoke for 90 days using scanning electron microscope. Scanning electron micrographs for the interior surface of the bronchioles demonstrated an increase in the thickness of some septal walls of alveoli, and the appearance of spherical bodies attached to the surface of the bronchioles lining cells. IM addition, Clara cells appeared in different shapes and sizes and they either connected together or separated from each other. As well as this current study was demonstrated the appearance of white collagen fibers in the lungs lobule of mice exposed to 6gm of Incense smoke for 90 days and left for recovery for 60 days.

Conclusion:

Based on the recent findings and other related studies, we conclude that exposure to Incense smoke causes harmful effects due to sever changes in pulmonary ultrastructures, such effects do not disappear even when Incense smoke inhalation was stopped. Therefore, we recommend that Incense smoke should use only in open places to reduce its harms.

Competing Interests: "The authors declare that they have no competing interests"

Corresponding author
Sahar Ragab El Hadad.
[1]Biology Department, Faculty of Science, King Abdulaziz University, Jeddah, KSA
[2]Genetic Engineering Research and Bioinformatics Center, VACSERA, Egypt
saharelhadad@hotmail.com

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Microbiological Studies on *Aeromonas* and *Pseudomonas* Species Isolated From Contaminated Fish Foods

Mohamed T. Shaaban

Botany Department, Faculty of Science, Menoufiya University, Egypt
Dr_mohamedtawfiek@yahoo.com

Abstract: Three bacterial isolates related to the Genus *Aeromonas* and eleven isolates related to the Genus *Pseudomonas* isolated from contaminated fish foods were studied in this work. The study included the identification of these isolates and their susceptibility to the crude extracts of five weeds and four new synthesized thiazolidinone derivatives. The isolates were identified as *Aeromonas hydrophila*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, and *Pseudomonas stutzeri*. The antibacterial activities investigations of the extracts and the thiazolidinone derivatives were performed on solidified growth plates and in liquid media. On solid plates, the highest inhibitory effects (inhibition zones) were recorded to the crude extract of *Brassica tournefortii* inhibiting the growth of all tested species with inhibition zones 8-18mm diameters. The lowest effects were recorded to the extract of *Convolvulus arvensis* inhibiting only the growth of *Pseudomonas putida*. Among the four thiazolidinone derivatives tested, the highest effects were recorded to the derivatives (1d) and (2d) and the lowest effects were recorded to the derivative (4a) inhibiting the growth of *Aeromonas hydrophila* only. In liquid media, the growth of different *Aeromonas* and *Pseudomonas* species-in terms of optical densities at 660nm-was affected by most of the crude extracts and thiazolidinone derivatives.
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Key words *Aeromonas, Pseudomonas*, fish food, plant extract, thiazolidinone.

1. Introduction

Some fishermen use contaminated fish foods in bottom traps and barriers in Nile river branches to attract fish. Microbiological investigations showed that such fish foods are highly contaminated by bacteria and fungi. Bacterial contaminants were found to be related to the bacterial Genera *Aeromonas*, *Pseudomonas*, *Bacillus* and actinomycetes. *Aeromonas species* and *Pseudomonas species* were the most abundant (**Alne-na-ei and Shaaban, 2003**).

Many species of *Aeromonas* and *Pseudomonas* are known as fish pathogens causing severe diseases to different fishes. Skin diseases of fish in polluted water are very important mortal diseases caused by microorganisms, the most important of them are *Aeromonas* species and Pseudomonas species (**Ventura and Grizzle, 1988; Alne-na-ei and Shaaban, 2003**). Species of *Aeromonas* and *Pseudomonas* infecting fish isolated from fish by **Twiddy (1995)** were found to be highly resistant to antibiotics, and many of them were found to be listed as human pathogens.

Plants and their extracts have been used many centuries ago as remedies for curing diseases caused by microorganisms. **Lucas and Lewis (1944)** extracted plant substances active against Gram +ve bacteria, Gram –ve bacteria and fungi. The application of these biologically active extracts provides new, safe source of chemical control against many pathogenic microorganisms and diseases caused by them (**Balandrin *et al.*, 1985**).

With many of constrains on using herbs in curing diseases, several species of wild or cultivated plants all over the world have been examined. Among different types of extracts of eleven wild weeds collected from the cultivated lands in Menoufiya Governorate, Egypt, extracts of *Urtica urens* and *Anagallis arvensis* showed antimicrobial (inhibitory) activities against Gram +ve bacteria, Gram –ve bacteria and yeasts (**El-Abyad *et al.*, 1990**).

Shaaban (1988) investigated the antimicrobial potency of the extracts of some weeds extracted by different methods and different solvents. The study showed inhibitory effects caused by extracts of *Anagallis arvensis*, *Brassica tournefortii*, *Convolvulus arvensis*, *Rumex dentatus* and *Urtica urens* against Gram +ve bacteria, Gram –ve bacteria and yeasts. Extracts and purified substances extracted from many other plants are known in folk medicine uses due to their antibacterial activities against Gram +ve bacteria and Gram –ve bacteria (**Shaaban *et al.*, 2011; 2012**).

Thiazolidinones are thiazole derivatives (**El-Sayed *et al.*, 2008; 2009**). Thiazole derivatives in general, are considered as one of the most important classes of heterocyclic compounds, characterized by high biological activities of great applications in

medicine, being antibacterial (**Tsuji and Ishikawa, 1944**) and antifungal (**Lopez-Garcia *et al*., 2003**).

Thiazolidinone derivatives were reported to show a variety of pharmacological properties (**El-Sayed *et al*., 2008**) being antibacterial and antifungal (**DeLima *et al*., 1992**), cyclooxygenase and lipoxygenase inhibitors (**Unangst *et al*., 1993**), cardiotonic (**Anderiani *et al*., 1993**) and anthelmentic (**Vagdevi *et al*., 2006**).

Some thiazolidinone derivatives (1,3 thiazolidinones) were recorded as potent antimicrobial agents, showing clear inhibitory effects against many Gram +ve bacterial pathogens (**Singh *et al*., 1981**). **Nasr *et al*. (2003)** reported that chemical modifications at different positions of thiazolidine ring increases the antimicrobial activities of the compound. Similar results obtained by **El-Sayed *et al*. (2009).**

Different methods have been used in the investigation of antimicrobial potencies of plant extracts and chemicals. These methods included the investigation of the antimicrobial or inhibitory effects on solidified plates and in liquid cultures (**Shaaban and El-Sharif, 2001; Shaaban *et al*., 2012**)

So, this study was interested in the identification of the *Aeromonas* isolates and *Pseudomonas* isolates isolated from contaminated fish foods and investigation of susceptibility of the identified *Aeromonas species* and *Pseudomonas species* to the crude extracts of some weeds collected from the cultivated lands in Menoufiya Governorate, Egypt and four new synthesized thiazolidinone derivatives on solidified growth plates and in liquid cultures.

2. Material and Methods

Test microorganisms

The test microorganisms included three *Aeromonas* isolates (A_1, A_2, and A_3) and eleven *Pseudomonas* isolates (P_1, P_2, P_3, P_4, P_5, P_6, P_7, P_8, P_9, P_{10} and P_{11}) were isolated from contaminated fish foods in our study, **(Al-ne-na-ei and Shaaban, 2003).**

Test Plant Extracts

The crude extracts of five weeds collected from the cultivated lands in Menoufiya Governorate, Egypt. The weeds which were investigated for their antibacterial and antifungal potencies in our study, **(Shaaban, 1988)** are *Anagallis arvensis*, *Brassica tournefortii*, *Convolvulus arvensis*, *Rumex dentatus* and *Urtica urens*.

Test new chemical derivatives

Four new 2,4 disubstituted thiazolidinone derivatives (1d), (2d), (4a) and (4d)- soluble in 12.5% Dimethyl sulfoxide (DMSO) which were synthesized and tested for their inhibitory effects against Gram +ve bacteria, Gram –ve bacteria and actinomycetes in our study, (**El-Sayed *et al*., 2009**).

Identification of the bacterial isolates

The bacterial isolates A_1, A_2, A_3, P_1, P_2, P_3, P_4, P_5, P_6, P_7, P_8, P_9, P_{10} and P_{11} were identified according to **Bergey's Manual** of Systematic Bacteriology (1984). The identification criteria included the growth features (colony, shape, color, elevation and margin), growth conditions (aerobiosis, optimum temperatures, ability to grow at different temperatures, special requirements in their growth media, suitable growth media), morphology of cells (shape and dimensions), physiological characteristics (motility, number and position of flagella and Gram reaction), production of enzymes (oxidase, catalase and lecithinase), production of starch, hydrolysis of starch, denitrification and utilization of different carbon and nitrogen sources.

Investigation of the antibacterial activity

Different techniques have been applied including the investigation a- on solidified plates using filter paper discs method. Nutrient agar plates in Petri-dishes were inoculated with test species, allowed to dry for 15-20 minutes. Nine test substances could be tried against one species in one Petri-dish. Filter paper discs (8mm in diameter) were prepared and sterilized in an autoclave (1 atm for 20 minutes). Sterile filter paper discs were soaked in the crude plant extracts and thiazolidinone derivatives for four hours to reach saturation. The saturated filter paper discs were then taken and placed at the top of agar seeded with the test organism. The Petri-dishes containing the test microbe and the filter paper discs were then incubated at the optimum temperature for 24-48 hours. The inhibitory effect of a substance was detected by the inhibition zone appeared surrounding the discs of +ve activity; the diameter of this inhibition zone is a measure of the antibacterial potency (**Egorov, 1985; Izzo *et al*., 1995**). b- In liquid cultures. 45 ml of nutrient broth in 100 ml conical flasks were autoclaved, cooled and then 5 ml of sterilized and filtered crude plant extract or the thiazolidinone derivatives were added to each flask, inoculated by the test *Aeromonas* or *Pseudomonas* species, incubated at the optimum temperature of the species handled, in an incubator with shaker (at 150 rpm). The growth was followed up at standard intervals (4 hours). Changes in turbidity (in terms of the optical densities OD) were taken as the growth measure, measured in a spectrophotometer at wave length of 660nm (**Farag *et al*., 1989; Lortie *et al*., 1992; Shaaban, 2001; Shaaban and El-Sharif, 2001**).

3. Results

As shown in table 1, the three isolates relating to the Genus *Aeromonas* (A_1, A_2 and A_3) were found to have the same morphological, physiological and biochemical characteristics. All of them form white circular convex colonies with entire margin. All of them are Gram –ve, non capsule formers, motile by one, polar flagellum. The cells of all of the three isolates are of straight rod shape with cell lengths of 2.0-2.5µm and cell width of 0.5µm. The optimum temperature for growth was the same for all (28˚C), but all of them were able to grow at 37˚C in nutrient broth, sodium was not required for growth of any of them. The three isolates were able to produce oxidase enzyme, lipase enzyme, gas from glucose and H_2S from cystiene, able to ferment mannitol and sucrose, able to reduce nitrate NO_3 to nitrite NO_2. All of them were able to utilize D-mannitol, histidine, L-arginine and L-arabinose. According to these characteristics and according to Bergey's Manual for Systematic Bacteriology (1984), the *Aeromonas* isolates no. A_1, A_2 and A_3 were identified as *Aeromonas hydrophila*.

Eleven *Pseudomonas* isolates (P_1, P_2, P_3, P_4, P_5, P_6, P_7, P_8, P_9, P_{10} and P_{11}) were characterized for their morphology of colonies, physiological activities and biochemical behaviors listed in Bergey's Manual for Systematic Bacteriology. The results classified the eleven isolates to three groups. The first group included the isolates P_1, P_5, P_6 and P_8; the second group included the isolates P_2, P_7, P_9 and P_{10}; the third group included the isolates P_3, P_4 and P_{11} (Table 2).

The isolates P_1, P_5, P_6 and P_8 were found to form white colonies. The cells are rod shaped with cell length of 3µm and cell diameter of 0.8µm, Gram –ve, aerobic, motile by one polar flagellum. The optimum temperature for them was 37˚C but all of them were able to grow at 41˚C. All of the isolates showed +ve oxidase test, +ve catalase test and –ve lecithinase test. None of them was able to form levan, produce or hydrolyse starch. None of them accumulated poly-β-hydroxyputyrate PHB. All of the isolates were able to denitrify or reduce nitrate NO_3 to nitrite NO_2. All of them produced fluorescent pigments. All of isolates were able to utilize mannitol, N-butanol, D&L-arabinose. These characteristics were coincident to the characteristics of *Pseudomonas aeruginosa* listed in Bergey's Manual for Systematic Bacteriology, so the isolates P_1, P_5, P_6 and P_8 were identified as *Pseudomonas aeruginosa*.

Isolates P_2, P_7, P_9 and P_{10} formed white colored colonies. The cells were rod shaped with 2.5µm cell length and cell diameter of 0.8µm, Gram –ve, aerobic, motile with a polar taft of flagella. The optimum temperature was found to be 30˚C, but all of them were able to grow at 4˚C, unable to grow at 41˚C. All of the isolates showed +ve oxidase test, +ve catalase test and –ve lecithinase test. None of the isolates formed levan, neither produced or hydrolyzed starch and none of them accumulated poly-β-hydroxybutyrate PHB in their cells. None of the isolates were denitrifying bacteria. All of them produced fluorescent pigments but none of them produced diffusible pigments in the growth medium. All the isolates were able to utilize sorbitol, mannitol, N-butanol, D&L-alanine, lactate acetate, pyruvate, succinate and L-arabinose. These characteristics were coincident to the characteristics of *Pseudomonas putida* listed in Bergey's Manual for Systematic Bacteriology, accordingly, the isolates P_2, P_7, P_9 and P_{10} were all identified as *Pseudomonas putida*.

The isolates P_3, P_4 and P_{11} formed white colonies. The cells of the isolates were found to be rod shaped with cell length of 2.3µm and cell diameter of 0.7µm, Gram –ve, aerobic, motile by one polar flagellum. The optimum temperature for growth was 36˚C, able to grow at 41˚C, but unable to grow at 4˚C. The isolates showed +ve oxidase test, +ve catalase test and –ve lecithinase test. None of the isolates was able to form levan, produce starch or accumulate poly-β-hydroxybutyrate PHB inside their cells. The isolates were able to denitrify nitrogen compounds and hydrolyze starch. Neither fluorescent pigments nor diffusible pigments produced. They were able to utilize mannitol, N-butanol, D&L-alanin, lactate, acetate, pyruvate and succinate, but none of them was able to utilize sorbitol, sucrose, or L-arabinose. These characteristics were coincident with that listed in Bergey's Manual for Systematic Bacteriology for *Pseudomonas stutzeri*. Thus P_3, P_4 and P_{11} were identified as *Pseudomonas stutzeri*.

The antimicrobial potencies of the plant crude extract of five weeds were handled in this study against the growth of *Aeromonas hydrophila*, *Pseudomonas aeruginosa*, *Pseudomonas putida* and *Pseudomonas stutzeri*. The inhibitory effects in terms of inhibition zone diameter as shown in table 3 were different from one extract to the other, and the responsibility of bacteria tested differed from one species to the other. The crude extract of *Brassica tournefortii* inhibited the growth of *Aeromonas hydrophila*, *Pseudomonas aeruginosa*, *Pseudomonas putida* and *Pseudomonas stutzeri* with inhibition zones of 18mm, 8mm, 8mm and 10mm diameter respectively. The highest effect of this extract was against *Aeromonas hydrophila*, followed by *Pseudomonas stutzeri*. The crude extract of *Anagallis arvensis* exhibited its inhibitory effects against three only of the test organisms, *Aeromonas hydrophila* with inhibition zone diameter of 22mm, *Pseudomonas aeruginosa* with 9mm diameter

inhibition zone and *Pseudomonas atutzerii* with inhibition zone diameter of 6mm. the growth of *Pseudomonas putida* showed no susceptibility to the crude extract of *Anagallis arvensis* (no inhibition zone appeared). The crude extract of *Convolvulus arvensis* inhibited the growth of *Pseudomonas putida* only showing an inhibition zone of 6mm diameter. No inhibition zones appeared on the plates of *Aeromonas hyrophila*, *Pseudomonas aeruginosa* or *Pseudomonas stutzeri*. The extract of *Rumex dentatus* inhibited the growth of *Aeromonas hydrophila* exhibiting inhibition zones of 15mm and 10mm diameters respectively. *Pseudomonas putida* and *Pseudomonas stuzerii* were found to be resistant to this extract (no inhibition zones appeared). The extract of *urtica urens* inhibited the growth of *Aeromonas hydrophila*, *Pseudomonas putida* and *Pseudomonas stutzeri* exhibiting inhibition zones of 17mm, 11mm and 8mm diameter, respectively. *Pseudomonas aeruginosa* was resistant to the extract of *Utrica urens* (no inhibition zones appeared).

In regard to the inhibitory effects of the four thiazolidinone derivatives (1d), (2d), (4a) and (4d) against *Aeromonas hydrophila*, *Pseudomonas aeruginosa*, *Pseudomonas putida* and *Pseudomonas stutzeri*, the results are shown in table 4. The thiazolidinone derivative (1d) inhibited the growth of *Aeromonas hydrophila*, *Pseudomonas aeruginosa* and *Pseudomonas putida* showing inhibition zones on the growth plates of 21mm, 11mm and 7mm diameters, respectively, while *Pseudomonas stutzeri* was resistant to this derivative. The derivative (2d) showed inhibitory effects against the growth of *Aeromonas hydrophila*, *Pseudomonas aeruginosa* and *Pseudomoans stutzeri* exhibiting inhibition zones of 24mm, 8mm and 7mm diameters, respectively. *Pseudomonas putida* was resistant to the derivative (2d). The thiazolidinone derivative (4a) affected only the growth of *Aeromonas hydrophila* showing an inhibition zone of 8mm diameter. No inhibition zones appeared on the growth plates of *Pseudomonas aeruginosa*, *Pseudomonas putida* or *Pseudomonas stutzeri*. The derivative (4d) inhibited the growth of *Aeromonas hydrophila*, *pseudomonas stutzeri*. The derivative 4d inhibited the growth of *Aeromonas hydrophila*, *Pseudomonas putida* and *Pseudomonas stutzeri* causing inhibition zones of 6mm, 8mm and 7mm diameters, respectively. No inhibitory effect was exhibited by the derivative (4d) against the growth of *Pseudomonas aeruginosa* i.e. no inhibition zone appeared. The solvent (12.5% DMSO) was used as a control. No inhibition zones appeared around the filter paper discs saturated by (DMSO) on any of the bacterial species studied.

In liquid cultures, following up the bacterial growth- in terms of optical densities at wavelengths 660nm- in presence of crude extracts and thiazolidinone derivatives. The results obtained showed different effects of different crude extracts and different thiazolidinone derivatives upon the growth of different bacterial isolates. A matter depending on the agent used and the bacterial species studied. A control to which no crude extracts or thiazolidinone derivatives were added was investigated in parallel to that containing the agents (crude extract or thiazolidinone derivatives).

In liquid cultures, crude extracts exhibited inhibitory effects of different degrees against different microbes.

As shown in Fig.1 the highest inhibitory effects against the growth of *Aeromonas hydrophila* was that caused by the extract of *Anagallis arvensis*, followed by *Brassica tournefortii*, *Urtica urens*, *Rumex dentatus*. The lowest effects was recorded the extract of *Convolvulus arvensis*. Figure 2 shows the inhibitory effects exhibited by different plant extracts upon the growth of *Pseudomonas aeruginosa*. No clear differences were observed between the effects of different extracts i.e. all the extracts were inhibitory to the *Pseudomonas aeruginosa* by approximately the same potency. In regard to *Pseudomonas putida* the growth curves as shown in Fig. 3 indicate that the organisms were affected by extracts of *Urtica urens* followed by *Brassica tournefortii* and *Convolvulus arvensis*. The inhibitory effects of *Anagallis arvensis* and *Rumex dentatus* were very low. The growth curves of *Pseudomonas stutzeri* shown in Fig.4 show that extracts of *Anagallis arvensis* and *Brassica tournefortii* have approximately the same and the highest effects followed by the extracts of *Urtica urens*, *Rumex dentatus* and *Convolvulus arvensis*.

The inhibitory effects of different thiazolidinone derivatives against *Aeromonas hydrophila*, *Pseudomonas aeruginosa*, *Pseudomonas putida* and *Pseudomonas stutzeri* are represented by the growth curves in figures 5,6,7 and 8 respectively. Figure 5 shows that all the derivatives were of moderate inhibitory effects against the growth of *Aeromonas hydrophila*. The highest effects were recorded to the thiazolidinone derivatives (2d) and (1d) respectively followed by the derivative (4a) and the lowest effect was recorded to the derivative (4d).

Growth curves of *Pseudomonas aeruginosa* shown in figure 6, reflect the inhibitory effects exhibited by different thiazolidinone derivatives upon the growth of the organisms. The highest inhibition was that caused by the derivative (2d) followed by the derivative (1d). Moderate to limited inhibitory effects caused by the derivatives (4d) and (4a) respectively. Figure 7 shows that the highest inhibitory effect against the growth of *Pseudomonas*

putida was that of thiazolidinone derivative (2d) followed by the derivative (1d), while the derivative (4a) showed a limited inhibitory effect. No observable inhibitory effect was recorded to the derivative (4d) against the growth of *Pseudomonas putida*.

Table (1): The identification criteria of the *Aeromonas* isolates A1, A2 and A3 according to Bergey(1984).

Criterion	**Isolates no.**		
Colonies on nutrient agar:	**A_1**	**A_2**	**A_3**
Color	White	White	White
Shape	Circular	Circular	Circular
Elevation	Convex	Convex	Convex
Margin	Entire	Entire	Entire
Cell morphology:			
Cell shape	Straight rod	Straight rod	Straight rod
Cell length	2.5µm	2.5µm	2µm
Cell diameter	0.5µm	0.5µm	0.5µm
Gram reaction	-ve	-ve	-ve
Capsule formation	-	-	-
Motility	+	+	+
Flagella	One (Polar)	One (Polar)	One (Polar)
Optimum temperature	28˚C	28˚C	28˚C
Oxidase test	+	+	+
Lipase production	+	+	+
Sodium required	-	-	-
Growth at 37˚C in nutrient broth	+	+	+
Production of :			
Gas from glucose	+	+	+
H_2S from cystiene	+	+	+
Fermentation of mannitol	+	+	+
Fermentation of sucrose	+	+	+
Reduction of NO_3 to NO_2	+	+	+
Utilization of :			
D-mannitol	+	+	+
L-histidine	+	+	+
L-arginine	+	+	+
L-arabinose	+	+	+

Table (2): Identification criteria of the *Pseudomonas* isolates P1-P11

Criterion	Isolates no.		
	P_1,P_5,P_6 & P_8	**P_2,P_7,P_9 & P_{10}**	**P_3,P_4 & P_{11}**
Colony color	White	White	White
Cell shape	Rod	Rod	Rod
Cell length	3µm	2.5µm	2.3µm
Cell diameter	0.8µm	0.8µm	0.7µm
Gram reaction	-ve	-ve	-ve
Aerobiosis	+	+	+
Motility	+	+	+
No. of flagella	1	taft	1
Oxidase test	+	+	+
Catalase test	+	+	+
Levan formation	-	-	-
Starch Production	-	-	-
Starch hydrolysis	-	-	+
Lecithinase	-	-	-
Denitrification	+	-	+
Fluerscent Pigment	+	+	-
Diffusable pigment	-	-	-
PHB accumulation	-	-	-
Growth at 4˚C	-	+	-
Growth at 41˚C	+	-	+
Optimum temperature	37˚C	30˚C	36˚C
Utilization of sorbitol	-	+	-
Utilization of mannitol	+	+	+
Utilization of N-butanol	+	+	+
Utilization of D&L-alanin	+	+	+
Utilization of sucrose	-	+	-
Utilization of lactate	+	+	+
Utilization of acetate	+	+	+
Utilization of pyruvate	+	+	+
Utilization of succinate	+	+	+
Utilization of L-arabinose	-	+	-

Table (3): Diameters (mm) of inhibition zones around the filter paper discs saturated by different test plant extracts on the growth plates of different *Aeromonas* and *Pseudomonas* species. Data presents the mean values ± Standard errors.

Test Plant	*A. hydrophila*	*P. aeruginosa*	*P. putida*	*P. statzeri*
Anagallis arvensis	22±2.00	9.33±0.67	0±0.00	6±0.00
Brassica tournefortii	18±1.53	8±1.15	8±1.15	10±1.15
Convolvulus arvensis	0±0.00	0±0.00	6±0.00	0±0.00
Rumex dentatus	15±1.00	10±1.15	0±0.00	0±0.00
Urtica urens	16.67±0.67	0±0.00	11±1.00	8±0.00

Table (4): Diameters of inhibition zones (mm) produced around the filter paper discs saturated by 1d, 2d, 4a and 4d derivatives of thiazolidonine on the growth plates of different test *Aeromonas* and *Pseudomonas* species. The solvent DMSO (12.5% in water) used as a control. Data presents the mean values ± Standard errors.

Thiazolidonine derivative	*A. hydrophila*	*P. aeruginosa*	*P. putida*	*P. stutzeri*	Control
1d	20.67±0.67	10.67±0.67	6.67±0.67	0±0.00	0±0.00
2d	24±2.31	8±1.15	0±0.00	7.33±0.67	0±0.00
4a	8±1.15	0±0.00	0±0.00	0±0.00	0±0.00
4d	6±0.00	0±0.00	8±0.00	6.67±0.67	0±0.00

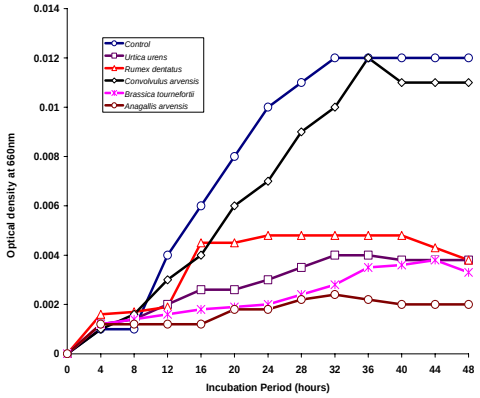

Fig.1: The growth curves of *Aeromonas hydrophila* growing in liquid media containing the plant extracts. Control is free from plant extract.

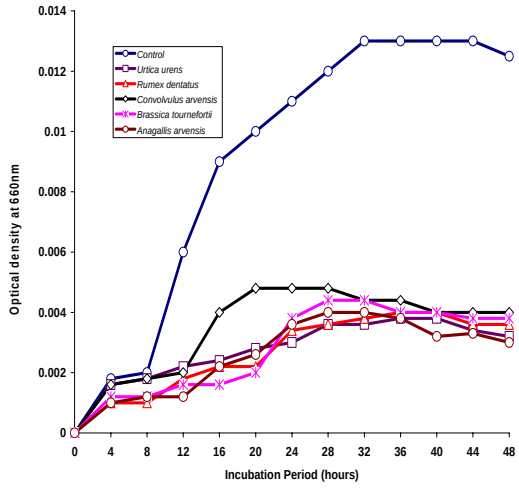

Fig.2: The growth curve of *Pseudomonas aeruginosa* growing in liquid medium containing the plant extracts. Control is free from plant extracts.

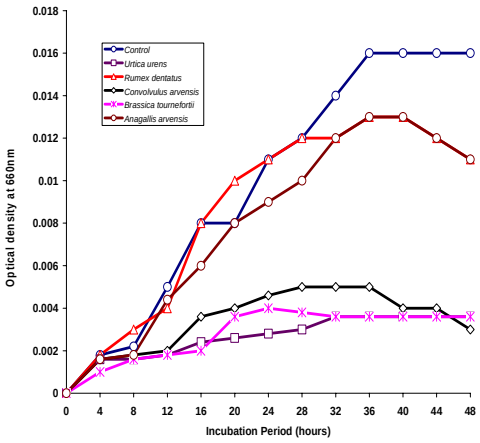

Fig.3: The growth curve of *Pseudomonas putida* growing in liquid medium containing plant extracts. Control is free from the plant extracts.

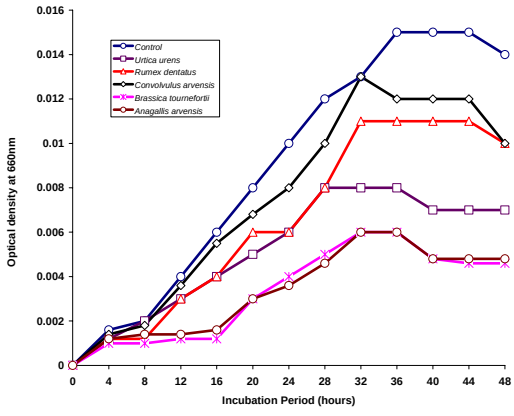

Fig.4: The growth curve of *Pseudomonas stutzeri* growing in liquid media containing plant extracts. Control is free from plant extracts.

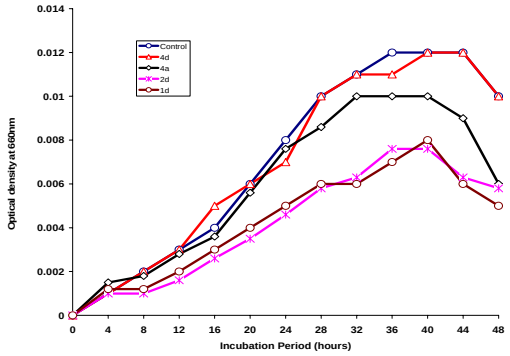

Fig.5: The growth curve of *Aeromonas hydrophila* growing in liquid medium containing the thiazolidinone derivative 1d, 2d, 4a and 4d. The control contains the solvent DMSO (12.5% in water).

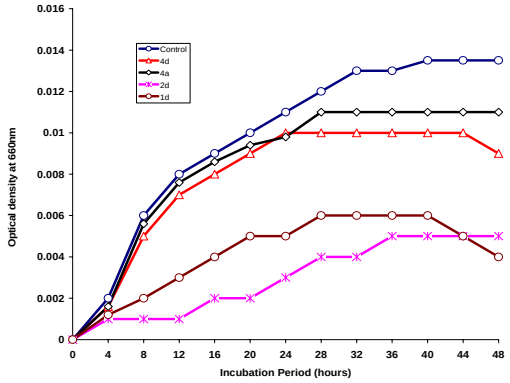

Fig.6: The growth curve of *Pseudomonas aeruginosa* growing in liquid medium containing thiazolidinone derivatives 1d, 2d, 4a and 4d. The control contains the solvent DMSO (12.5% water).

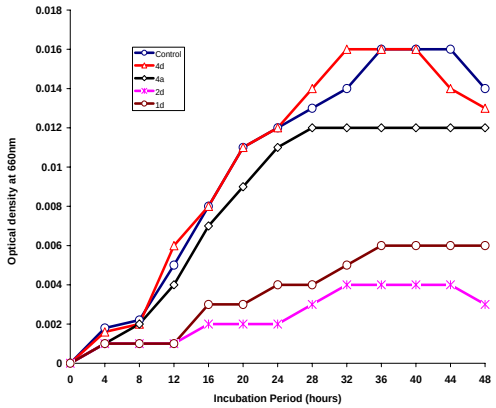

Fig.7: The growth curve of *Pseudomonas putida* growing in liquid medium containing the thiazolidinone derivatives 1d, 2d, 4a and 4d. The control contains the solvent DMSO (12.5% in water).

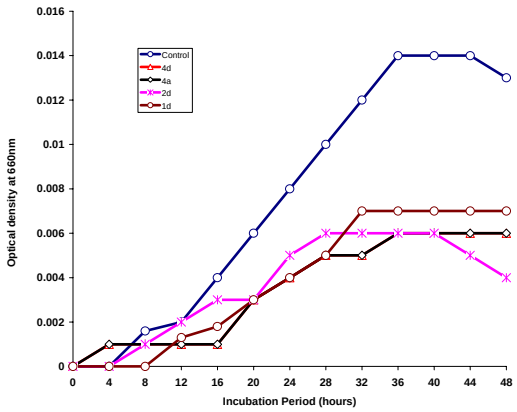

Fig.8: The growth curve of *Pseudomonas stutzeri* growing in liquid medium containing the thiazolidinone derivatives 1d, 2d, 4a and 4d. The control contains the solvent DMSO (12.5% in water).

4. Discussion

This study dealt with bacterial isolates (three *Aeromonas* isolates and eleven *Pseudomonas* isolates) isolated from contaminated fish foods. These contaminated foods are used by some fishermen in waters of Nile River to attract fish to traps and barriers, caused bacterial contamination of water and fish surfaces (**Alne-na-ei and Shaaban, 2003**).

Aeromonas isolates and *Pseudomonas* isolates investigated in this study belong to two important genera in regard to their high frequency as fish food contaminants, their high frequency in fresh water and their pathogenicity to many species of fish (**Nedoluha and Westhoff, 1993**).

Aeromonas isolates A_1, A_2 and A_3 were identified according to **Bergey (1984)** as *Aeromonas hydrophila*, an *Aeromonas* species of great importance and activities in fresh water and fish ponds water, and effects on the biological aquatic life (**El-Gammal, 2000**). *Aeromonas hydrophila* is one of the virulent fish pathogens causing severe infections and mortal diseases to fresh water fish (**Shotts *et al.*, 1972; Plumb *et al.*, 1976; Boulanger *et al.*, 1977; Twiddy, 1995; El-Gammal, 2000**).

Pseudomonas isolates P_1, P_5, P_6 and P_8 were identified as *Pseudomonas aeruginosa*, a *Pseudomonas* species known for its great frequency in fresh and marine aquatic life, causing some mortal fish diseases (**Fluchter, 1979; El-Gammal, 2000**). The isolates P_2, P_7, P_9 and P_{10} were identified as *Pseudomonas putida* and the isolates P_3, P_4 and P_{11} were identified as *Pseudomonas stutzeri*. Both, the species *Pseudomonas putida* and *Pseudomonas stutzeri* were recorded as important species of the genus *Pseudomonas* found in substantial numbers as

free-living saprophytes in fresh water and marine environments causing many chemical transformations affecting the aquatic life (**Shaaban, 1992; Ghanem *et al*., 1993**).

The specific importance of the four identified species, *Aeromonas hydrophila*, *Pseudomonas aeruginosa*, *Pseudomonas putida* and *Pseudomonas stutzeri* (1- as fish food contaminants able to denaturize the food components, producing toxic substances, 2- as fish pathogens causing severe infections which may transfer to human beings) made it very important to look for natural and chemical nontoxic substances of antibacterial activities against these harmful bacterial species.

Substances chosen in this study included the crude extracts of five wild weeds grassed by animals i.e. have no toxicity to animals (*Anagallis arvensis*, *Brassica tournefortii*, *Convolvulus arvensis*, *Rumex dentatus* and *Urtica urens*) and previously recorded as having antimicrobial activities (**Shaaban, 1988; El-Abyad *et al*., 1990**) and four new derivatives of thiazolidinone, synthesized compounds of no toxicity and potent antimicrobial activities against the growth of Gram +ve bacteria, Gram –ve bacteria and actinomycetes (**Abdel-Rahman *et al*., 2008; El-Sayed *et al*., 2008; 2009**).

It was found important to carry out the antimicrobial activities tests of the test substances on both the solidified nutrient agar plates and in liquid cultures, to avoid the disadvantage of each technique (**Egorov, 1985; Shaaban and El-Sherif, 2001**).

On solid media the results showed, in regard to the crude weed extracts, that, the crude extract of *Brassica tournefortii* was the most potent as antimicrobial agent inhibiting the growth of all the test organisms followed by the extract of *Anagallis arvensis*, which inhibited the growth of *Aeromonas hydrophila*, *Pseudomonas aeruginosa* and *Pseudomonas stutzeri*. The crude extract of *Urtica urens* inhibited the growth of *Aeromonas hydrophila*, *Pseudomonas putida* and *Pseudomonas stutzeri*, while the extract of *Rumex dentatus* was found inhibitory against the growth of two microbes only (*Aeromonas hydrophila* and *Pseudomonas aeruginosa*). The extract of *Convolvulus* was absolutely the weakest, inhibiting only the growth of *Aeromonas hydrophila* with an inhibition zone of 8mm diameter. Antimicrobial activities of plant extracts were investigated on solid media in many studies. Extracts of different plants showed different antimicrobial potencies against different bacterial species i.e. the effect is correlated to the chemical composition of the extract and susceptibility or resistant of the tested bacterial species (**Shaaban, 1988; El-abyad, 1990; Shaaban and El-Sharif, 2001; Khattab, 2009**).

Thiazolidinone derivatives (1d, 2d, 4a and 4d) showed also different inhibitory effects against the growth of the tested bacterial species. These derivatives and similar compounds showed high inhibitory effects against Gram +ve bacteria and actinomycetes, and weak inhibitory effects against the growth of Gram –ve bacteria (**Singh *et al*., 1981; Nasr *et al*., 2003; El-Sayed *et al*., 2009**).

The results obtained from the investigation of the antimicrobial potencies of either the plant extracts or the thiazolidinone derivatives in liquid cultures showed that most of the examined plant extracts and the tested thiazolidinone derivatives exhibited inhibitory actions against most of the bacterial species investigated. The results here may be not coincident with that obtained on the solid plate technique. Some substances showed inhibitory affects against a certain species in liquid cultures, while the same substance caused no inhibition zones on the plates planted by the same species. This could be explained on the bases of the correlation between inhibitory potency (inhibition zone formation, and its diameter) and the ability of the tested substance to diffuse in the solid growth medium (**Egorov, 1985; Shaaban and El-Sharif, 2001**).

5. Conclusion:

We can conclude that studies should be directed towards nontraditional antimicrobial agents from natural sources and safe cheap chemicals (**El-Sayed *et al*., 2009, Shaaban 2011; 2012**) to be added to the fish foods to prevent or minimize the bacterial contamination in these foods.

Correspondence author name:
Mohamed T. Shaaban
Botany Department, Faculty of Science, Menoufiya University, Egypt
Dr_mohamedtawfiek@yahoo.com

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Direct Boundary Element Method for Calculation of Hyperbolic Flow Past a Sphere

Muhammad Mushtaq[1], Nawazish Ali Shah[1] & G. Muhammad[2]

[1]Department of Mathematics, University of Engineering & Technology Lahore – 54890, Pakistan
[2]Department of Mathematics, GCS, Wahdat Road, Lahore – Pakistan
Corresponding Author, e-mail: mushtaqmalik2004@yahoo.co.uk

Abstract: In this paper, direct method is applied for calculating the hyperbolic flow past a sphere. The surface of the body is discretised into boundary elements on which the velocity distribution is found. The comparison of computed and exact results is also made.
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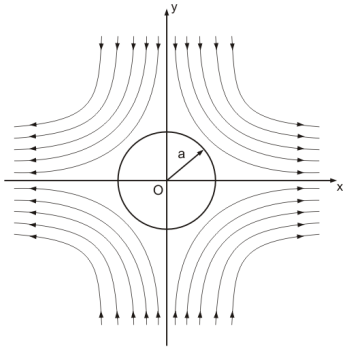
Keywords: Direct boundary element method, hyperbolic flow, Sphere

Introduction

Direct method is a numerical method which is in the form of a statement which gives the values of unknown variables at the field point under discussion in terms of a complete set of the entire boundary data. This method is used in different areas like solid and fracture mechanics, fluid dynamics and potential theory etc. [1]. The initial work for potential flow calculations was done by Hess and Smith ([2], [3]). Indirect method is popular due to its simplicity because the discretisation only takes place on the surface of the body. The direct method was applied for potential flow calculation in the past by Morino [4], Muhammad [8] and Mushtaq [9].

Calculation of Hyperbolic Flow Past a Sphere

Let a sphere of radius 'a' be taken as stationary and let U be the velocity of a uniform stream flowing in the positive direction of x – axis as shown in figure 1 [5].



Hyperbolic flow past a sphere

Figure (1)

The stream function in this case given by

$$\psi = \Omega x (y^2+z^2)\left\{1-\frac{5}{2}\left(\frac{a}{R_1}\right)^3+\frac{3}{2}\left(\frac{a}{R_1}\right)^5\right\} \quad (1)$$

Since $y^2+z^2 = r^2$

$$\psi = \frac{U x}{\sqrt{4x^2+y^2+z^2}}(y^2+z^2)\left\{1-\frac{5}{2}\left(\frac{a}{R_1}\right)^3+\frac{3}{2}\left(\frac{a}{R_1}\right)^5\right\}$$

$$= \frac{U x r^2}{\sqrt{4x^2+r^2}}\left\{1-\frac{5}{2}\left(\frac{a}{R_1}\right)^3+\frac{3}{2}\left(\frac{a}{R_1}\right)^5\right\} \quad (2)$$

Since $v_x = -\frac{1}{r}\frac{\partial \psi}{\partial r}$

and $v_r = \frac{1}{r}\frac{\partial \psi}{\partial x}$

$$\frac{\partial \psi}{\partial r} = U\left[\left\{\frac{2xr}{\sqrt{4x^2+r^2}}-\frac{xr^2(2r)}{2(4x^2+r^2)^{3/2}}\right\}\left\{1-\frac{5}{2}\left(\frac{a}{R_1}\right)^2+\frac{3}{2}\left(\frac{a}{R_1}\right)^5\right\}+\frac{xr^2}{\sqrt{4x^2+r^2}}\left\{-\frac{15}{2}\left(\frac{a}{R_1}\right)^2\frac{\partial}{\partial r}\left(\frac{a}{R_1}\right)+\frac{15}{2}\left(\frac{a}{R_1}\right)^4\frac{\partial}{\partial r}\left(\frac{a}{R_1}\right)\right\}\right]$$

$$= U\left[\left\{\frac{2xr}{\sqrt{4x^2+r^2}}-\frac{xr^3}{(4x^2+r^2)^{3/2}}\right\}\left\{1-\frac{5}{2}\left(\frac{a}{R_1}\right)^2+\frac{3}{2}\left(\frac{a}{R_1}\right)^5\right\}+\frac{xr^2}{\sqrt{4x^2+r^2}}\left\{-\frac{15}{2}\left(\frac{a}{R_1}\right)^2\left(-\frac{a}{2R_1^3}2r\right)+\frac{15}{2}\left(\frac{a}{R_1}\right)^4\left(-\frac{a}{2R_1^3}2r\right)\right\}\right]$$

$$= U\left[\left\{\frac{2xr(4x^2+r^2)-xr^3}{(4x^2+r^2)^{3/2}}\right\}\left\{1-\frac{5}{2}\left(\frac{a}{R_1}\right)^3+\frac{3}{2}\left(\frac{a}{R_1}\right)^5\right\}+\frac{xr^2}{\sqrt{4x^2+r^2}}\left\{\frac{15ar}{2R_1^3}\left(\frac{a}{R_1}\right)^2-\frac{15ar}{2R_1^3}\left(\frac{a}{R_1}\right)^4\right\}\right]$$

$$= U\left[\frac{x\, r\,(8x^2+2r^2-r^2)}{(4x^2+r^2)^{3/2}}\left\{1-\frac{5}{2}\left(\frac{a}{R_1}\right)^3+\frac{3}{2}\left(\frac{a}{R_1}\right)^5\right\}+ \frac{x\,r^2}{\sqrt{4x^2+r^2}}\ \frac{15\,r}{2}\left(\frac{a^3}{R_1^5}-\frac{a^5}{R_1^7}\right)\right]$$

$$= U\left[\frac{x\, r\,(8x^2+r^2)}{(4x^2+r^2)^{3/2}}\left\{1-\frac{5}{2}\left(\frac{a}{R_1}\right)^3+\frac{3}{2}\left(\frac{a}{R_1}\right)^5\right\} + \frac{x\,r^2}{\sqrt{4x^2+r^2}}\ \frac{15\,a^3 r}{2R_1^7}\left(R_1^2-a^2\right)\right]$$

$$= U\left[\frac{x\,r\,(8x^2+r^2)}{(4x^2+r^2)^{3/2}}\left\{1-\frac{5}{2}\left(\frac{a}{R_1}\right)^3+\frac{3}{2}\left(\frac{a}{R_1}\right)^5\right\}+\frac{15}{2}\frac{a^3 x\, r^3\left(R_1^2-a^2\right)}{R_1^7\sqrt{4x^2+r^2}}\right] \quad (3)$$

$$v_x = -\frac{1}{r}\frac{\partial \psi}{\partial r}$$

$$= -\frac{U}{r}\left[\frac{x\,r\,(8x^2+r^2)}{(4x^2+r^2)^{3/2}}\left\{1-\frac{5}{2}\left(\frac{a}{R_1}\right)^3+\frac{3}{2}\left(\frac{a}{R_1}\right)^5\right\}+\frac{15}{2}\frac{a^3 x\, r^3\left(R_1^2-a^2\right)}{R_1^7\sqrt{4x^2+r^2}}\right]$$

$$= -U\left[\frac{x\,(8x^2+r^2)}{(4x^2+r^2)^{3/2}}\left\{1-\frac{5}{2}\left(\frac{a}{R_1}\right)^3+\frac{3}{2}\left(\frac{a}{R_1}\right)^5\right\}+\frac{15}{2}\frac{a^3 x\, r^2\left(R_1^2-a^2\right)}{R_1^7\sqrt{4x^2+r^2}}\right]$$

$$\frac{\partial \psi}{\partial x} = U$$

$$\left[\left\{\frac{r^2}{\sqrt{4x^2+r^2}}-\frac{x\,r^2(8x)}{2(4x^2+r^2)^{3/2}}\right\}\left\{1-\frac{5}{2}\left(\frac{a}{R_1}\right)^3+\frac{3}{2}\left(\frac{a}{R_1}\right)^5\right\} + \frac{x\,r^2}{\sqrt{4x^2+r^2}}\left\{-\frac{15}{2}\left(\frac{a}{R_1}\right)^2\frac{\partial}{\partial x}\left(\frac{a}{R_1}\right)+\frac{15}{2}\left(\frac{a}{R_1}\right)^4\frac{\partial}{\partial x}\left(\frac{a}{R_1}\right)\right\}\right]$$

$$= U\left[\left\{\frac{r^2(4x^2+r^2)-4x^2r^2}{(4x^2+r^2)^{3/2}}\right\}\left\{1-\frac{5}{2}\left(\frac{a}{R_1}\right)^3+\frac{3}{2}\left(\frac{a}{R_1}\right)^5\right\} + \frac{x\,r^2}{\sqrt{4x^2+r^2}}\left\{-\frac{15}{2}\left(\frac{a}{R_1}\right)^2\left(-\frac{a}{2R_1^3}2x\right)+\frac{15}{2}\left(\frac{a}{R_1}\right)^4\left(-\frac{a}{2R_1^3}2x\right)\right\}\right]$$

$$= U\left[\left\{\frac{r^2(4x^2+r^2-4x^2)}{(4x^2+r^2)^{3/2}}\right\}\left\{1-\frac{5}{2}\left(\frac{a}{R_1}\right)^3+\frac{3}{2}\left(\frac{a}{R_1}\right)^5\right\} + \frac{x\,r^2}{\sqrt{4x^2+r^2}}\left\{\frac{15}{2}\frac{a\,x}{R_1^3}\left(\frac{a}{R_1}\right)^2-\frac{15}{2}\frac{a\,x}{2R_1^3}\left(\frac{a}{R_1}\right)^4\right\}\right]$$

$$= U\left[\frac{r^4}{(4x^2+r^2)^{3/2}}\left\{1-\frac{5}{2}\left(\frac{a}{R_1}\right)^3+\frac{3}{2}\left(\frac{a}{R_1}\right)^5\right\} + \frac{x\,r^2}{\sqrt{4x^2+r^2}}\ \frac{15\,x}{2}\left(\frac{a^3}{R_1^5}-\frac{a^5}{R_1^7}\right)\right]$$

$$= U\left[\frac{r^4}{(4x^2+r^2)^{3/2}}\left\{1-\frac{5}{2}\left(\frac{a}{R_1}\right)^3+\frac{3}{2}\left(\frac{a}{R_1}\right)^5\right\} + \frac{x\,r^2}{\sqrt{4x^2+r^2}}\ \frac{15\,x\,a^3}{2R_1^7}\left(R_1^2-a^2\right)\right]$$

$$= U\left[\frac{r^4}{(4x^2+r^2)^{3/2}}\left\{1-\frac{5}{2}\left(\frac{a}{R_1}\right)^3+\frac{3}{2}\left(\frac{a}{R_1}\right)^5\right\}+\frac{15}{2}\frac{a^3x^2r^2\left(R_1^2-a^2\right)}{\sqrt{4x^2+r^2}}\right]$$

$$v_r = -\frac{1}{r}\frac{\partial \psi}{\partial x}$$

$$= U\left[\frac{r^4}{(4x^2+r^2)^{3/2}}\left\{1-\frac{5}{2}\left(\frac{a}{R_1}\right)^3+\frac{3}{2}\left(\frac{a}{R_1}\right)^5\right\}+\frac{15}{2}\frac{a^3x^2r^2\left(R_1^2-a^2\right)}{\sqrt{4x^2+r^2}}\right]$$

$$= U\left[\frac{r^3}{(4x^2+r^2)^{3/2}}\left\{1-\frac{5}{2}\left(\frac{a}{R_1}\right)^3+\frac{3}{2}\left(\frac{a}{R_1}\right)^5\right\}+\frac{15}{2}\frac{a^3x^2r\left(R_1^2-a^2\right)}{R_1^7\sqrt{4x^2+r^2}}\right] \quad (4)$$

Equation of Direct Boundary Element Method

The equation of direct boundary element method for three – dimensional problems is given by (see [6, 7, 8, 9]).

$$c_i \phi_i = \phi_\infty - \frac{1}{4\pi}\iint_S \frac{1}{r}\frac{\partial \phi}{\partial n}\, d S + \frac{1}{4\pi}\iint_{S-i} \phi \frac{\partial}{\partial n}\left(\frac{1}{r}\right) d S \quad (5)$$

Discretization of Sphere:

The surface of the sphere is discretized into quadrilateral elements. The scheme of discretization is as shown in the figure (2).

The direct boundary element method is applied to calculate the hyperbolic flow solution around the sphere for which the analytical solution is available

Consider the surface of the sphere in one octant to be divided into three quadrilateral elements by joining the centroid of the surface with the mid points of the curves in the coordinate planes as shown in figure (2) [7, 8, 9].

Then each element is divided further into four elements by joining the centroid of that element with the mid–point of each side of the element. Thus one octant of the surface of the sphere is divided into 12 elements and the whole surface of the body is divided into 96 boundary elements. The above mentioned method is adopted in order to produce a uniform distribution of element over the surface of the body .

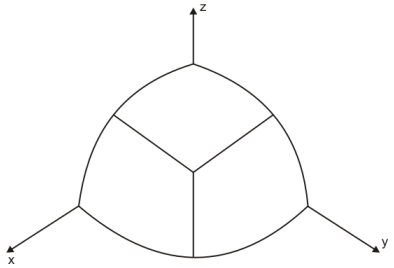


Figure (2)

Figure (3) shows the method for finding the coordinate (x_p, y_p, z_p) of any point P on the surface of the sphere.

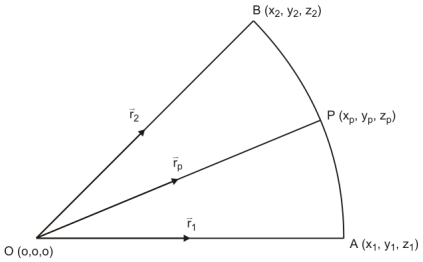


Figure (3)

From above figure, we have the following equation

$$|\vec{r}_p| = 1$$

$$\vec{r}_p \cdot \vec{r}_1 = \vec{r}_p \cdot \vec{r}_2$$

$$(\vec{r}_1 - \vec{r}_2) \cdot \vec{r}_p = 0$$

or in cartesian form

$$x_p^2 + y_p^2 + z_p^2 = 1$$

$$x_p (x_1 - x_2) + y_p (y_1 - y_2) + z_p (z_1 - z_2) = 0$$

$$x_p (y_1 z_2 - z_1 y_2) + y_p (x_2 z_1 - x_1 z_2) + z_p (x_1 y_2 - x_2 y_1) = 0$$

As the body possesses planes of symmetry, this fact may be used in the input to the program and only the non–redundant portion need be specified by input points. The other portions are automatically taken into account. The planes of symmetry are taken to be the coordinate planes of the reference coordinate system. The advantage of the use of symmetry is that it reduces the order of the resulting system of equations and consequently reduces the computing time in running a program. As a sphere is symmetric with respect to all three coordinate planes of the reference coordinate system, only one eighth of the body surface need be specified by the input points, while the other seven–eighth can be accounted for by symmetry.

The sphere is discretised into 96 and 384 boundary elements and the computed velocity distributions are compared with analytical solutions for the sphere using Fortran programming.

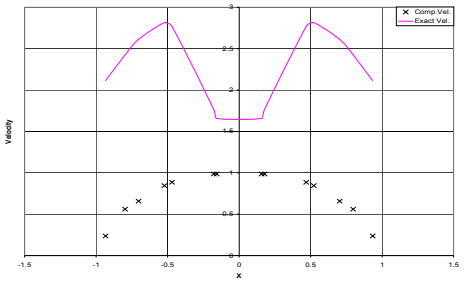


Figure (4): Comparison of computed and analytical velocity distributions over the surface of the sphere using 96 boundary elements.

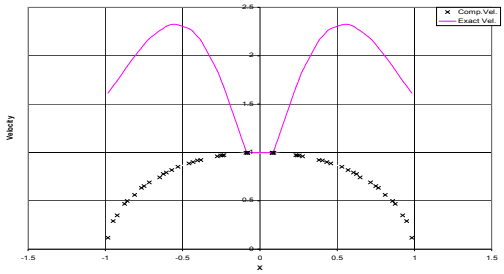


Figure (5): Comparison of computed and analytical velocity distributions over the surface of the sphere using 384 boundary elements.

Conclusion

Direct boundary element method has been applied to calculate the hyperbolic flow past a sphere. The improvement in results gained by taking 384 can be seen from figures (4) and (5) and such improvement increases with increase in number of boundary elements. Moreover, the computed results are in good agreement with exact results at the top of a body under consideration.

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Effect of Electromagnetic Mobile Radiation on Chick Embryo Development

Fatma Al-Qudsi and Solafa Azzouz

Biology Department, Science Faculty, King Abdulaziz University, Jeddah, Saudi Arabia
P.O. Box 42650, Jeddah 21551, Saudi Arabia
falqudsi@kau.edu.sa

Abstract: The widespread use of mobile phones in the last decade has increased the concern about its potential effects on human body. This research aims to study the effect of electromagnetic waves emitted from mobile phones on chick embryos development. Fertile hen eggs were divided into two groups control and treated group. Both groups were incubated at 37.5 ◦C. The treated group had an active mobile device (900MHz- 1800MHz) during incubation. The mobile was rang 4 times daily for 15 minutes each time. Embryos were extracted on days 7, 10, and 14 of incubation. Congenital malformations were seen in treated embryos (bigger embryos, subcutaneous bleeding, and brain malformation) compared to the controls. Also increased eye growth in 7 and 10 days, significant increase in neural retina thickness in all studied ages, significant increase in retina lipid peroxidase and significant decrease in glutathione level in 10 and 14 days treated chick embryo retina compared to the controls. It was concluded that mobile phone electromagnetic waves (900MHz- 1800MHz) might induce embryonic eye growth till 10 days of incubation, and then it might cause brain malformation with reduced body and eye growth in chick embryo.
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Key words: mobile phones, chick embryo, eye development, retina thickness

1. Introduction

The number of mobile phone users worldwide is increasing tremendously. This led to increase the worries about possible health risks, related with mobile phone use and position near the body. Inhabitants living near mobile phone base stations. Suffered from; headache (23.5%), memory changes (28.2%), dizziness (18.8%), tremors (9.4%), depressive symptoms (21.7%), and sleep disturbance (23.5%) (Abdel-Rassoul *et al.,* 2007). Studies showed reproductive side effects caused by the use of mobile phones. Extremely low frequency electromagnetic field was able to reduce the fertilization rate in swine animal model, and negatively affect early embryo development. **(Bernabo *et al.*, 2010).** Some effects showed negative alterations of the nervous tissue in the brain and some sensory organs. Rapid cellular molecular alterations was seen in the rat brain after exposure to 900-MHz pulsed microwaves and power of 6 W/kg for 15-min. **(Bonnefont *et al.*, 2004).** Also electromagnetic waves emitted from mobile phones (900 MHz) reduced glutathione level in brain tissue and blood of exposed guinea pigs **(Meral *et al.*, 2007).** Electromagnetic field (EMF) emitted by a mobile phone with frequency 900 MHz caused derangement of chick embryo retinal differentiation **(Zareen, *et al.*,2009).** Low frequency electromagnetic fields caused chick embryos to have abnormal brain ventricles, spina bifida, eye malformation, and growth retardation **(Lahijani *et al.*, 2007).** The thermal effects of mobile phones on the eye induced cataracts, corneal edema, endothelial cells loss and retinal degeneration **(Vignal *et al.*, 2008).**

On the other hand electromagnetic waves were found to stimulate proliferation and differentiation of embryonic cells **(Parivar *et al.*, (2006).**

When using mobile phones the person is exposed to electromagnetic fields from the mobile phone and the work station. These electromagnetic fields might alter the cell structure beginning with the plasma membrane and its receptors to the different biomolecules present within the cell which might cause genotoxicity. This alteration might have its effect on cell proliferation in terms of increasing or reducing proliferation rate thus playing an important role during early embryonic development **(Panagopoulos, *et al.*, 2004, Zareen, *et al.*, 2009).**

Regarding the widespread of mobile phone use during pregnancy, it was very important to conduct series of studies to detect, analyze and understand the potential teratogenic effects of the use of mobile phones before and during pregnancy. Chick embryos were used as a model in this study for the ease of exposing them to a stable dose of EMF.

2. Material and Methods

This study was given approval for the methodology and other ethical issues concerning the work by Biology Department, – Science Faculty - King abdulaziz University.

Fertilized chicken eggs were obtained from a private home farm chicken raised by the researcher.

Average weight of eggs was (39.5) gm. The mobile phone used in the present study was on Extended Global System for Mobile Communication (EGSM) 900 MHz and 1800 MHz networks and Specific energy Absorption Rate (SAR) 2.0 watts/kilogram which is the limit stated by International Commission on Non-Ionizing Radiation (ICIRP) guidelines. Fertile hen eggs were incubated in an electrical incubator from Al-sloli foundation Kingdom of Saudi Arabia model number ME4A / ME4M in two batches. One for control group and the other for treated group. Each batch contained 30 eggs. Both were incubated under identical standard conditions temperature 37.5^{o}C, and suitable ventilation and humidity. For the treated group a mobile phone on silent was placed at the upper center of the eggs which were organized as a square circumference having the mobile at the center of the square. The distance between the mobile and eggs was ranging from 10.5 to 19 cm (.See Figure 1). The mobile phone was rang for 15 minutes every 6 hours daily from any other mobile phone, which is 60 minutes per-day. Embryos were collected from all groups at the following incubation days 7, 10 and 14. The embryos were extracted and washed in warm saline solution then they were dried and weighed, and photographed.

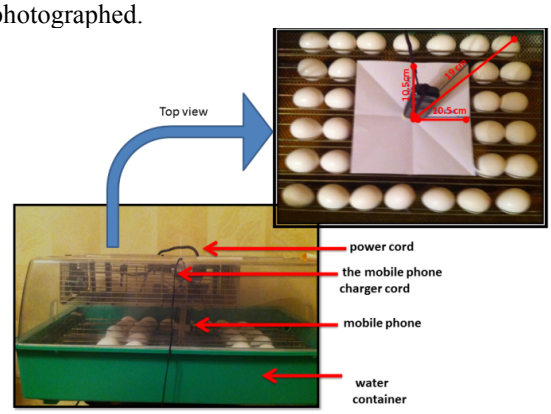

Figure 1: Showing the experimental set-up

At least 10 embryos from each group of each age were fixed in 10% formalin their eyes were removed for histological studies. The eyes were then dehydrated, cleared in xylene, embedded in soft paraffin wax and cut at 5µm thickness then stained by Haematoxylin and eosin (to examine the general structure of the retina), also 5 embryos of age 10 and 14 days of each group were frozen for Glutathione (GSH) and Lipid peroxide (LPO) assays.

Photographing:

Each embryo was photographed using a Lumix Panasonic camera as shown in Figure (2). a ruler was put near the embryo to be used as a scale when performing morphometrics using the photos. The camera zooming and distance between the camera and specimen was the same for all whole body photos. The head and left and right eye of each embryo were photographed using an Olympus SZx10 stereo microscope with DPZ-BSW camera.

Slides were photographed using a compound Olympus Bx51 microscope connected to an Olympus DP72 camera and a compound Nikon DS-Fil microscope connected to a Nikon ECLIPSC 80i camera.

Figure 2 : Showing the photographing methodology
Morphometric studies

Measurements of all alive control and treated specimens were taken. Full embryonic length measurements and head length were taken from the photos taken by the Lumix Panasonic camera using a computer program "Image tool" (http://ddsdx.uthscsa.edu/dig/itdesc). Length of the beak, and eye measurements, were taken by DPZ-BSW software. The measurements of the eye retina were taken from the histological slide photographs using Image tool program for measurements. See figure 3 for measurement method. At least 18 slides were done from 20 eyes of controls and each treatment. Each slide contained 3-4 sections. From each section 3 measurements were taken from 3 different areas. All readings were saved in Excel 2003.

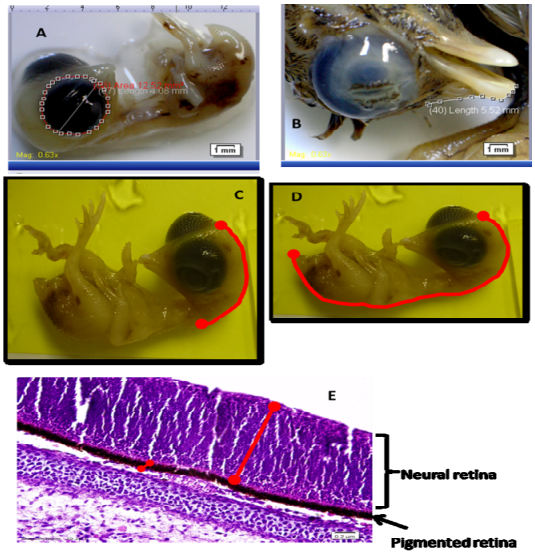

Figure 3: Showing morphometric measurements methodology. (A) eye measurements, (B) beak length, (C) head length, (D) whole body length, (E) retina thickness.

Glutathione (GSH) and Lipid peroxides (LPO) Assays:

Embryos of age 10 and 14 days were frozen immediately after extraction Eye retina of the frozen embryos was removed by making a slit at the back of each eye extracting the vitreous body and lens. The retina was then detached from the sclera. Retinas of the same age treatment were gathered till reaching the weight of 0.1 mg for each batch.

Reduced glutathione assay

Reduced GSH estimation was performed by the method of (Beutler *et al*., 1963). Retinas were homogenized in 1 ml of 1.1% KCl cooled, then homogenate (100 ll) was mixed with 750 ll of precipitate solution (1.67 g of glacial meta-phosphoric acid, 0.2 g of EDTA and 30 g of NaCl in 100 ml of D.W) and 900 ml of D.W. Homogenated tissue were centrifuged at 2000g for 15 min to precipitate proteins. Protein-free supernatant (250 ml) was added to 1 ml of Na^2 HPO4 (0.3 M) solution and the reaction was initiated by adding 125 ml of DTNB (6 mM) and the absorbance of 5-thio-2-nitrobenzoic acid (TNB) formed was measured at 412 nm. The level of GSH was obtained by standard curve and expressed as mmole per g eye tissue.

Lipid peroxidation assay:

The extent of LPO was estimated as the concentration of thiobarbituric acid (TBA) reactive product malondialdehyde (MDA) by using the method of Ohkawa *et al.* (1979). Two hundred fifty microliters of eye tissue homogenate were added to 1.5 ml of 1% phosphoric acid (pH 2.0) and 1 ml of 0.6% of TBA in air–light tubes and were placed in a boiling water bath for 25 min. After incubation, the sample was cooled to room temperature and MDA-TBA was extracted with 2.5 ml of butanol. Organic phase was separated by centrifugation for 5 min at 2000g and measured at 532 nm. MDA concentrations were determined using 1,1,3,3-tetraethoxypropane as standard and expressed as l mol/g eye tissue.

Statistical analysis:

Data was analyzed using SPSS 13. The test used with normal distribution was Anova, Student-Neuman Keul test. In case of abnormal distribution Man-Whiteney U test was used from the non parametric test. Significance was at $p<0.05$.

3. Results

Subcutaneous bleeding was seen in several treated embryos of age 7 and 10 days (Figure 4). The 14 days embryos were covered with feathers so it was not possible to examine them for subcutaneous bleeding. However some congenital malformations were seen in 14 days embryos these were; development of one eye or no eye development (anophthalmia),brain malformation, abdominal hernia and beak malformation(. See Figure 5).

An increase in whole body weight and whole body length in 7 and 10 days treated embryos was seen compared to the controls, however the increase was only significant in 10 days treated embryos (P=0.012) for both weight and length. On the other hand 14 day treated embryos showed a non significant decrease in whole body weight and whole body length compared to the controls (See Figure 6).

Treated embryonic beak length showed an increase in 7 day embryos compared to the controls, however the increase was not significant. On the other hand 10 and 14 days treated embryos showed a significant increase in embryo beak length P=0.049 for both compared to the controls (See Figure 6)

Treated embryo right and left eye growth parameters (eye weight, eye diameter, eye area, eye perimeter) showed an increase in 7 and 10 day embryos compared to the controls, however the increase was only significant in 10 days treated embryos (p= 0.000 for right and left eye weight, p= 0.041. p= 0.049 for right and left eye diameter respectively, p= 0.001. p = 0.000 for right and left eye area respectively, p=0.002, p= 0.007 for right and left eye perimeter respectively. On the other hand left and right eye growth parameters of 14 day treated embryos decreased non-significantly (See Figure 7).

Significant increase in neural retina thickness was seen in all studied ages 7,10, and 14 day of

treated chick embryo compared to the controls *P*=0.000. (See Figure 8)

No difference in pigmented retina thickness was seen in 7 and 10 day treated embryos. On the other hand 14 day treated embryos showed a significant increase in pigmented retina thickness compared to the controls *P*=0.020 (See Figure 8).

A significant decrease in retina glutathione was seen in treated embryos in days 10 and 14 of incubation compared to the controls *P*=0.000. See (Figure 9).

A significant increase in retina lipid peroxidation was seen in treated chick embryos in days 10 and 14 of incubation compared to the controls *P*=0.000. See (Figure 9).

On statistically comparing neural retina thickness of treated 7 day chick embryo and control 10 day chick embryo no significant difference was seen between neural retina thickness of treated 7 day chick embryo and control 10 day chick embryo *P*=0.63, on the other hand when statistically comparing between neural retina thicknesses of controls 7 day chick embryo and control 10 day chick embryo a high significant different was seen between neural retina thickness of control 7 day chick embryo and control 10 day chick embryo *P*=0.000.

Also a high significant difference was seen between neural retina thickness of treated 10 day chick embryo and control 14 day chick embryo. *P* = 0.000

An examination of the cross sections of eye Retina (R) in 7 day chick embryos in treated group showed that the thickness of the Neural zone (NZ), Ganglion Cell layer (GCL), and Optic Fiber layers (OFL), increased in treated compared to the controls. See (Figure 4.21-B).

An examination of the cross sections for eye Retina in 10 day chick embryos in treated group showed that

The neural retina in the treated embryos seemed to be thicker compared to the control. Rods and Cones were larger. Henl's membrane seemed to be disintegrated.

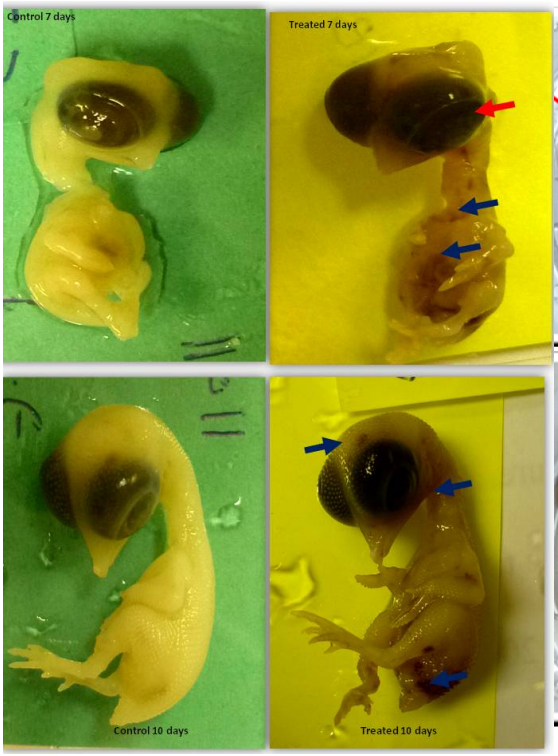

Figure 4: showing the sites of subcutaneous bleeding in 7 day (upper images) and 10 day (lower images) chick embryos (blue arrows), and the bigger eyes (red arrow)

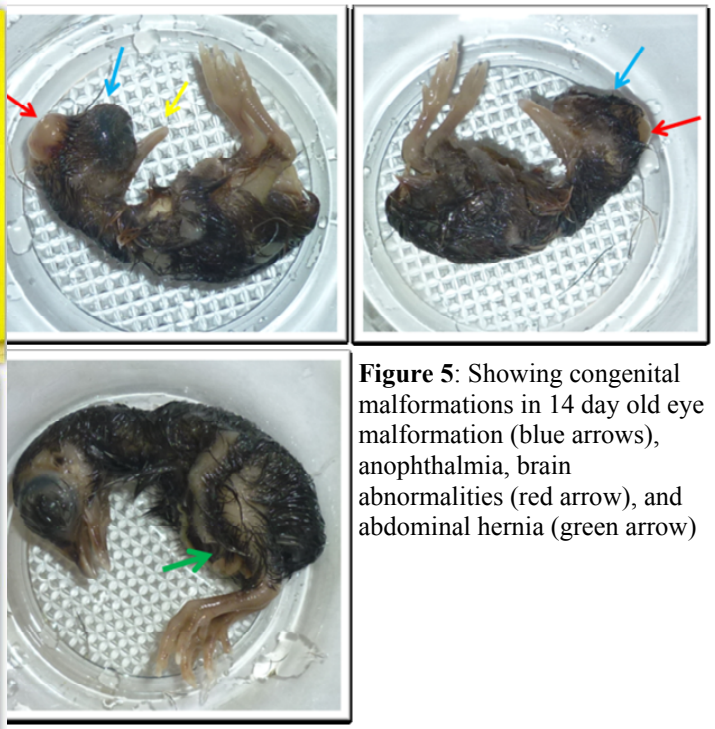

Figure 5: Showing congenital malformations in 14 day old eye malformation (blue arrows), anophthalmia, brain abnormalities (red arrow), and abdominal hernia (green arrow)

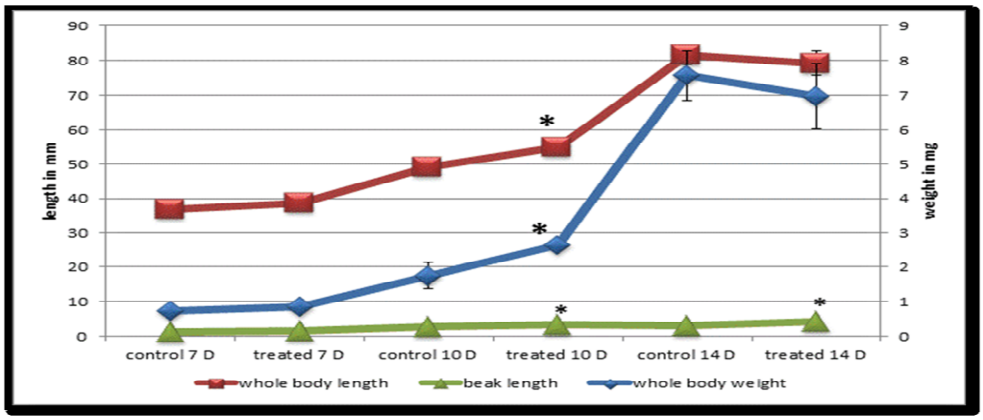

Figure 6: Graph showing the effect of mobile electromagnetic waves on chick embryo whole body length, whole body weight and beak length. Values are means ±SE, taken from 10 samples. For control and each treatment (*) $p< 0.05$

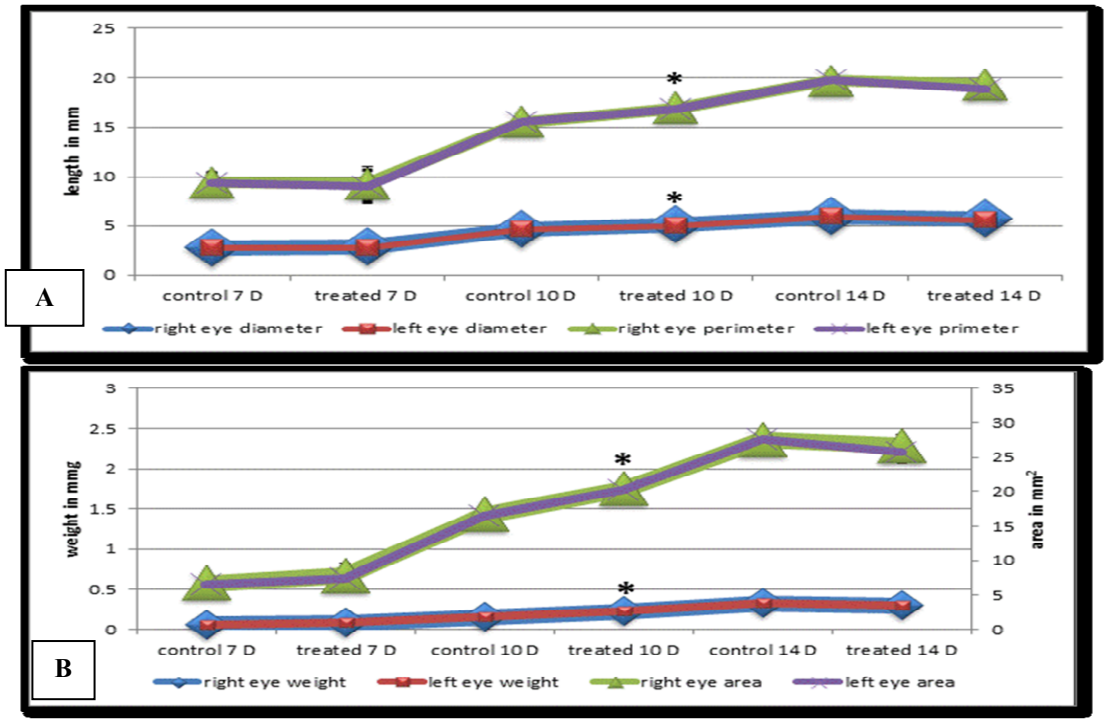

Figure 7: Graphs showing the effect of mobile electromagnetic waves on chick embryo right and left eye parameters. **(A)** Showing eye weight and eye area and **(B)** showing eye diameter and eye perimeter. Values are means ±SE, taken from 10 samples for control and each treatment (*) $p< 0.05$

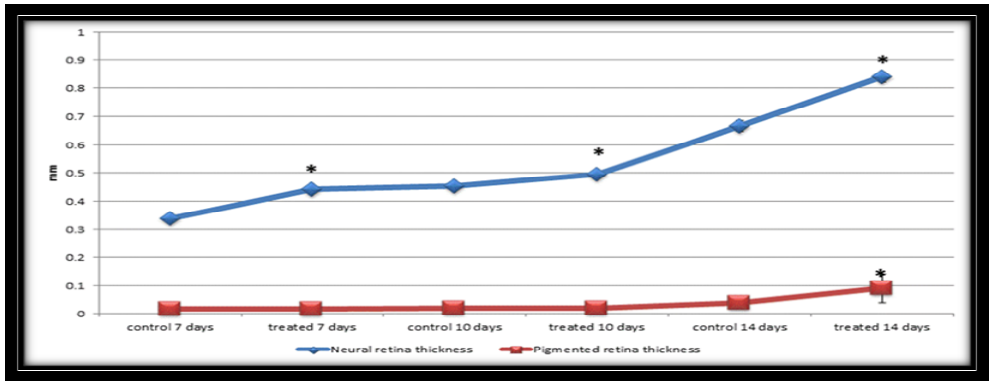

Figure 8: Graph showing the effect of mobile electromagnetic waves on chick embryo retina thickness. Values are mean ± SE (no of readings; 7 days 70, 10 days 52, 14 days 57(*) $p< 0.05$.

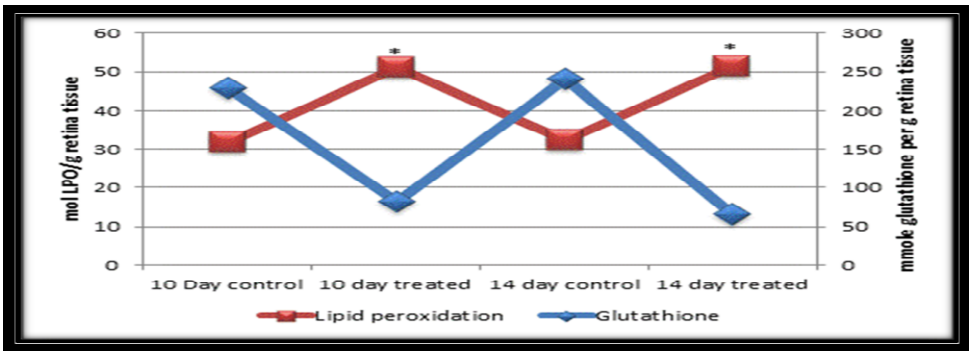

Figure 9: Graph showing the effect of mobile electromagnetic waves on chick embryo retina content of glutathione and lipid peroxidase. Values are mean ± SE (no of readings; 3 for each treatment taken from 5 embryos of each treatment. (*) $p < 0.05$

The Inner nuclear layer was less intensive. Inner Plexiform layer and ganglion cell layer seemed larger and the optic fiber layer seemed disintegrated compared to control See (Figure 10).

An examination of the cross sections of eye Retina (R) in 14 day chick embryos treated group showed that the neural retina in the treated seemed thicker compared to control. Also the rods and cones seemed more intensive compared to control. The inner nuclear layer seemed thicker and cells appeared disorganized. The Ganglion cell layer appeared also disorganized and thinner compared to control.

The optic fiber layer appeared disintegrated See (Figures 4.23-B; 4.24-B)

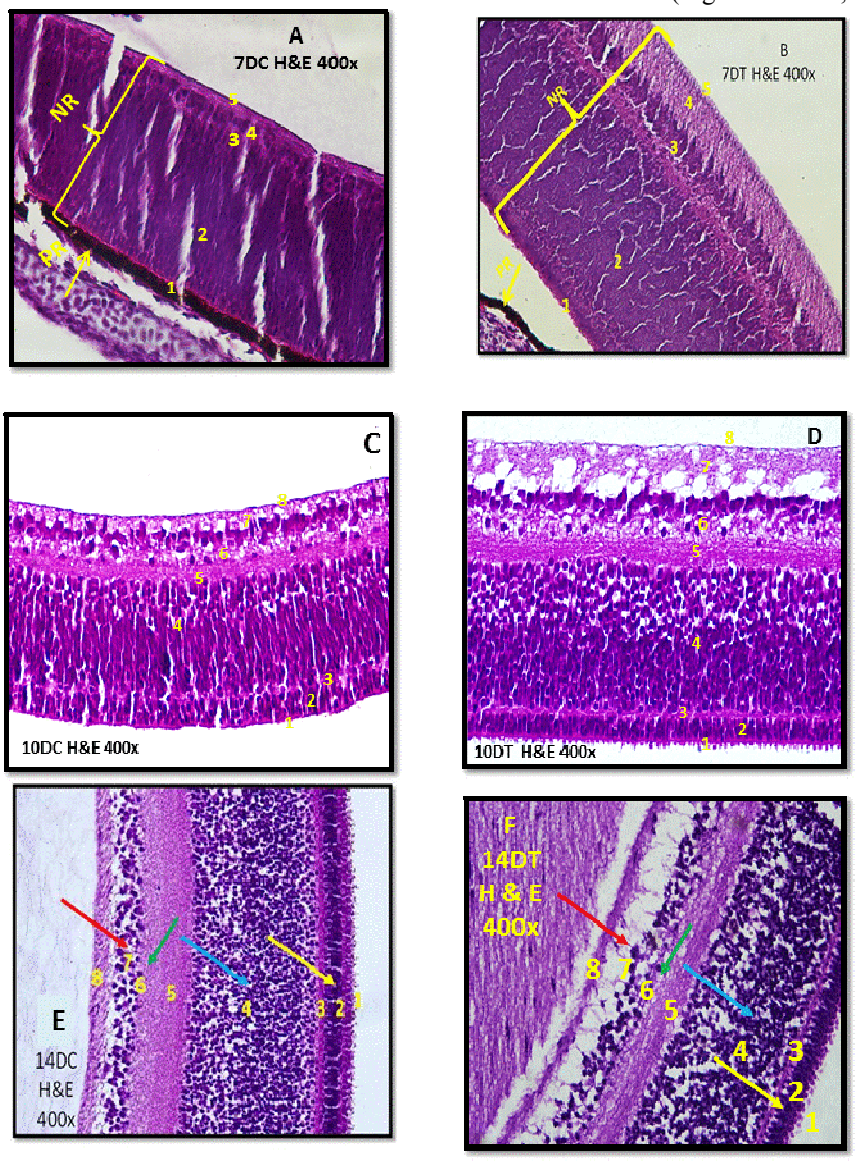

Figure (10): Histological sections of chick embryo retinas. (mag. 400X, H&E.)
(A) Control 7 day
(B) Treated 7 days, (PR) Pigmented Retina. (NR) Neural Retina. (1) External Limiting Membrane. (2) Neural Zone. (3) Ganglion Cell Layer. (4) Optic Fiber Layer. (5) Internal Limiting Membrane. Note that (NR) seems to be thicker and more differentiated on (B) compared to (A).
(C) Control 10 days
(D) treated 10 days
(E) control 14 days
(F) treated 14 days. (1)External Limiting Membrane. (2)Rods and Cones. (3) Henl's Membrane. (4) Inner Nuclear layer. (5)Inner Plexiform layer. (6)Ganglion Cell layer. (7) Optic Fiber layer. (8) Internal Limiting Layer. Note that rods and cones (2) are more differentiated in (D and F) compared to (C and E) (yellow arrow), layer (3) seems more distinct in (D and F) compared to (C and E) , layer (4) seems to have more spaces between cells in (D and F) compared to (C and E) (blue arrow), layers (5&6) seems to be thicker in (D and F) compared to (C and E) (green arrow), layer (7) in (D and F) seems to be disintegrated having many clear spaces compared to (C and E) (red arrow).

4. Discussion

The hazardous or beneficial biological effects of electromagnetic field on human and animals is the subject of many studies nowadays **(Lotifi *et al.*, 2012).** Mobile phone culture is spreading rapidly. The technology is quickly penetrating not only the lifestyles of adults, but is also becoming commonly used by children. **(Zareen *et al*., 2009).**

In this study the exposure of chick embryo to electromagnetic radiation (EMR) 900-1800 MHz during incubation period caused embryos to have different congenital malformation. Such as subcutaneous bleeding, anophthalmia, head abnormalities and abdominal hernia. Subcutaneous bleeding was reported in other studies. Several reports indicated harmful effects of 50-60 Hz electromagnetic waves on biological organs. Electromagnetic waves are able of causing hemorrhages, sinusoid denaturation, and an increase in lymphoidal tissues in white leghorn chick embryos exposed for 24 hour before incubation to 50-6- Hz. **(Lahijani *et al.*, 2011).**

Some of the abnormalities seen in this study were seen in other studies; **Lahijani *et al*., (2007)** studied the effect of electromagnetic waves with frequencies of 50 Hz on white leghorn chick embryo. Eggs were exposed for 24 hours before incubation. Results showed larger and abnormal brain structure, spina bifida, monophthalmia, microphthalmia, anophthalmia and growth retardation were observed in exposed embryos **(Lahijani *et al.*, 2007).** In this study anophthalmia was seen in 14 day embryos having abnormal brain tumors. Any malformation in the anterior part of brain may influence inductive activities of neural ectoderm **(Lahijani *et al.*, 2009).** Rapid cellular and molecular alterations in the rat brain were seen after exposure to mobile phone radiation **(Bonnefont *et al*., 2004).** Electromagnetic waves emitted by mobile phones are absorbed in the brain within range that could influence neuronal activity. Although the intensity of waves is very low, the oscillatory frequencies correspond to some of the oscillation frequencies recorded in neuronal tissue and could interfere with neuronal activity. **(Volkow *et al*., 2011).**

In this study the exposure of chick embryo to electromagnetic radiation (EMR) 900 - 1800 MHz during incubation period caused increased growth in 7and 10 day embryos as seen through growth parameters (whole body weight, whole body length, beak length). Increased eye development was also seen (eye weight, eye diameter, eye area, and eye perimeter). This increase was significant on day 10 of incubation. Increase in growth usually indicates an increase in cell multiplication and, increase metabolism leading to an increase in glycogen consumption within the cell. Some studies showed greater embryonic growth and body mass in EMF exposed groups.

Parivar *et al*. (2006) reported a significant increase of cell division in the limb of mouse embryo due to electromagnetic waves 50Hz. **Volkow *et al*. (2011)** reported that electromagnetic waves of 0.901 W/kg emitted by mobile phones significantly increased brain glucose metabolism in the region closest to the antenna in human.

Ahijani and Ghafoori (2000) reported that there was no significant difference in measurement of body weight, length of crown to rump, length of tip of the beak to occipital bone, heart and liver weight to chick embryos exposed to 50Hz electromagnetic fields for 24hrs of before incubation. The difference between these results and the results of this study might be due to the difference in the electromagnetic field and the time of exposure, in the present study the electromagnetic field was higher and the time of exposure was more, which indicates that the field strength and exposure durance could be important factors determining the effect of electromagnetic radiation on chick embryos.

In the present study the extended exposure of chick embryo to electromagnetic waves 900-1800MHz till 14 days of incubation period caused a non-significant decrease in growth parameter. This might be due to different cellular responses to EMF during different embryological periods as cells might be trying to rebalance their growth and differentiation rate. **Batellier *et al*. (2008)** reported that chick embryo mortality of fertile eggs exposed to 900MHz was about 4.5% during the first 4 day of incubation, 1% from days 5 to 7, less than 1% from day 7 to 14, and 6.1% from day 18 to 21.

Zareen *et al.* (2009B) studied the effect of electromagnetic radiation emitted by mobile phones on chick embryos during incubation for 10 and 15 days. The eggs were exposed to different doses of mobile phone electromagnetic radiation. The exposure levels were as follows; Grade I: Low dose, short time duration exposure level, which resulted in significantly less weights and lengths. Grade II: High dose, short time duration exposure level, where weight increased. Grade III: Low dose, long time duration exposure level and Grade IV: High dose, long time duration exposure level.

Grade III and Grade IV resulted in significantly higher weight and length compared to the controls. This might be due to increased growth linked with higher dose of electromagnetic radiation. however it seems that The exposure grading might have been one of the methodological limitations of the study. As the amount of exposure was not mentioned. The study referred growth enhancement to several factors

such as EMF acting as soluble growth factors, or that EMF might be stimulating cell proliferation and differentiation and activating metabolic process, the study also mentioned that such growth could induce the probability of an uncontrolled neoplastic cellular proliferation induced **(Zareen, *et al.*, 2009).**

Lotifi *et al.* (2012) found that exposing chick embryos to 50 Hz electromagnetic field during the 21 day of incubation decreased significantly the amount of glucose in the blood serum of newly hatched chicks.

In this study the exposure of chick embryo to electromagnetic radiation (EMR) 900 - 1800 MHz during incubation period caused a significant increase in embryonic retina lipid peroxidation and a significant decrease in glutathione level in days 10 and 14 of incubation compared to the controls. Electromagnetic waves of mobile phone may affect biological systems by increasing free radical (enhancing lipid peroxidation), and by changing the antioxidant defense system of tissue, thus leading to oxidative stress. Studies indicated that electromagnetic waves emitted by mobile phone of 900-MHz was associated with increased free radical production and lipid peroxidation levels and a decrease in glutathione level in blood and brain tissue in guinea pigs. **(Meral, *et al.*, 2007).**

In this study the exposure of chick embryo to electromagnetic radiation (EMR) 900 and 1800 MHz during incubation period caused a significant increase in neural retina thickness in all ages of treated chick embryos compared to the controls. Many studies reported similar results under different exposures of electromagnetic radiation.

Zareen, *et al,* (2009A) reported that retinal development was delayed as a result of exposing chick embryos to 1800 MHz emitted by a mobile phone, however this delay was then transformed into a growth enhancement as the duration to EMF was expanded to 15 days of incubation. **Khaki *et al.* (2011)** studied the effect of electromagnetic waves with frequencies from 50 Hz to 60 Hz in rat. The experimental group was under electromagnetic waves for 4 weeks. Total retina thickness was significantly increased compared to the control.

In this study when statistically comparing the thickness of neural retina of treated 7 day chick embryo with that of control 10 day chick embryo no significant different was seen, on the other hand comparing control 7 day chick embryo with control 10 day chick embryo showed highly significant results P=0.000. This indicates that electromagnetic field EMF used in this study induced neural retina growth of 7 day treated chick embryo to reach that of control 10 day chick embryo.

This was not the case when comparing neural retina thickness of treated 10 day chick embryo and control 14 day chick embryo as there was a significant different, which indicates that between day10 and 14 embryo's neural retina did not response to EMF as did the neural retina of 7 day embryos. It might be that some factors were changed within the cells or cell environment.

This study showed that EMF seems to induce embryonic growth and differentiation during chick embryonic development till 10 days of incubation. The effect then turns to be depressive. More studies should be done on earlier development stages covering all aspects of developmental biology in order to understand the real effect of EMF on cellular behavior during early embryonic stages. There might be hope that EMF at might help regenerate loss body parts the induce cell dedifferentiation.

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Corresponding author
Fatma Al-Qudsi
Biology Department, Science Faculty, King Abdulaziz University, Jeddah, Saudi Arabia P.O. Box 42650, Jeddah 21551, Saudi Arabia
falqudsi@kau.edu.sa

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Hazardous Effects of Hyperthermia on Brain and Testicular Responses in Rats

Faisal A. Bughdadai

Dean of University College Umm Al-Qura Univ.Makkah – Saudi Arabia
fabughdadi@uqu.edu.sa

Abstract: Hyperthermia was investigated in adult male albino rats exposed to high temperature (40 ± 1°C) for 12 hours. Twenty male rats 3 months old were used. They were divided into two groups and separated into two rooms. The rats in the first room were subjected to hyperthermia (40±1°C), while rats of the control group were maintained under normal environmental conditions (25±5°C). Neural response to heat exposure was assessed by determining the levels of dopamine, nor adrenaline and serotonin in the brain homogenate. Serum electrolytes (sodium, potassium, magnesium and calcium) were also evaluated. Testes response to heat exposure was also assessed by determining the levels of LH and testosterone by (EIA). Histopathological examination of brain and testes were evaluated after heat exposure. Brain content of dopamine, nor adrenaline and serotonin showed significant increase, there was an increased potassium and calcium levels while sodium and magnesium concentrations decreased. Also LH testosterone levels decreased after heat exposure.
Histopathological examination revealed hyperemic capillaries and blood vessels allover the cerebral and disorganization of semineferous tubules of the testes. In conclusion, avoidance of hyperthermia is very important, the body temperature must be maintained in a safe normothermic range. It is recommended to assess the role of nutritional supplementation to alleviate the hazardous effects of heat stress.

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Key Words: Brain, testicular, Biochemical changes, Hyperthermia, rats.

1. Introduction

Hyperthermia is a condition of abnormal rise of core (intraperitoneal) body temperature resulting from exceeded heat gain over heat loss (Robergs and Roberts, 2000). They added that the increasing hyperthermia can lead to a series of system and cellular changes that increase risk for exhaustion, organ failure (especially the brain and testes), and death. Although hyperthermia can occur without severe dehydration, dehydration exacerbates the condition. Mickley et al., (1997) stated that hyperthermia or heat shock or hyperpyrexia is mostly achieved following prolonged exposure to high atmospheric temperature. This occurs usually in the tropic regions during summer. Heat generation is also occurred by injection of pyrogens or during exposure to hyperthermic agents as microwaves, ultrasonic and radiofrequency radiation.

Guyton and Hall (2006) noted that when the body temperature rises beyond a critical temperature into the range of 105°F to 108°F, the individual is likely to develop heatstroke. They added that the hyperpyrexia itself is also exceedingly damaging to the body tissues, especially the brain, and is responsible for most of the effects. In fact, even a few minutes of very high body temperature can sometimes be fatal. A complicating fact to achieve hyperthermia is the variety of normal body temperature between mammalian species, eg. 37°C in human, 38°C in rats, 39°C in pigs and 39°C in rabbits and sheep (Miller and Ziskin, 1989). Hyperthermia intensified the processes of lipid peroxidation in many organs (Kokuta et al., 2002).

Zaghloul (2009) reported that spermatozoa are subjected to different stresses during thawing, it should be slow enough to prevent intracellular ice formation which is lethal.

Several noxious effects on the central nervous system were induced by hyperthermia. It caused disruption of the blood brain barrier, increased intracranial pressure and induced an upregulation of nitric oxide synthases in brain which causing cell injury (Sharma and Alm, 2002). Gourine et al., (2004) reported that fever, which means a body temperature above the usual range of normal, can be caused by abnormalities in the brain itself or by toxic substances that affect the temperature regulating centers. Some causes of fever include bacterial diseases, brain tumors and environmental conditions that may terminate in heatstroke.

The mechanisms involved in cellular injuries caused by hyperthermia deserve more attention. Therefore, the purpose of this study was to elucidate the neural and testicular changes involved when adult male rats exposed to high temperature (40°C) for 12 hours.

2. Materials and Methods

Animals and treatment: Twenty adult male albino rats, aged 3 months, weighing 121±9 g. Rats were acclimatized for 3 days. The animals were housed, one per cage in wire bottomed stainless steel cages in a temperature controlled room (25±5°C) with relative humanity (50±10) and with 12 h. light/dark cycle. Rats were allowed to a standard commercial chow diet.

The rats were divided randomly into two groups each comprising 10 rats, a control group were left in a temperature controlled room (25±5°C) and the experimental group were subjected to high temperature (40±1°C) for 12 hours. During the time course of heat exposure rectal temperature was monitored using thermometer unit reached to 40°C.

The animals were sacrificed after overnight fasting under ether anesthesia. The relative humidity of experimental group was 90%. Blood samples were collected for estimation of serum LH and testosterone levels in control and heat exposed rats by (EIA) together with Calcium, Magnesium, Sodium and Potassium brain and testes samples were taken for histopathological examination.

Biochemical analysis of the brain:

1- Estimation of dopamine, noradrenaline and serotonin, the brain of experimental and control group were removed immediately and frozen in dry ice. The frozen tissues were weighed and homogenized in butanal acidified with 0.1 NHCl and centrifuged at 1500 rpm for 10 min. Catecholamines and serotonin were measured in the clear supernatant spectroflurometer as described by Jacabwitz and Richardson (1978).

2- Specimens of brain and testes were taken for histopathological examination, and after zenker fixation for 24 hours they were embedded in paraffin wax, sections were cut 5-8 µm thick and stained with hematoxylin and eosin.

Estimation of serum electrolytes:

Sodium and potassium were determined by using flame photometer according to Zilversmit (1965). Magnesium and calcium, were determined using spectrophotometer according to Grindler and Health (1971) and Grindler and King (1972).

Statistical analysis :

Data was expressed as means ± SD using Snedecor and Cochran (1990) statistical analysis.

3. Results

Hyperthermia induced by exposure to elevated temperature (40°C) for 12 hours, produced significant changes in the levels of dopamine, nor adrenaline and serotonin (Table 1), significant increases in the levels of neurotransmitters were recorded in brain homogenate. Features of diffuse hyperemic changes were prominent in brain tissues in examined samples (Fig. 1).

Serum electrolyte imbalance was observed in the hyperthermia group. There was marked hyperkalemia with decreased concentration of sodium and magnesium. Calcium level showed significant increases when compared to the control group (Table 2). Serum level of LH and testosterone were diminished in experimental rats compared with the control group (Table 3).

Semineferous tubules of experimental group showed disorganization and slight damage of germinal cells (Fig. 4).

Table (1) Effect of hyperthermia on the concentrations of Dopamine, Noradrenaline and sertonin (ng/g tissue) in the brain homogenate of rats

Parameters	Control group	Hyperthermic group
Dopamine	993±5.10	1068±5.4*
Noradrenaline	266±4.2	312±4.6*
Serotonin	232±2.9	259±2.4*

Values are expressed as means ± SE (n = 10)
* Significant different ($p < 0.05$)

Table (2) Effect of hyperthermia on the concentrations of electrolytes in rats

Parameters	Control group	Hyperthermic group
Sodium (mEq/L.)	138±7.4	106±5.2*
Potassium (mEq/L.)	4.18±0.30	5.9±0.42*
Magnesium (mg%)	4.56±0.51	3.09±0.22*
Calcium (mg%)	6.90±0.58	8.86±0.48*

Values are expressed as mean ± SE (n = 10)
* Significant different ($p < 0.05$)

Table (3) Effect of hyperthermia on testosterone and LH in rats.

Parameters	Control group	Hyperthermic group
Testosterone ng/ml	2.74±0.7	1.2±0.2*
LH mIU/ml	0.46	0.22*

Values are expressed as mean ± SE (n = 10)
* Significant different ($p < 0.05$)

Fig. (1) : Brain of heat exposed rat showing hyperemic capillaries and blood vessels allover the cerebral tissue (H & E X 40)

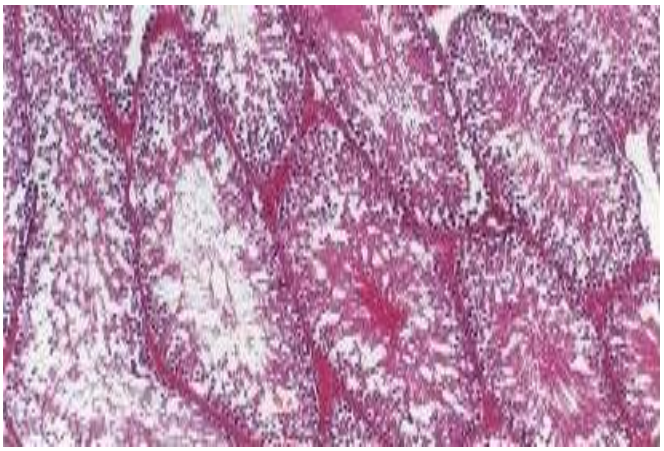

Fig. (2) : Transverse section in testis showing normal structure (H&E X 100)

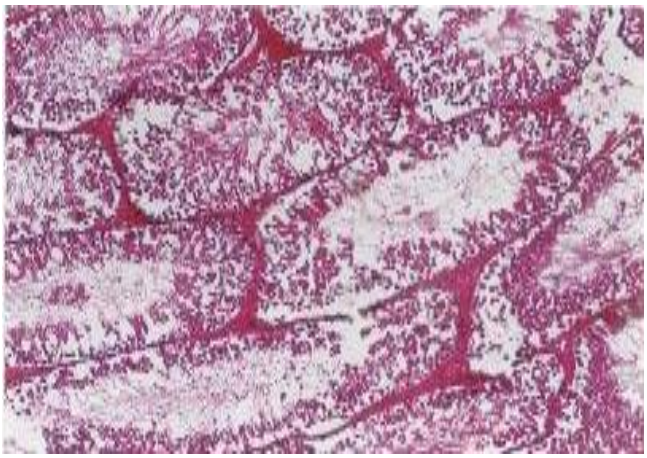

Fig. (3) : Transverse section in testis showing degenerative changes in all series of spermatogenic cells as well as some tubules are empty from spermatozoa. (H&E X 100)

4. Discussion

Core temperature : the temperature of the deep tissues of the body, remains constant within ±1°F (±0.6°C). an individual can be exposed to temperature as low as 55°F or as high as 130°F in dry air and still maintain an almost constant core temperature (Guyton and Hall, 2006). They also added that experiments have been performed in which minute areas in the brain of an animal have been either heated or cooled by use of a thermode. The thermode affects body temperature, control the preoptic and anterior hypothalamic nuclei of the hypothalamus, they contain large numbers of heat-sensitive neurons and cold – sensitive neurons. These neurons function as temperature sensors for controlling body temperature. Xi et al., (2001) stated that whole body hyperthermia is a distinctive pathological condition with significant impact on tissue metabolism and organ function. Frosini et al., (2000) mentioned that, hyperthermia raise all inhibitory and excitatory neurotransmitters.

Table (1) revealed a significantly increased Dopamine, Noradrenaline and serotonin was mainly related to increased body temperature. Increased levels of these neurotransmitters could be due to their important role in temperature regulation. Noradrenaline and adrenaline are chemical transmitters at most sympathetic post ganglionic endings, stored in the synaptic knobs of adrenergic neurons. Dopamine is the immediate precursor of noradrenaline. It is found in the dopaminergic neurons in certain parts of the brain, particularly in the hypothalamus. Serotonin is a synaptic mediator formed from hydroxylation and decarboxylation of tryptophan. Distribution of nor adrenaline in the brain, parallels that of serotonin, it appears to be involved in the regulation of body temperature (Nylo and Nielsen, 2001).

The increased brain content of catecholamines and serotonin was in agreement with the findings of Zhao et al., (2001) and Blatteis et al., (2004). They found that the level of dopamine and serotonin were higher in hypothalamus and frontal cortex in heat stressed animals. However, there are marked species variations in the temperature responses to these amines. Ganong (2000) mentioned that increased catecholamine secretion is an important endocrine response. Drug have antidopaminergic properties and those capable of stimulating serotonin release are responsible for hyperthermia (Nimmo et al., 1993).

Fig. (1) revealed brain of heat exposed rat showing hyperemic capillaries and blood vessels and focal hemorrhage in cerebral tissue. Boulant (2004) reported that the pathological findings in cases that dies of hyperpyrexia are local hemorrhages and parenchymatous degeneration of cells throughout the entire body, but especially in the brain.

Table (2) revealed serum electrolyte variables in the hyperthermic group. There was a marked hyperkalemia with decreased concentration of sodium and magnesium, while calcium level showed a significant increase compared to control group. The marked potassium increase with decreased sodium concentration might be due to potassium that leak out the cells and the entrance of the sodium leading to dysfunction of the central nervous system and failure of the vital centers. In case of increased calcium quantities in extracellular fluid, this might cause stoppage of the heart in systole and act as a mental depressant (Chatterjea and Shinde, 2006) (Korner and Leibel, 2003), while magnesium which is about one sixth as plentiful in cells as potassium, it is required as catalyst for many intracellular enzymes, particularly those related to carbohydrate metabolism, and low magnesium concentration causes increased of the nervous system and cardiac arrhythmias and affects badly the brain (Guyton and Hall, 2006).

Table (3) revealed a decreased LH and testosterone concentrations after hyperthermia as compared with control rats. Histopathological examination (Fig. 2, 3) showed disorganization of semineferous tubules, with slight damage of germinal cells. The histological damage may also attribute to the biochemical alteration. Thoreux – Manlay et al., (1995) stated that a high level of exposure can impair testosterone production that could later have secondary reproductive consequence such as the impairment of spermatogenesis, because testosterone is clearly essential for the maintenance of established spermatogenesis. The reduced LH concentration may lead to leidig cell receptor inadequate and drop of secretory capacity after hyperthermia (Kemprinas et al., 1990). De Kretser (2004) reported that testosterone is secreted by the interstitial cells of Leydig in the testis, but only when they are stimulated by LH from the anterior pituitary gland. Furthermore, the quantity of testosterone secreted increases approximately in direct proportion to the amount of LH available.

Heat stress is one of the most challenging environmental conditions. Thus, substantial attention has been paid to the role of nutritional additives to minimize the effects of heat stress (Bollengier-Lee et al., 1998). Wilczek (2005) added that the physiological condition of an organism under stress can be assessed using different biochemical and molecular markers,

like stress protein expression and antioxidant activities.

Sahin et al. (2003) found that, dietary supplementation of vitamin C and E as a combination increased total protein but decreased corticosterone, glucose and cholesterol concentrations. El-Shaieb et al., (2009) concluded that ascorbic acid supplementation alleviate detrimental effects of heat stress.

It may be concluded that : avoidance of hyperthermia is very important, the body temperature must be maintained in a safe normothermic range. It is recommended to assess the role of nutritional supplementation to alleviate the hazardous effects of heat stress.

Corresponding author
Faisal A. Bughdadai
Dean of University College Umm Al-Qura Univ.Makkah – Saudi Arabia
fabughdadi@uqu.edu.sa

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Reactive Power Planning and Voltage Control using Particle Swarm Optimization

A. Memaripour [1], Mostafa Abdollahi [2], Asadollah Salimi [3] , E. Behzadipour [4]

[1, 2, 3, 4.] Department of Electrical Engineering, Boroujen Branch, Islamic Azad University, Boroujen, Iran
memarpor@yahoo.com

Abstract: A new tool for planning reactive power compensation is presented. It is based on the capability chart of the power system, which describes the domain of allowable operation of the system in the plane of total active and reactive load demand. Power flow concepts are used to describe the ability of the power system to face the load; the optimization approach is adopted because load system is fundamentally nonlinear. Results for the IEEE-24 bus test system are presented.

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Keywords: Reactive Power Planning; Loss Minimization; Voltage Control; Particle Swarm Optimization

1. Introduction

The losses are naturally occurring in electrical system components such as transmission lines, power transformers, measurement systems, etc. due to their internal electrical resistance. It is not possible to achieve zero losses in a power system, but it is possible to keep them at minimum. The losses are becoming higher when the system is heavily loaded and transmission lines are transmitting high amount of power. The transmitted power for this case consists of active and reactive power. Necessity of reactive power supply together with active power is one of the disadvantages of the power generation, transmission and distribution with alternating current (AC). Reactive power can be leading or lagging. It is either generated or consumed in almost every component of the power system. In AC system Reactance can be either inductive or capacitive, which contribute to reactive power in the circuit. In general most of the loads are inductive and they should be supplied with lagging reactive power. We need to release the power flow in transmission lines for partially solving of problem of supply the reactive power locally where it is highly consumed in a system. In this way the loading of lines would decrease. It would decrease the losses also and with this action the problem of voltage drops could be solved also. By means of reactive power compensation transmission system losses can be reduced as shown in many papers in the literature [1-4]. It has also been widely known that the maximum power transfer of the transmission system can be increased by shunt reactive power compensation, typically by capacitors banks placed at the end of the transmission lines or a the load terminals [5]. Therefore, planning of reactive power supports would give benefits to the users of the transmission systems, in terms of loss reduction, among other technical benefits, such as improving steady-state and dynamic stability, improve system voltage profiles, etc. which are documented in [6]. The reactive power planning problem involves optimal allocation and sizing of reactive power sources at load centers to improve the system voltage profile and reduce losses. However, cost considerations generally limit the extent to which this can be applied.

This paper presents an optimal reactive power planning of power system using the Static Var Compensator (SVC). The proposed planning optimizes several objective functions at the same time within one general objective. The optimized objectives are minimization of average voltage deviation, minimization of total system loss and total system cost. particle swarm optimization (PSO) is used to solve the optimization problem. Simulation results emphasis on the validity of the proposed method.

2. Problem formulation

As referred before, in this paper three different parameters are considered as objective function. These parameters are: total investment cost, average voltage deviation and total system loss. Also the power system constrains such as generation reactive limits, voltage limits and etc, should be incorporated in planning. Therefore, the objective functions are as follows:

$$J_1 = \sum_{k\in\Omega_1} (c_{0k} + c_{1k} q_k) u_k \qquad (1)$$

Where, c_0 and c_1 are fixed and variable costs of locally reactive sources. q is amount of locally reactive source in bus K and u_k is a binary vector that indicates whether or not to install reactive power sources at bus k.

$$J_2 = P_{loss} \qquad (2)$$

$$J_3 = \sum_{i=1}^{n} (V_{ref} - V_i)^2 \quad (3)$$

Where, J_1 shows the investment cost due to locally reactive sources. J_2 shows the system losses and J_3 presents the voltage deviation. These objective functions should be converted to a unique unit. The coefficients ω convert the proposed functions to a unique unit. Eventually, reactive power planning formulation can be represented as follows:

$$\text{Min } \omega_1 J_1 + \omega_2 J_2 + \omega_3 J_3 \quad (4)$$

Subject to

$$P(V,\Theta,n) - P_G + P_D = 0 \quad (5)$$

$$Q(V,\Theta,n) - Q_G + Q_D - q = 0 \quad (6)$$

$$P_G^{min} \le P_G \le P_G^{max} \quad (7)$$

$$Q_G^{min} \le Q_G \le Q_G^{max} \quad (8)$$

$$V^{min} \le V \le V^{max} \quad (9)$$

$$(N+N0)S^{from} \le (N+N0)S^{max} \quad (10)$$

$$(N+N0)S^{to} \le (N+N0)S^{max} \quad (11)$$

$$q^{min} \le q \le q^{max} \quad (12)$$

Equations (5) and (6) introduce the conventional equations of AC power flow and (7) and (8) show the limits for real and reactive power for generators. Equation (9) presents the limits for voltage magnitude. Capacity limits of the line flows are presented by (10) and (11). Equation (12) presents the limit for locally reactive sources.

The proposed formulation in used to find the best place of SVCs. In this paper particle swarm optimization (PSO) is used to solve the optimization problem. In the next section a brief introduction about PSO is presented.

3. Particle swarm optimization

PSO was formulated by Edward and Kennedy in 1995. The thought process behind the algorithm was inspired by the social behavior of animals, such as bird flocking or fish schooling. PSO is similar to the continuous GA in that it begins with a random population matrix. Unlike the GA, PSO has no evolution operators such as crossover and mutation. The rows in the matrix are called particles (same as the GA chromosome). They contain the variable values and are not binary encoded. Each particle moves about the cost surface with a velocity. The particles update their velocities and positions based on the local and global best solutions as shown in (13) and (14) [8]:

$$V_{m,n}^{new} = w \times V_{m,n}^{old} + \Gamma_1 \times r_1 \times (P_{m,n}^{local\ best} - P_{m,n}^{old}) + \Gamma_2 \times r_2 \times (P_{m,n}^{global\ best} - P_{m,n}^{old}) \quad (13)$$

$$P_{m,n}^{new} = P_{m,n}^{old} + \Gamma\, V_{m,n}^{new} \quad (14)$$

Where:

$V_{m,n}$ = particle velocity

$P_{m,n}$ = particle variables

W= inertia weight

r_1, r_2 = independent uniform random numbers

$\Gamma_1 = \Gamma_2$ = learning factors

$P_{m,n}^{local\ best}$ = best local solution

$P_{m,n}^{global\ best}$ = best global solution

The PSO algorithm updates the velocity vector for each particle then adds that velocity to the particle position or values. Velocity updates are influenced by both the best global solution associated with the lowest cost ever found by a particle and the best local solution associated with the lowest cost in the present population. If the best local solution has a cost less than the cost of the current global solution, then the best local solution replaces the best global solution. The particle velocity is reminiscent of local minimizes that use derivative information, because velocity is the derivative of position. The advantages of PSO are that it is easy to implement and there are few parameters to adjust. The PSO is able to tackle tough cost functions with many local minima [8].

4. Illustrative system

Figure 1 shows a typical electric power system. IEEE-24 bus test system is considered as illustrative system. The system data are presented appendix [7]. The fixed and variable costs of locally reactive sources are as c_0 = 100$ and c1 = 0.3$/kvar, respectively. To implement PSO, initial population size, cross over rate and mutation rate are chosen as 24, 0.5 and 0.1 respectively. Also 110% and 90% of the nominal value are used for the maximum and minimum voltage magnitude limits.

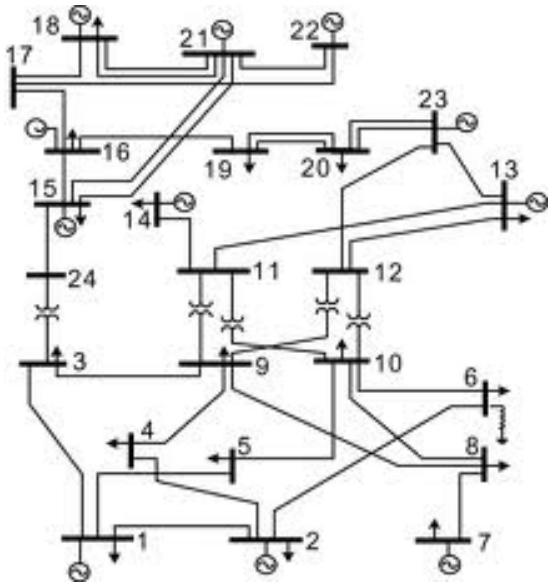

Figure 1. IEEE 24 bus test system

5. Results and discussions

In this section the SVC placement based on the particle swarm optimization is presented. The SVC places are accuracy calculated using PSO and the results are listed in Table 1. The locally reactive sources are places near to load buses and it is due to compensation of reactive demands. In this way, the current in transmission lines are reduced and the total loss is reduced. Also, because of locally supply of reactive demands, the congestion of lines is reduced.

Table 1. Optimal SVC places

Bus	Locally Reactive Source (MVAR)
3	300.0
4	43.60
9	97.11
12	200.8
24	149.2

6. Conclusion

The particle swarm optimization (PSO) approach has been developed for solving the Reactive Power Planning (RPP) problem in large-scale power systems. The application studies on the IEEE 24 bus system show that PSO gives suitable results and always leads to the global optimum points of the multi-objective RPP problem. By the PSO approach, more savings on the energy and installment costs are achieved and the violations of the voltage and reactive power limits are eliminated.

Corresponding Author:
Ahmad Memaripour
Department of Electrical Engineering,
Islamic Azad University,
Boroujen Branch, Boroujen, Iran.
E-mail: memarpor@yahoo.com

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Titanium Three Dimensional Miniplate versus Conventional Titanium Miniplate in Fixation of Anterior Mandibular Fractures

Mahmoud E. Khalifa[1], Hesham E El-Hawary[2] and Mohamed M. Hussein[3]

[1]Lecturer of Oral and Maxillofacial Surgery, Faculty of Dentistry, Tanta University, Egypt, Umm Al Qura University, KSA
[2]Lecturer of Oral and Maxillofacial Surgery, Faculty of Oral and Dental Medicine , Cairo University, Egypt, Umm Al Qura University, KSA
[3] Professor of Oral and Maxillofacial Surgery, Faculty of Dentistry, Tanta University, Egypt

Abstract: The optimal management of symphyseal and parasymphyseal fractures continues to evolve. Fractures in this area of the mandible predispose the patients to malocclusion and widening of the face if not properly treated. The current understanding of the biomechanics and fracture healing of the mandible has influenced the modern approach to the open reduction and internal fixation of these fractures. A total of 20 patients were managed by open reduction and internal fixation utilizing the conventional titanium mini-plate and 3dimensional mini-plate for fractures of anterior mandible. The patients were randomly divided into two equal groups according to the type of hardware used for fracture fixation. Group I: (10 patients) were treated with two 2.0 mm titanium mini-plates, Group II: (10 patients) were treated with 3D rectangular mini-plates. Intraoperatively duration of surgery was measured from the time of incision till the closure of wound. Subsequent postoperative clinical follow up for malocclusion, neurosensory deficit, wound breakdown, infection and presence of mal-union/non union was performed. Postoperative radiographs were taken to assess the gap between fracture segments. All patients were followed up clinically and radiographically for 6 months postoperatively. The mean duration of surgery (hours) was 1.88 ± 0.38 for group I and 1.61 ± 0.27 for group II. The difference was found to be statistically significant (p value 0.001). There was no clinical evidence of neurosensory deficits due to surgery in all cases. No problems of wound healing, swelling, discoloration, or discharge were seen during follow-up expect one patient in group I showed slight wound dehiscence with exposure of upper plate at the second post-operative week. Post-operative radiographic examination revealed that the fracture line could not be detected on the radiographs after 6 months in six patients (60%) patients in group I and eight patients (80%) patients in group II. The 3D mini-plate system is a better and easier method for fixation of mandibular fractures, compared to the conventional mini-plate. But there is limitation to use in cases of oblique fractures and those involving the mental nerve as well as there is excessive implant material because of the extra vertical bars.

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Key words: symphyseal mandibular fracture, parasymphyseal mandibular fracture, titanium miniplate, titanium 3D miniplate.

1. Introduction

Fractures through the mandible at the level of the Symphysis and or parasymphysis are relatively common and account for approximately 20% of mandibular fractures [1]. These fractures are often associated with a second fracture of the mandible, especially in the subcondylar region [2]. Fractures of the symphyseal region are often associated with the clinical findings of a widened intragonial distance with resultant malocclusion [1].

Fractures of the anterior mandible lack two of the stabilizing factors provided to fractures of the posterior tooth-bearing mandible: the splinting effects of the masseter and internal pterygoid muscles, which form a natural sling, and the interlocking cusps and fossae of bicuspid and molar teeth [3].

Although the techniques of fracture management have changed, the goals have not changed significantly. Accurate reduction of the fractures, maintenance of premorbid occlusion, and early return to function are the keys to successful management of these fractures. The technique of fracture repair and hardware choice will depend on the fracture pattern, fracture severity, and patient factors, such as residual dentition, coexistent lacerations, and associated injuries [4].

The treatment of symphyseal and parasymphyseal mandibular fractures has evolved significantly over the past few years. Historically, mandibular fractures were treated with closed reduction and a course of prolonged maxillomandibular fixation. The next phase of mandibular fracture management involved open reduction and internal fixation using wire osteosynthesis, then titanium hardware including lag screws and plates [5, 6].

Aim of the Study

This study was aimed to compare titanium 3D miniplates versus conventional miniplate in fixation of anterior mandibular fractures.

2. Patients and Methods

Twenty patients with anterior mandibular fractures were included in this study. The management started with immediate resuscitation following the principles of advanced trauma life support (ATLS). Plain anteroposterior (AP), lateral cephalometry radiographs and Orthopantomogram (OPG) were made for all the cases. An axial, coronal and 3-D CT scan were obtained for patients with associated mandibular condyle or subcondylar fractures.

An accurate assessment of the fractures was performed including the site and type of fracture, amount of displacement, amount of pain or discomfort, paraesthesia in the distribution of inferior alveolar nerve, marginal mandibular nerve paresis, status of dental occlusion, any associated temporomandibular joint (TMJ) dislocation, or any other functional deficits. All the selected patients were entailed about the surgical procedure. They were informed about the surgical procedure including prognosis, potential hazards and complications. They gave their approval to participate in a written informed consent. The study protocol was reviewed and approved by the central regional ethics committee.

Surgical Technique

All operations were performed under general anesthesia by nasotracheal intubation. The surgeries were performed by the same surgeon with same operating team. Erich-type arch bars were first applied to the upper and lower dentition. The fracture was approached through a vestibular incision between the mental foramina. (Fig.1). The segments were reduced and fixed temporarily using a special reduction forceps[9]. Once the fracture has been reduced to the anatomic position, intraoperative maxillomandibular fixation was obtained. The patients were randomly divided into two equal groups according to the type of hard ware used for fracture fixation. Group I: (10 patients) were treated with two 2.0 mm titanium mini-plates system and Group II: (10 patients) were treated with 3D rectangular mini-plates system.

The operating time taken for plate adaptation and fixation to last screw placed was recorded and also the total operating time of surgery was measured from the time incision was placed till the closure of wound. Principles of 3D plates fixation was followed; the horizontal bar perpendicular to the fracture line while the vertical bars parallel to it. (Fig.2 A&B)

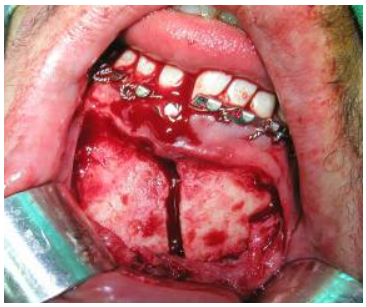

Figure 1: The surgical approach to the anterior mandible

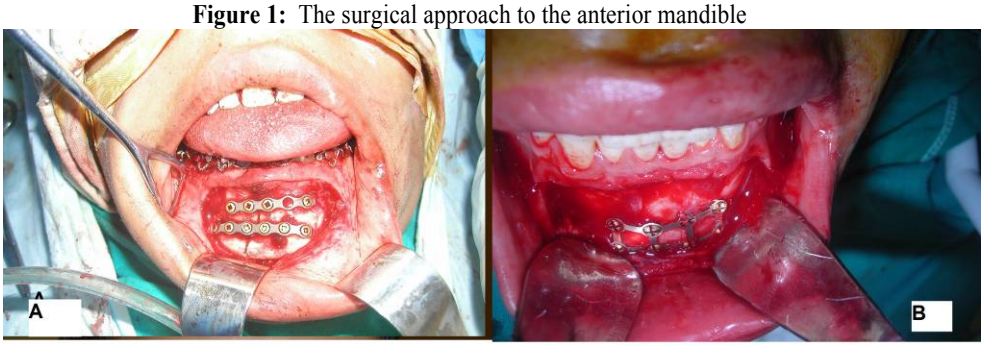

Figure 2: A, patient in GI with 2miniplates fixation. B, patient in G II with 3D miniplates fixation.

Once the hardware has been placed, the occlusion was checked and attention was turned to closure. After copious irrigation, the intraoral incision was closed with care taken to reattach the mentalis muscle. A watertight closure of the mucosa was achieved with absorbable sutures.

Postoperatively; the patients were assessed clinically for wound breakdown, neurosensory deficit, mobility of fractured segments occlusion discrepancy, and mal-union/non-union. Postoperative radiographs were taken to assess the gap between fracture segments. Patients were followed for 6 months to insure accurate reduction and proper occlusion during the fracture healing.

3. Results

Twenty patients (14 Males and 6 Females) with anterior mandibular fractures were included in this study. The patient age ranged from 15-50 years with mean of 32.5

y. Road-Traffic accidents were the cause of fractures in 16 patients out of total 20 patients, 2 from fall and 2 resulted from interpersonal violence.

Parasymphysis was the most frequent site of fracture, 17 out of 20 patients had parasymphyseal fractures, out of which 7 patients had isolated parasymphyseal fracture and rest 10 patients had other associated fracture (4 angles and 6 condyles) of the mandible. Symphysis fractures were present in 3 patients; all of them had associated condylar fracture of the mandible

In this study, the mean duration of plate adaptation and fixation (minutes) was 19.4±2.75 for group I and10.8±1.93for group II. The mean duration of surgery (hours) was 1.88 ± 0.38 for group I and 1.61 ± 0.27 for group II. The difference was found to be statistically significant (p value 0.001).

All patients were followed up clinically and radiographically for 6 months postopertively.

No problems of wound healing, swelling, discoloration, or discharge were seen during follow-up expect in one patient in group I showed slight wound dehiscence with exposure of the upper plate started at the second post-operative week. This patient was treated by continuous irrigation with warm normal saline, antiseptic mouthwash and keeping good oral hygiene until complete wound healing was achieved in ten days.

There was no clinical evidence of neurosensory deficits due to surgery in all cases. Paresthesia of the lower lip, before surgery, encountered in three patients one in group I and two in group II, these patients were followed up until regained normal neurosensory function spontaneously after four weeks in two patients and after six weeks in the other patient.

Three patients in group I and two in group II had mobility of the fracture fragments at 2 weeks follow up that was not detected at the end of the first month.

Postoperatively, two patients in each groups had slight occlusal discrepancy which was successfully corrected by simple selective coronoplasty in two patient and guiding elastics with selective girding in two cases.

In immediate post-operative radiographs taken within two days, reduction of the anterior fractures was assessed as exact in all cases in both groups Radiolucencies representing the fracture lines were still noted in all cases.

Radiographic examination at the first month post-operatively revealed no changes in the position of the fractured segments and the fracture lines are hardly detected (Fig.3). At the end of the follow up period none of the patients showed any signs of non-union in both groups. The fracture line could not be detected on the radiographs after 6 months in six patients (60%) patients in group I and eight patients (80%) patients in group II . No signs of adverse effects were seen around the screws. No external callus was detected in the both groups.

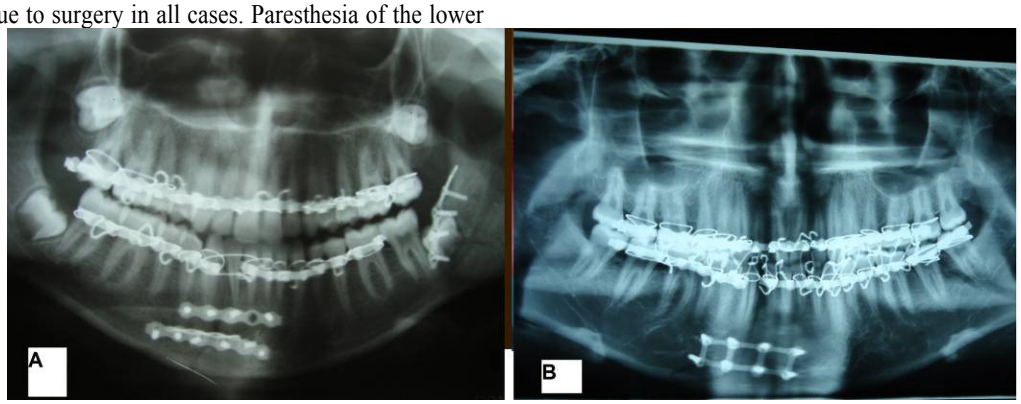

Figure 3: Six months postoperative radiographs A, patients in B, patient in GII

4. Discussion

Methods of osteosynthesis may be evaluated not only by the reduction achieved and the stability of fixation, but also by their technical application, economic aspects involved, and increasingly by the extent of trauma resulting from the used surgical approach. Methods should be selected only when they ensure early full rehabilitation of the patient in combination with minimally invasive surgery and economic use of materials and time. The less technical input required for a particular method, the more it will be accepted. Also, adequate knowledge of biomechanics and static and dynamic forces acting in the region being restored are important factors for successful management. Many factors are usually taken in consideration when selecting the methods of fixation of mandibular fractures. The nature of injury, presence of other associated fractures, medical and economic status of the patient and surgeons experience are some of these factors. Also the site of injury dictates to great extent the selected method of fixation [10].

Treatment of mandibular fractures using open reduction and internal fixation (ORIF) through an intraoral approach in our study provided the advantage of simultaneous visualization of the fracture line and occlusion relation. It also eliminated extraoral incision and the risk of scar formation [11-14].

Rigid internal fixation with metal plates and screws is used extensively to secure bone fragments in fracture

surgery. Development of more biocompatible osteosynthesis materials such as titanium has led some to recommend leaving these materials *in situ* forever. [15]

In a recently published survey of 104 North American and European AO/ASIF surgeons, only 6% stated that they use 3D plates. Moreover, only a few follow-up series are presented in the literature, with few studies [16-18] emphasizing the hardware-related advantages over conventional miniplates and reconstruction plates. These advantages include easy application, simplified adaptation to the bone without distortion or displacement of the fracture, simultaneous stabilization at both superior and inferior borders, and hence less operative time.

In this study the time required for the adaptation and fixation of the plate at the fracture site (Parasymphysis & symphysis region) was recorded for both the groups. The total time was also recorded. The operating time for adaptation and fixation for 3D plating was short when comparing with time for conventional mini-plates this lead to reduction the total operating time in group II comparing with group I this result was in agreement with Guimond et al., 2005[19] and Babu et al., 2007. [20]

In group I, conventional mini-plates required longer time because these are linear plates and two plates are required for fixation at parasymphysis or symphysis region. On the other hand in group II, 3D plate is geometric configured plate which consists of two horizontal bars interconnected with two vertical bars. So single 3D plate stabilized the fracture both at superior and inferior border at a time, hence time is saved in plate fixation. This is in agreement with Jain et al., [21] as he stated that a geometric plate is much broader and has to be bent in 3 dimensions, whereas a linear plate has to be bent only in 2 dimensions and so it is trying to adapt a "plane" rather than a "line" to a curved surface.

In cases of oblique fracture or the fracture running through the mental foramina required more time in placement of 3D plate. This might be due to difficulty in achieving principles of 3D plate fixation (horizontal bar perpendicular and vertical bar parallel to fracture line) which result in limitation in using 3D plate in such cases. In such cases the plate was placed either inferior or superior to the foramina, and care was taking while placing the plate superior to the foramina is that the screws are placed between the roots of the teeth. Another limitation of 3D plates as we saw in this study was excessive implant material resulting from extra vertical bars incorporated for countering the torque forces which is in agreement with Babu et al.[20] and Wittenberg, [22] in a prospective study, reported the stabilization of 20 fractures of the mandibular angle; 12 were associated with additional fracture of the body using 3D plates.

Wound dehiscence occurred in one patient in group I. The improvement of plate stability might be a way to minimize wound healing complications. Guimond et al., [19] also experienced the low incidence of wound dehiscence and plate exposure with 3D plate in comparison to conventional miniplate that might be as a result of reduced operating time in 3D miniplate fixation.

Paresthesia of the lower lip, before surgery, encountered in three patients one in group I and two in group II, nerve was entrapped in the fracture fragment which was retrieved during the operation .These patients were followed up until regained normal neurosensory function spontaneously after four weeks in two patients and after six weeks in the one patient.

Preoperatively all patients of Group I and Group II had mobility of fracture fragment. In our study, it was observed that two cases (20%) out of 10 cases of group I had mobility after conventional mini-plate osteosynthesis at 2 weeks postoperative this mobility decreased over a period of one month postoperatively,. In Group II, one of ten patients had mobility at 2 weeks postoperative. By the end of the 3rd month postoperatively none of the patients in both groups showed any mobility in fractured segments.

Postoperatively two patients in each groups had slight occlusal discrepancy which were successfully corrected by simple selective coronoplasty in two patients and guiding elastics with selective girding in two cases. All patients with post-operative occlusal discrepancy had some other associated fracture of the mandible. The occlusal discrepancy was seen as a result of the imbalance between the muscular activities of the muscles of mastication after the trauma and due to the edema at the TMJ region post operative. By using guiding elastics this problem was solved. This incidence of occlusal discrepancy was compared between the two groups and the results showed no statistically significant difference. 3D plates and mini-plates (semi rigid type of fixation) reported less occlusal disturbances. Whatever post operative occlusal discrepancy encountered in the patients of both groups, were the fracture associated with other part of the mandible such as condyle and the angle. As these plates are self adaptable and non-compressive, they do not fix the fragments rigidly, hence self correction due to action of oro- facial musculature can take place.

Conclusion

Methods of fixation for anterior mandibular fractures should be selected only when they ensure early full rehabilitation of the patient in combination with minimally invasive surgery and economic use of materials and time.

To conclude, 3D miniplate system is a better and easier method for fixation of mandibular fractures, compared with the conventional miniplate. The 3D miniplate system provides good stability in most cases and operative time is shorter because of simultaneous stabilization at both superior and inferior borders. But there is limitation to use in cases of oblique fractures and those involving the mental nerve as well as there is excessive implant material because of the extra vertical bars incorporated for countering the torque forces.

Correspondence author
Mahmoud E. Khalifa
[1]Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Tanta University, Egypt. Umm Al Qura University, KSA

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Successful resuscitation of a patient With electrical storm over 7 days: a case report

Yue-Ling Guo[1], Hai-ying Zhang[2], Wei Zhang[2], Yun-Feng Han[2], Shu-Lin Zhang[2]

[1.] Department of Medical Engineering, The Military General Hospital of Beijing PLA, Beijing 100070, China;
[2.] Department of Geriatrics, The Military General Hospital of Beijing PLA, Beijing 100070, China;
Correspondence author: Yun-Feng Han (hanyf001@126.com)
(The first two authors contributed equally to this work)

Abstract: A 75 year old woman with atrial fibrillation-related tachycardia-induced cardiomyopathy suffered ventricular fibrillation induced by dobutamine and was successfully defibrillated into atrial fibrillation in hospital. During the following 7 days, she suffered a cardiac electrical storm with 55 episodes of ventricular tachycardia rapidly degenerating to ventricular fibrillation and was converted with a total of 51 defibrillations and 4 chest compressions. Sinus rhythm was restored by electric cardioversion in the second episode of ventricular fibrillation. There was no response to the use of any recommended anti arrhythmic drugs. However, the use of chlorpromazine and promethazine surprisingly stabilized her heart rhythm. During a two-year follow-up period, the patient has remained free of ventricular fibrillation episodes and maintained sinus rhythm.
[Guo YL, Zhang HY, Han YF, Zhang SL. **Successful resuscitation of a patient With electrical storm over 7 days: a case report**. Life Sci J 2012;9(2):1011-1014] (ISSN:1097-8135). http://www.lifesciencesite.com. 150

Key Words: electrical storm; tachycardia-induced cardiomyopathy; dobutamine

Introduction

The term tachycardia-induced cardiomyopathy (TIC) or tachycardiomyopathy refers to impairment in left ventricular function secondary to chronic tachycardia, which is partially or completely reversible once the tachyarrhythmia is controlled. The incidence of TIC is unknown, but in selected studies of patients with atrial fibrillation, approximately 25% to 50% of those with left ventricular dysfunction had some degree of TIC[1,2]. Data on the prognostic significance of electrical storm strongly suggest that these patients have a poor outcome. Electrical storm might be an independent risk factor for cardiac death. In the AVID trial[3], patients with electrical storm had an increased risk of nonsudden cardiac death (risk ratio, 2.4). In the MADIT –II substudy, patients with electrical storm had a 7.4-fold higher risk of death than patients without electricalstorm[4].

The term electrical storm (ES) was introduced in the 1990s to describe a state of electrical instability of the heart characterized by a series of malignant ventricular arrhythmias in a short period of time[5]. This condition has been described in patients with post-infarction coronary artery disease, as well as in patients with various forms of cardiomyopathy, valvular disease, surgically corrected congenital heart disease, and genetically determined cardiac diseases without any apparent underlying structural disease, such as Brugada syndrome[6]. They need immediate resuscitative treatment, identification of the underlying cause and strategies for long term prevention of recurrence. We present a case of electrical storm when a woman with TIC was defibrillated 51 times over 7days but went on to make a good recovery.

CASE REPORT

A 75-year-old woman was admitted to our hospital due to worsening palpitation and dyspnoea. She had had hypertension for twenty years and atrial fibrillation for six month. The patient had no history of ischaemic heart disease, type 2 diabetes, hyperlipidaemia, chest pain, syncopal episode, or alcohol and drug abuse. She did not smoke and did not report any family history of syncope and sudden death. Blood pressure (BP) was 136/82 mmHg. Physical examination was unremarkable aside from a fast irregular pulse. Thyroid function tests were normal. Laboratory data were within normal limits. An electrocardiogram revealed atrial fibrillation with a ventricular rate of 107 beats per minute. The QT interval was 0.358s and no significant ST change was observed. The chest X-ray showed cardiomegaly (cardiothoracic ratio, 60%). The echocardiography revealed a dilated left ventricle with a diastolic diameter of 6.0cm and a reduced ejection fraction of 45%. A diagnosis of dilated cardiomyopathy was made. The patient was commenced on digoxin, valsartan, furosemide, spironolactone and metoprolol.

On the 5th hospital day, when dobutamine was infused at a rate of 1ug/kg/min for 3 hours, ECG monitoring revealed ventricular tachycardia (VT) rapidly degenerating to ventricular fibrillation (VF). Immediate chest compressions were started. Bolus infusion of 1 mg of adrenaline was given. Atrial fibrillation was restored by 3 electric cardioversion (200, 300, 360 Joule). Serum level of potassium was

in the normal range (4.1mmol/L). Initial laboratory findings revealed no significant abnormalities including electrolyte disturbance, troponin and CPK-MB enzymes. After 75mg of amiodarone was administered intravenously over 10 minutes, continuous intravenous administration (60mg per hour) was given.

Ten hours later after her first VF in the Ward, she suffered a new event of VT rapidly degenerating to VF and successfully defibrillated (200J) into sinus rhythm(70 times per minute). After intravenous bolus of 40mg of lidocaine and 75mg of amiodarone, continuous intravenous lidocaine (120mg per hour) and amiodarone (60mg per hour) was given. She suffered 2 episodes of VF and successfully defibrillated (200J) into sinus rhythm in next 4 hours. A diagnosis of ES was made. Immediately, metoprolol was administered intravenously 3 times (5mg/times) in one hour. Oral metoprolol was added to 50mg per day. The serum potassium level was kept at 4.0 mmol/L or higher.

The electrical storm persisted, despite the continuous infusion of lidocaine, amiodarone and repeated doses of metoprolol, magnesium and potassium. Her hemodynamic condition was stable and serum electrolytes were normal. She suffered 15-20 episodes of VT and 5--10 episodes of VF each day in the next 5 days. Each event triggered immediate chest compressions for 20-30s while charging the defibrillator. Sinus rhythm was restored by electric cardioversion(150-200J, total 51 times) or chest compressions(4 times) in each shock. The QT/QTc interval was 0.428s/0.426s and no significant ST change was observed, but she never suffered the event of *torsade de pointes* (TdP). Five days later after her first VF in the Ward, she was sedated by intramuscular chlorpromazine and promethazine. Surprisingly, the occurrence of VF was markedly decreased to 1--2 episodes of VF each day. After two days the occurrence of VT and VF terminated.

Ten days later after her first VF, sedation was discontinued and she regained consciousness with intact cerebral function. Coronary angiography showed normal coronary arteries. There was only a slight increase in cardiac enzymes (Troponin I: 0.2 ng/mL) during ES. The echocardiography revealed a dilated left ventricle with a diastolic diameter of 5.5cm and a reduced ejection fraction of 50%. Chest x-ray showed cardiomegaly (cardiothoracic ratio, 60%) and Cardiac MR showed dilated cardiomyopathy. No premature ventricular contractions were detected by 24-hour Holter monitoring. An implantable cardioverter defibrillator was not implanted due to economic reason.

Interestingly, upon discharge from the hospital the patient had no ventricular arrhythmias. At follow up six months later, a repeat echocardiogram confirmed that the left ventricular dimensions had returned to normal(diastolic diameter of 5.0cm) and the estimated ejection fraction was 70%. A diagnosis of atrial fibrillation-related TIC was made. During a two-year follow-up period, the patient has remained free of ventricular fibrillation episodes and maintained sinus rhythm. She has continued long-term treatment with metoprolol and amiodarone. At present, she is in excellent condition.

Discussion

ES is defined as the recurrence of hemodynamically unstable VT and/or VF, twice or more in 24 hours, requiring electrical cardioversion or defibrillation[7,8]. With the arrival of the ICD (implantable cardioverter defibrillator) this definition was broadened, and ES is also defined as the occurrence of three or more distinct episodes of ventricular tachycardia (VT) or ventricular fibrillation (VF) in 24 hours, requiring the intervention of the defibrillator [9]. It should be noted that this latter definition does not include the presence of hemodynamic instability. This condition has been described in patients with post-infarction coronary artery disease, as well as in patients with various forms of cardiomyopathy, valvular disease, surgically corrected congenital heart disease, and genetically determined cardiac diseases without any apparent underlying structural disease, such as Brugada syndrome[6].

The patient reported to have atrial fibrillation six month prior to admission. The echocardiography revealed a dilated left ventricle with a diastolic diameter of 6.0cm and a reduced ejection fraction of 45% after admission. Six month after sinus rhythm was restored by electric cardioversion, a repeat echocardiogram confirmed that the left ventricular dimensions had returned to normal and the estimated ejection fraction was 70%. So, a diagnosis of atrial fibrillation-related TIC was made. Clinically, the most common cause of TIC is believed to be atrial fibrillation (AF), which is increasing in incidence with the aging of society. AF causes TIC via 2 distinct mechanisms: inadequate diastolic filling and tachycardia-induced systolic dysfunction. When AF develops, the active atrial contraction disappears in end-diastole, leading to a reduction in the cardiac output by 15–20%[10]. This insufficient diastolic filling is augmented by the shortening of diastolic filling time. Furthermore, sustained tachycardia results in impaired systolic function through a mechanism represented by TIC, which leads to a greater reduction in the cardiac output[10,11]. A recent report has suggested a risk of sudden death in this

particular group[12]. Our report is the first to document one patient with fibrillation-related TIC who suffered ES and has a good recovery.

It is noteworthy that, in spite of a detailed analysis of the electrocardiogram, haematological and biochemical examinations, and the patients'clinical symptoms, in only 36% was any triggering mechanism found that could provoke electrical storm. Those factors were acute ischemia, worsening heart failure, hypokalemia, hypomagnesemia, arrhythmogenic drug therapy, hyperthyroidism, and infection or fever. In this case, we found, CHF secondary to atrial fibrillation-related TIC, who developed ES during low-dose dobutamine infuse. Dobutamine is an inotropic pharmaceutical that improves the hemodynamic and clinical status of patients suffering from congestive heart failure (CHF) refractory to standard treatment[13]. The proarrhythmic effects of dobutamine are supported by several observations. It increases the dispersion of action potential duration in adjacent areas of ischemic and non-ischemic myocardium in experimental animals[14], and increases the incidence of VT in patients[15].

Electrical storm activates the sympathetic nervous system. β-Blockers play a key role in the management of ES. In a canine study[16], β-blockers increased the fibrillation threshold (that is, made the animals less susceptible
to fibrillation) 6-fold under ischemic and nonischemic conditions. Amiodarone is widely used in the treatment of electrical storm[6]. In acute amiodarone therapy, rapid intravenous administration blocks fast sodium channels in a use-dependent fashion (producing more channel blockade at faster heart rates), inhibits norepinephrine release, and blocks L-type calcium channels but does not prolong ventricular refractoriness. Conversely, in oral amiodarone therapy, prolonged ventricular refractory periods are seen over periods ranging from days to weeks[17]. Amiodarone has few negative inotropic effects and is safe in patients who have depressed systolic function. Amiodarone or β-blockers are generally accepted as the best available drugs for prevention of arrhythmic storm[18]. Class I antiarrhythmic drugs are used widely, with variable success rates, and can play a role in polymorphic ventricular arrhythmias[19]. But in our report, there was no response to the use of any above drugs in the patient.

The physical and emotional stress that patients experience in association with electrical storm and multiple electrical cardioversions often perpetuates arrhythmias. All patients who have electrical storm should be sedated. Short-acting anesthetics such as propofol, benzodiazepines, and some agents of general anesthesia have been associated with the conversion and suppression of VT[20]. Left stellate ganglion blockade and thoracic epidural anesthesia have also reportedly suppressed electrical storms that were refractory to multiple antiarrhythmic agents and β-blockade[21]. These therapeutic approaches directly target nerve fibers that innervate the myocardium, and a reduced adrenergic tone is most likely responsible for the reported efficacy[22]. In this article, we found, there was no response to the use of any recommended anti arrhythmic drugs. However, the use of chlorpromazine and promethazine surprisingly stabilized her heart rhythm.

The good neurological outcome in the patient was probably due to a number of positive factors. This case illustrates that immediate and high quality chest compressions is necessary. Each episode of VF initiated immediate manual chest compressions while charging the defibrillator, thus hands-off time was reduced to a minimum.

This case illustrates the importance of defibrillation, multiple anti-arrhythmic agents and sedation in management of electrical storm. Despite repeated defibrillations, she went on to make a good recovery. Every possible attempt should be made to reduce as far as possible the number of patients who undergo it.

Correspondence author:
Yun-Feng Han
Department of Medical Engineering,
The Military General Hospital of Beijing PLA,
Beijing 100070, China;
hanyf001@126.com.

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5/20/2012

Monitoring DNA Hybridization with a Simply Manufactured GMR Biosensor

Ting-Jou Ding[1], Jiann-Hwa Lue[1], Ya Lun Tsai[1], Tsung-Hsun Yang[1*], Jenq-Yang Chang[1,2], Wen-Yih Chen[3]

[1] Department of Optics and Photonics, National Central University, Chungli, Taiwan, ROC
[2] Optical Science Center, National Central University, Chungli, Taiwan, ROC
[3] Department of Chemical and Materials Engineering, National Central University, Chungli, Taiwan, ROC
thyang@dop.ncu.edu.tw

Abstract: In this letter, a real-time approach to identify the various DNA molecules hybridized of the integrated micro-fluid with the simplest manufactured guided-mode resonance (GMR) sensor is presented. By monitoring the resonant peak wavelength shift of GMR biosensor, the DNA hybridization experiment can be recognized and clarified. The biosensor exhibited sensitivities about 2.14nm in peak wavelength shift for the detection of the hybridizing of both the capture DNA and the probe DNA. Furthermore, the stability and reliability of nucleic acid hybridization on the GMR sensor are examined by combining the sensor with the fluidic elements. The results reveal that the dynamics of DNA hybridization on the GMR sensor can obviously be monitored in real time.
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Keywords: guided-mode resonance, biosensor, DNA hybridization, simply manufacture, real time monitoring, cost down, reliability, stability

1. Introduction

Label-free DNA biosensors and DNA microarrays tests based on optical technical has become the hottest topics in the field of biological molecule detection in the few years. Detecting the optical signals variety of the complementary (target) DNA strand and the immobilized single-stranded (ss) DNA, can analyze the hybridization effects of specific DNA sequences [1]. The applications of DNA-based diagnostic can be used in various fields of life sciences, such as gene analysis, fast detection of biological warfare agents, monitoring of environmental pollutants, and forensic applications [2].

Conventional biosensors require labeling with external reagents such as enzymes, fluorescent dyes, or both, to enhance the major sensitivity. But, the labeling process would increase additional time and cost demands. Furthermore, the labels on cells will interference the molecular interaction and the cell biology of the target receptor, which leads to false negatives (or positive) [3]. Label-free biosensor can not only avoid the suppression of external reagents (such like enzymes, fluorescent dyes) and time-consuming cause in labeling procedure, but also ensure the life-cell detected completely. Therefore, the free-tag technical has become more and more helpful in the practical applications of medical diagnosis and treatment.

Beside, the miniaturized DNA biosensor and biochip approach has become increasingly important in basic researches into the genetics of disease and also in the more practical applications of medical diagnosis and treatment. Integrated optical devices onto the biosensors such as prism, grating, waveguide, interferometric and ellipsometry possess high sensitivity and small-sized characteristics, which allow them easily, continue monitor and miniaturization [4-10]. Among these optical biosensors, Guided-mode resonant (GMR) biosensor is the most potential one.

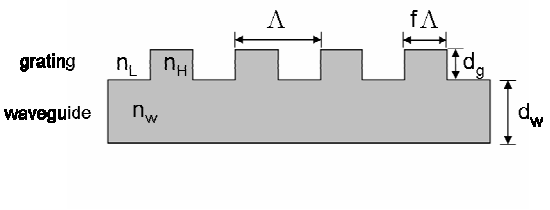
Physically, when one plane wave illuminates a period grating, the diffractive waves may be corresponding to a guided mode of the waveguide under the condition of resonance. Under this condition, the diffractive device would support a sharply reflective and transmittance spectra. With the changing of the refractive index or thickness of a resonant waveguide grating, its resonance wavelength will shift. By using such characteristics to bio- and chemical sensor, real time monitors can attach the biolayer on its surface without any fluorescent tags. This key feature also enables time resolved DNA or protein binding studies to be performed with very high accuracy. Since this technology can provide efficient reflection peaks with narrow linewidths, potentially very high signal to noise ratios can be achieved [11]. GMR sensors have been shown to exhibit high parametric sensitivity, rendering them extremely responsive to small amounts of trace chemicals or biological molecules. In 1998, Z. S. Liu et al reported a new type of narrow-band reflection filter based on guided-mode resonant effects [12-13]. A extremely narrow-

band and low side band reflection filter can be obtained when it is illuminated with TE (or TM) polarization light to a sub-wavelength grating.

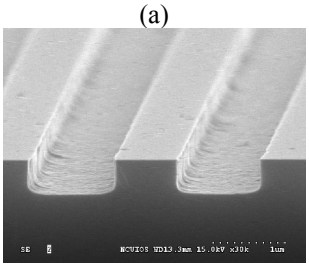
In this letter, a simply manufactured GMR device compound with a micro-fluidics channel biosensor was reported. The GMR optical device is a single dimension grating directly writing on waveguide layer by MEMS technology. By monitoring the reflective light spectrum shift, the DNA hybridization procedure can be detected in real-time. Beside, the experiments also discuss the repeatability of this sensor, by using fresh urea solutions to dehybridized the hybridized DNA. The results reveal that the GMR sensor has good detection sensitivity and good repeatability for DNA interaction dynamics experiments.

2. The Design and Fabrication of Elements

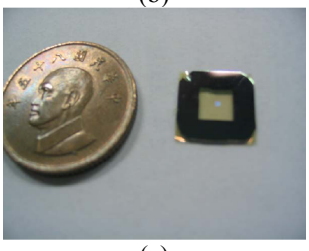
The two-layer GMR biosensor with a refractive index modulated periodic grating and a multiple-guided-mode waveguide layer are proposed to design the ultra-sensitive GMR sensor in this research by the use of simple structures and fabrication methods. The GMR structure under study is shown schematically in figure 1.



(a)



(b)



(c)

Figure 1. (a)The schematic drawing of GMR biosensor and (b) the SEM side view picture of grating and waveguide. (c) The picture of GMR biosensor.

The structure parameters of proposed GMR device are given as below: grating period Λ = 814 nm, grating depth d_g = 100 nm, waveguide depth d_{WG} = 900nm, grating filling factor f= 0.5, both refractive index of grating n_g and waveguide n_{Wg} are 2.05 (Si_3N_4). In consideration of the inducing guided-mode to the structure, the equivalent refraction index of the thin grating layer is design as n_{eff}, [12] which $n_{eff} = [f \times n_H^2 + (1-f) \times n_L^2]^{1/2}$.

There are four processing steps to fabricate the GMR device. First, deposit the Si_3N_4 thin film on both surfaces of the silicon substrate by using the low-pressure chemical vapor deposition (LPCVD). Second, transfer the sub-wavelength grating patterns on the top side of the Si_3N_4 film by using photolithography and e-beam lithography. Third, form square pattern on silicon substrate with photolithography and Inductively-Coupled-Plasma (ICP) dry etching. Finally, remove the silicon substrate beside the waveguide layer by KOH wet-etching.

Figure 1(b) shows the SEM photographs of the side profile of the GMR biosensor. The fabricated parameters of the GMR sensor have a little variation. For instance, the period of grating Λ=811nm, the depth of grating d_g=108.2nm, the thickness of waveguide d_{WG}=891.8nm. In figure 1(c) shows the picture of GMR biosensor.

3. DNA Hybridization Experiment and Results

3.1 Surface modification and DNA hybridization

For DNA hybridization experiment, four preload steps to immobilize DNA on GMR surface are necessary. First, we used plasma enhance chemical vapor deposition (PECVD) to deposit SiO_2 50nm on GMR surface. Second, steep the GMR device in the sulfuric acid and H_2O_2 solution for five minutes, to expose the OH^- group terminals on the SiO_2 surface. Third, immerse the device in the DETA (trimethoxysilylpropyldiethylenetriamine) solution for 30 minutes to a self-assemble monolayer. It provides the connectivity with the amino-groups to be attached to SMPB (4-(p-malemidophenyl) butyrric acid n-hydroxysuiimideester). Final, immerse the GMR sensor in the thiol-DNA solution to let the Capture DNA attached on the GMR biosensor surface. In addition to these immobilizing processes, it is also necessary to hybridize capture DNA complementary to one part of target DNA, and the probe DNA to the other part of target DNA. Figure 2 shows the processes of surface modification and DNA hybridization.

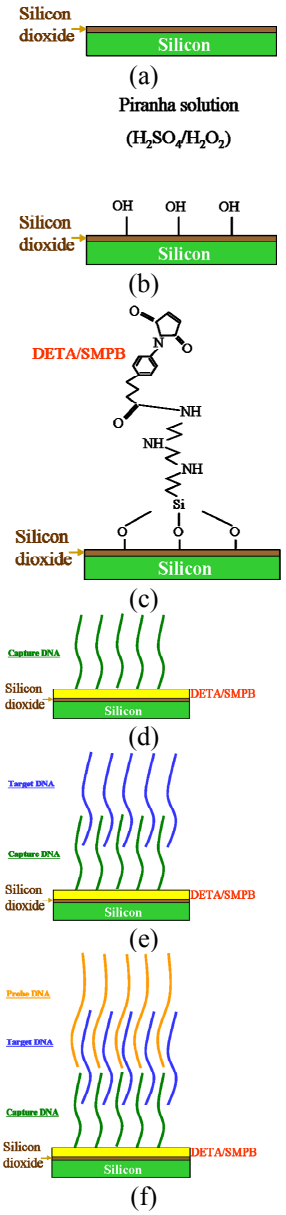


Figure 2. The DNA attaching processes are (a)-(d) and the DNA hybridization processes are (e)-(f).

3.2 Experimental setup

Figure 3 shows the fluid system of GMR biosensor. Figure 3(a) is the schematic diagram of this fluid system. And figure 3(b) is the picture of this system.

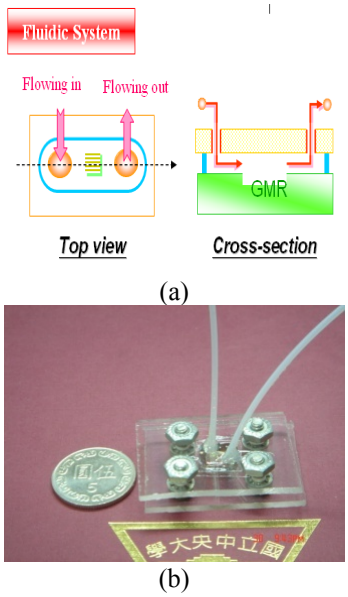
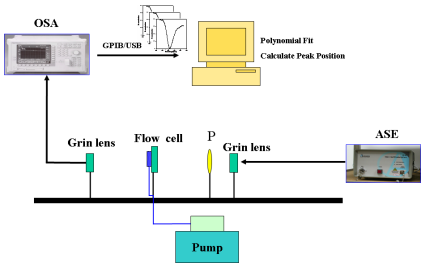
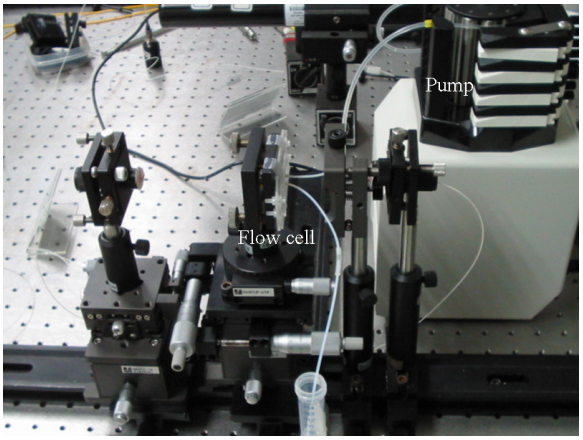


Figure 3. (a) Schematic diagram of GMR biosensor combined with fluid system. (b) The picture of this system.

Figure 4 shows the experiment setup of DNA hybridization. The GMR device is seated in a measurement system including light source, fluidic system, detection and analysis parts which were shown in figure 4(a). Amplified Spontaneous Emission (ASE, Amonics ALS15CL) is used to be a broad band light source. The range of wavelength is between 1525 and 1610 nm. The light emitted continuous waves from ASE light source with conventional communication band wavelengths and coupled into a single-mode fiber. The end of the single-mode fiber with GRIN lens that collimates parallel wave launches to the polarizer and fluidic system normally. The fluidic system includes two parts that are fluidic cell and syringe pump. By the use of the pumping of syringe pump, reaction reagents can flow into and out the fluidic cell through the tubing and react with the specific functional group on the surface of GMR device. Through the fluidic system, the transmission light was collected by GRIN lens and transmitted to Optical Spectrum Analyzer (OSA, Anritzu MS9710B). OSA was applied in this measurement system as a detector. Finally, those data were taken to analyze in personal computers which were connected to OSA with General Purpose Interface Bus (GPIB). Figure 4 (b) shows the picture of experimental setup.



(a)



(b)

Figure 4. The experiment setup of DNA hybridization.

4. Results and Discussion

Figure 5 shows the transmission spectrums of DNA hybridization. When DNA hybridizes, the resonance wavelength will shift to long wavelength apparently. According to Figure 5, molecules attached to the surface and the resonance wavelength shifted. The resonance wavelength of self-assembly monolayer and ligand, DETA/SMPB, is 1535.83nm. When capture DNA attached to SMPB, the resonance wavelength shifted about 1.44nm. Besides, capture DNA and target DNA hybridized target DNA and probe DNA, respectively. The resonance wavelength of hybridizing between capture DNA and probe DNA shifted about 2.14nm. Furthermore, the signal of hybridization is observed easily that the resonance wavelength with DNA hybridization is twenty times the frequency of which without DNA hybridization. We evaluated the total DNA length after hybridization to be 6.9nm, and the minimum resolution of OSA to be 0.07nm. The best resolution of our experiment is 0.23nm in thickness.

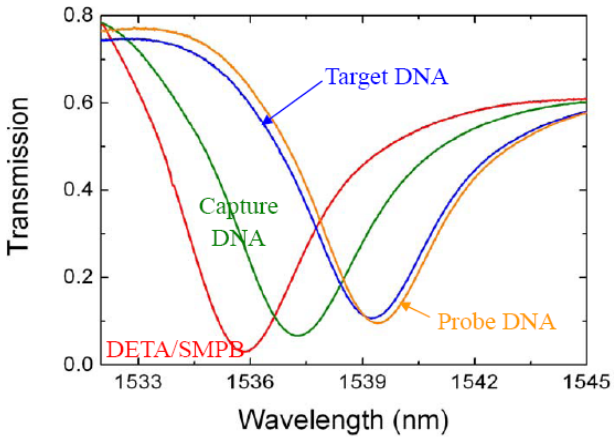
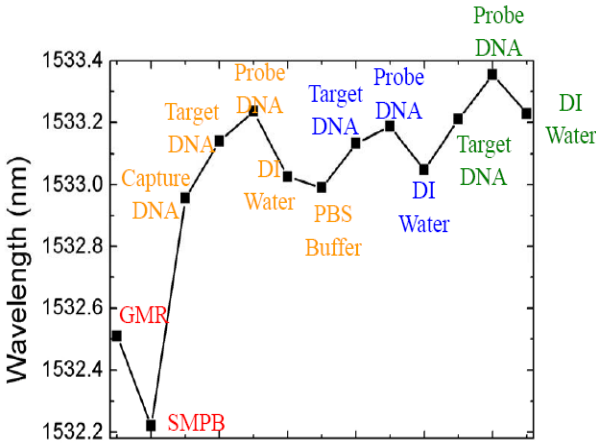
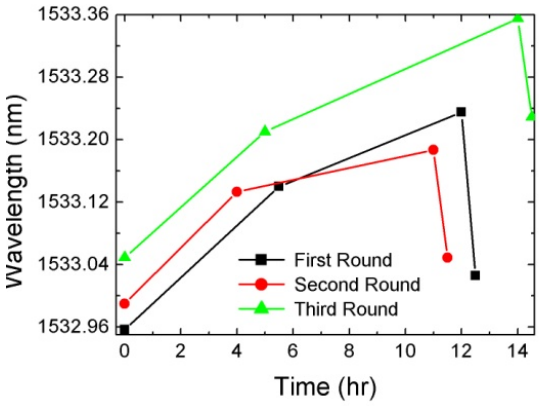


Figure 5. The transmission spectrums of DNA hybridization.

Figure 6 shows the result of DNA hybridization.



(a)



(b)

Figure 6. (a)The transmission spectrum of DNA hybridization and dehybridization. (b) The comparison of three repeat cycles. The result shows that three cycles have similar trends.

In this experiment, we process three cycles of DNA hybridization and dehybridization. The experimental steps show as followed. The GMR biosensor proceeded a sequence of hybridization and dehybridization steps. The hybridization steps were mentioned above and the dehybridization steps which remove target DNA and probe DNA were heated to 80°C for 20 minutes. Then, Put it into PBS buffer solution. With those methods, the reliability and the repeatability of GMR biosensor can be demonstrated.

As illustrated in figure 6(a), the three cycles have similar trends. Figure 6(b) shows the comparison of three cycles. For the repeatability test of the GMR biosensor, DNA can be hybridized and dehybridized successfully.

5. Conclusion

In this paper, we succeeded in applying the wave-guided resonance optical element on GMR biosensor. Differing from conventional sensors, GMR biosensor has many advantages, which include a label-free system, a more minimizing system, a high throughput system, a high sensitivity system, a real-time monitoring system, and, especially, high sensitivity and label-free. The result showed that GMR biosensor has great potential to detect DNA hybridization. We confirmed the feasibility of the detection of SiO_2 multilayer deposition on GMR element. Moreover, the application of DNA hybridization can be successfully applied on GMR biosensor. It provided a technical platform for studies of the bimolecular interaction. Also, the repeatability, DNA hybridization and de-hybridization of GMR biosensor are all demonstrated in this paper. As what's illustrated in figure 3, we combine GMR element with micro-fluid system to make real-time monitoring possible. Moreover, these advantages enables it a powerful biophysical tool to characterize quantitatively how biomolecular will complex form and dissociate apart over time in the future.

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Corresponding Author:
Tsung-Hsun Yang, Ph.D.
Department of Optics and photonics, National Central University, Chungli, Taiwan, ROC
No. 300, Jhongda Rd., Jhongli City, Taoyuan County 32001, Taiwan, ROC
E-mail: thyang@dop.ncu.edu.tw

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5/21/2012

Simple Embossing Process for Fabricating GMR Biosensor with Variable waveguide Thickness

Ting-Jou Ding, Jiann-Hwa Lue, Tsung-Hsun Yang*

Department of Optics and photonics, National Central University, Chungli, Taiwan, ROC
thyang@dop.ncu.edu.tw

Abstract: This work demonstrates a simple fabrication process for the guided-mode resonance biosensors with tunable waveguide thickness. For various waveguide thicknesses, the grating parameters can still keep the same. Although consisted of the waveguide layer and the grating layer, the GMR biosensor can be simply fabricated in a single step and of the same material by the conventional embossing technique. Moreover, the thickness of waveguide can be well controlled by applying various forces. It is shown that the tunable range of the waveguide thickness is approximately to 600nm and the deformation on the grating layer still keeps less than 10%.
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Keywords: soft lithography; spin-on glass; grating; waveguide; subwavelength; embossing

1. Introduction

The guided-mode resonance (GMR) sensor as one of the optical biosensor has presented several advantages including: (i) monitoring in real time, (ii) labeled-free detection to simplify assay, (iii) less analysis time, (iv) high potential for high throughput device, and (v) compact measurement system[1-9].

For the consideration of low cost and high throughput, simple and easy-made structure has more advantages in practical applications. In the construction of GMR sensor, it simply consists of one sub-wavelength diffraction grating and one waveguide layer. Figure 1 illustrates the schematic structure of GMR sensor. In the figure 1, the structure of GMR sensor can be subdivided into several parts: period, filling factor, grating depth, and waveguide thickness.

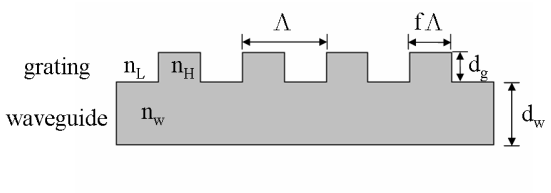


Figure 1. The schematic structure of GMR sensor. The GMR sensor, it simply consists of one sub-wavelength diffraction grating and one waveguide layer. It can be subdivided into several parts: period, filling factor, grating depth, and waveguide thickness in detail.

As being an excellent sensor, the capacity of high throughput in fabrication is an important factor. However, the conventional fabricating methods for GMR sensor can be sorted roughly by lithography and imprint techniques [10-15]. In the sub-micro or nanometer scale applications, conventional lithography techniques require more expensive equipments to increase resolution. For the high throughput fabrication objective, it is a major issue under the consideration of cost and commerce.

On the other hand, imprint techniques don't need expensive equipments in high throughput fabrication but have simple fabricating processes instead. Imprint techniques provide a powerful tool in micro and nanometer scale applications. Fabricating GMR sensor by nanoimprint is an excellent solution for low cost and high throughput consideration. In the conventional nanoimprint, a "hard" material, usually silicon, or glass based, is used to be the master mold. Generally, this master mold transfers designed patterns to UV-curing or heat-curing material. After stripping the mold, a high refractive index (or dielectric constant) layer was deposited on curing material to form GMR sensor. In general, low refractive index curing materials and high refractive index deposited layer are usually chosen to enhance the sensitivity. This method provides a cheap and powerful solution in nanofabricating. However, imprinting step in this fabricating process only defines and transfers patterns to curing materials. Nanoimprint does not build the GMR sensor. The higher refractive index deposited layer constructs GMR sensor's main structure after depositing step. In this method, two-step fabricating processes have room for improvement. If nanoimprint is the only needed fabricating process, the fabrication will not only be more efficient but cost less. For fabricating GMR sensor, simpler fabricating processes development is an important and attractive issue.

Soft lithography is a new concept in nanoimprint field [16-22]. The greatest feature of soft lithography is using an elastomer as a mold, a stamp,

or a mask. The elastomer mold replaces the rigid ones and is used to transfer designed patterns. Comparing to conventional imprint techniques, the soft mold is capable of larger-sized imprinting, such as tens centimeter-scale. Moreover, it has superiority in imprinting two-dimension and three-dimension structures [16]. Soft lithography technology approximately includes microcontact printing (μCP)[19,23-25], replica molding (REM)[26-27], microtransfer molding (μTM)[19,28], micromolding in capillaries (MIMIC)[29-31], embossing[32-33]. For the fabrication of GMR sensor by soft lithography, Magnusson fabricates GMR sensor by micromolding in capillaries (MIMIC) [34]. In their fabricating steps, GMR sensor was divided into two parts, grating and waveguide fabrications. The waveguide part was first deposited on the substrate with a high refractive index layer. Then, the grating part was formed by the micromolding in capillaries (MIMIC). In this method, the waveguide thickness is adjustable depending on the depositing thickness and not controlled by imprint step. It is a useful experimental design because the adjustable waveguide thickness is a key factor to GMR sensor. The thickness of waveguide has great influence on sensitivity. MIMIC technique is a powerful protocol in nanoimprint but has several limitations for GMR sensor fabrication. In MIMIC, the capillaries are the domain force to make the imprint materials fill into whole patterns. It needs much more time when the imprinting area increases. Embossing, one of soft lithography technology, has great superiority in GMR sensor fabrication. The outstanding characteristics of embossing are include a single procedure and high throughput. Embossing also makes the waveguide thickness adjustment possible due to its intrinsic properties.

In our work, we developed a cheap and stable imprint system to realize the fast fabrication of GMR sensor. This cheap imprint system only cost about 400 dollars. Utilizing this imprint system, we demonstrated a single procedure method successfully for fabricating adjustable waveguide thickness GMR sensor by embossing technique. In the practical fabrication, the liquid spin-on glass (SOG) was used as imprinting material.

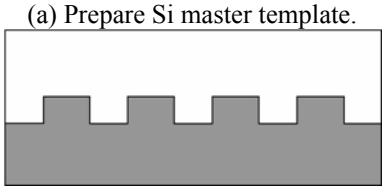
2. Soft Lithography Process

Elastomer mold: Polydimethyl siloxane (PDMS) is the major material used as an elastomer. In the elastomer mold constituent, SylgardR 184 silicone elastomer from Dow Corning was used. The characteristic of low surface energy makes mold stripping easily without any released agents.

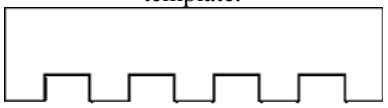
Pattern material: In this experiment, the material to pattern is Spin-on glass (SOG). SOG is a mixture of SiO_2 and dopants suspending in a solvent solution. We use SOG (IC1-200) containing 7.2% solids content from Futurrex.

Figure 2 shows the schematic fabrication process of GMR sensor by the embossing technique. The waveguide and grating of GMR sensor were produced at the same in imprinting step.

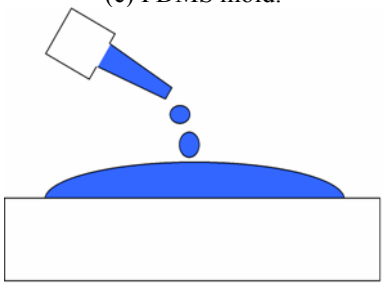
(a) Prepare Si master template.



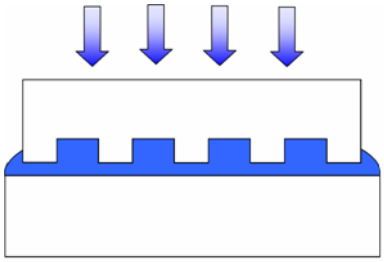
(b) Apply and cure PDMS and Remove Si master template.



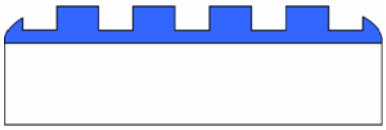
(c) PDMS mold.



(d) Apply liquid SOG solution on a ITO glass substrate.



(e) Press PDMS mold on and heat. Then, Stripping PDMS mold.

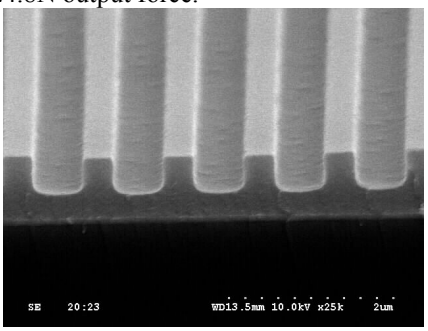


(f) Fabricated element.

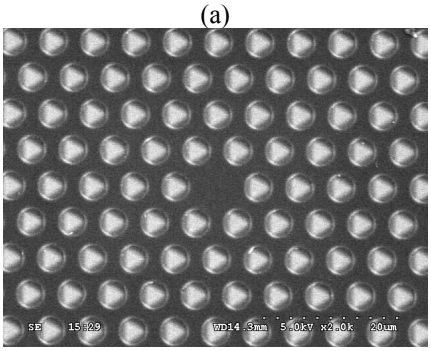
Figure 2. Scheme of the fabrication processes for producing one-dimension grating and waveguide structure by embossing. The elastomer mold production and imprint procedure composes of the whole processes.

In the beginning of procedures, a patterned silicon plate is prepared as the master template. This template was patterned of some sub-wavelength gratings by conventional e-beam lithography. The pattern size of sub-wavelength grating is 2 mm^2. Next, transfer the patterns and structures from the template to the elastomer PDMS mold. The SylgardR 184 silicone elastomer was mixed with the curing agent of 10:1 ratio by weight and standing until the air bubbles escaped from mixture. Applying PDMS on the template and heating 10 minutes at 100°C to cure PDMS. After curing step, the PDMS mold was stripped from silicon template. This PDMS mold plays an important role in embossing soft lithography. PDMS mold was taken as major mold replacing of rigid silicon template in soft lithography technology. In the imprint steps, the SOG liquid was applied on the surface of the glass substrate and pressed with PDMS mold. After impression step, the imprint stage heated from the room temperature to 90°C and made the solvent vapor. Two hours later, the PDMS mold was stripped from glass substrate.

This imprint method is not only designed for GMR sensor fabrication but also capable of two-dimension arbitrary patterns transference and adjustable waveguide thickness fabrication. Figure 3 shows the SEM pictures of one-dimension grating and two-dimension microcaves fabricated at 90°C with 24.8N output force.



(a)



(b)

Figure 3. The SEM pictures of (a)one-dimension grating and (b)two-dimension microcaves. Both of them were fabricated at 90°C with 24.8N output force.

In our experiment setup, the size of the PDMS mold is 1cm×1.3cm and the volume of SOG solution is 6 microliter. As maintaining at 90°C, the output force from the pressure cylinder was set approximately to 3.4N, 24.8N, 37.5N, and 50.2N, respectively.

3. Instruments and Setup

We develop an imprint system to fabricate waveguide thickness adjustable GMR sensor by embossing technique. Figure 4 shows the construction of the major imprint device. It is composed of several parts: frames, heaters, thermal sensors, thermal insulators, pressure cylinder, air valve controller, imprint stage, leveling adjustment components, and a plastic cover box. The heaters, thermal sensors, and the thermal insulators construct temperature control system. It provides stable and controllable surrounding temperature. In this temperature control system, power supply provides all the needed energy. The pressure cylinder and air valve controller provide the controllable output force in the imprinting processes. The outside cover prevents the disturbance of heat convention and helps to keep surrounding temperature stable.

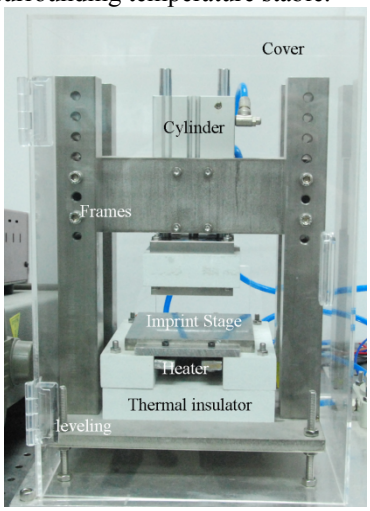


Figure 4. The picture of imprint device which is the major part included in self-developed imprint system. This imprint device composes of several parts including stainless frames, heater, thermal sensor, thermal insulator, pressure cylinder, air valve controller, imprint stage, leveling adjustment components, and a plastic cover preventing the disturbance of any airflow.

Figure 5 show the self-developed imprint system. This imprint system is composed of major imprint device, temperature control system, power supply, and pressure control system. For the low cost consideration, it costs only approximately 400 dollars

expect power supply. Simple construction makes assembly and operation easier. In this self-developed imprint system, low cost, stable, and practical are the notable characteristics of our design. This imprint system provides heating range from room temperature to 160°C and pressing force approximately up to 78 N.

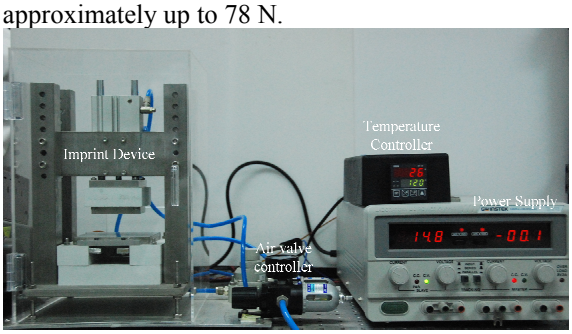


Figure 5. The picture of self-developed imprint system. This imprint system includes major imprint device, pressure controller system, temperature controller system, and power supply. The whole imprimt, expect the power supply, costs approximately 400 dollars. It is a cheap, stable, and practical design for imprinting.

4. Characterization

Embossing technique has two key factors, heating temperature and pressure (or output force) during the fabricating processes. In the functionality considerations, heating mainly evaporates solvent of imprinting material. The total amount of solutes in liquid imprinting material has small variation during the heating process. Heating only influences the evaporator rates but changes the total amount of solutes. The higher temperature makes faster evaporator. The experimental result of different heating temperature at constant press force (50.2N) condition shows in figure 6. In figure 6, the waveguide thickness keeps approximately in the order of 100 nm from room temperature to 120°C.

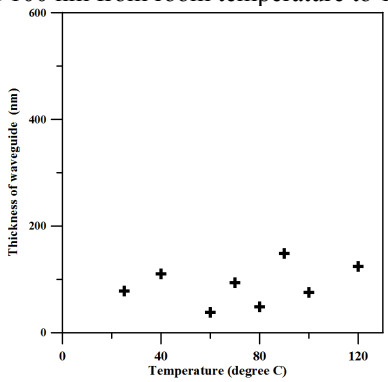
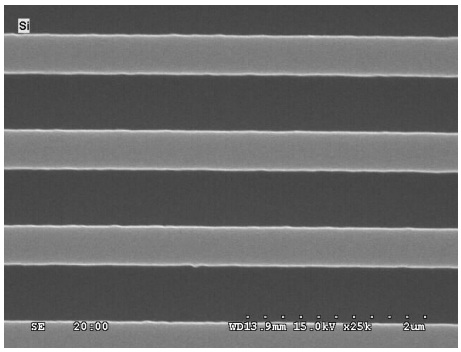


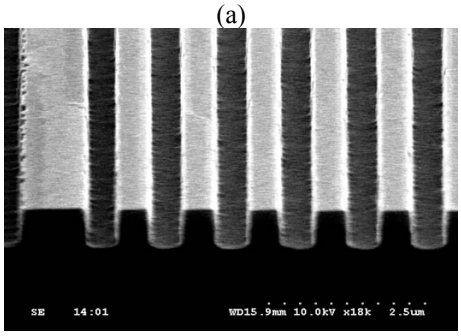
Figure 6. The variation of waveguide thickness at different temperature. The temperature range is form room temperature to 120°C and the output force in all cases is 50.2N.

5. Results and Discussion

Figure 7 shows the scanning electron microscope (SEM) picture of silicon template in top view. The grating period of this template is 1,013 nm and the filling factor is approximately 0.4. The grating depth is 520nm.



(a)



(b)

Figure 7. Scanning electron microscope (SEM) picture of silicon template in top view (a) and side view (b). The grating period of this template is 1013nm and the filling factor is approximately 0.4. The grating depth is 520nm.

Figure 8 shows the SEM pictures of each one-dimension grating and waveguide structure fabricating under different compressed force conditions at 90°C.

In those pictures, the thickness of waveguide decreases with the increments of compressed force. Those pictures also show that the characteristics of grating have less variation than thickness of waveguide. The variations of the period, the depth, and the filling factor of the grating are all less than 10%. It reveals that the grating characteristics have been reproduced very well.

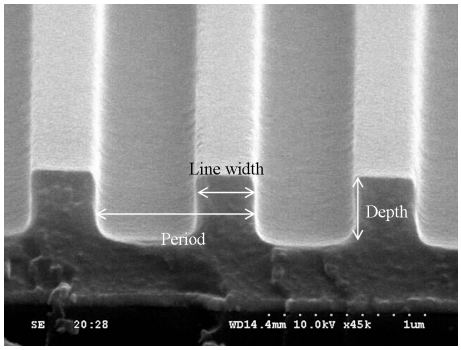


Figure 8. SEM pictures of one-dimension grating and waveguide structure fabricating under different compressed force conditions at 90°C. The picture of output force shows (b) 24.8N.

Figure 9 shows the relationship of output force and thickness of waveguide. Without doubts, the larger the output force is, the thinner the waveguide layer of the grating is. In the range of applicable force, the thickness of the waveguide layer of the grating reduces 10 nm per N pressing force.

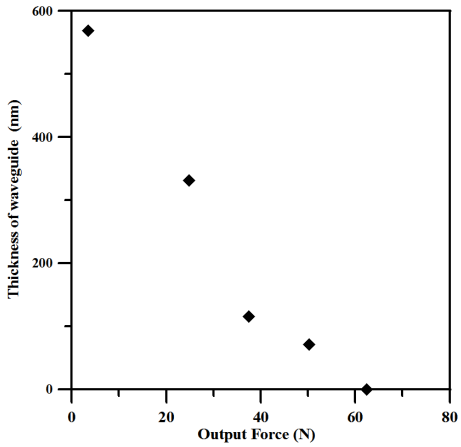


Figure 9. Relation between the output force and the resulting thickness of waveguide. The resulting thickness of the waveguide is inversely proportional to the output force.

Figure 10 shows the shrinkage between imprint sample and silicon mold. The graph shows the shrinkage rates of grating depth, line width, and period. In this case, the shrinkage rates of depth, line width and period are approximately 31.2, 10.5 and 3.2 respectively.

In the design of this experiment, the solvent vaporization time was set up to two hours which is enough for entire formation. This solvent vaporization time relies on the intrinsic characteristics of solvent. For our used SOG type, this time can reduce to 10 minutes in the condition of 24.8N applied force and 90°C.

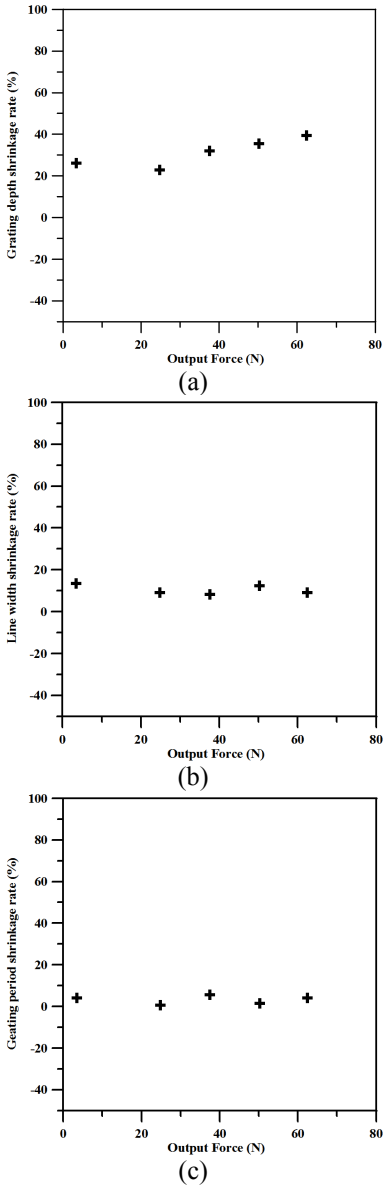


Figure 10. Shrinkage rates of (a) grating depth (b) grating line width (c) grating period versus the output force. In this case, the shrinkage rates of grating depth, line width and period are around 31.2, 10.5 and 3.2, respectively.

6. Conclusions

In conclusion, we present a method for fabricating adjustable waveguide thickness guided-mode resonance (GMR) biosensor successfully. The waveguide and grating can be fabricated in a single step and of the same material, spin-on glass (SOG), by embossing technique. In our case, the adjustable waveguide thickness range is around 600nm and the grating deformation can still keep less than 10%. For GMR biosensor design and fabrication, the stable grating structure and changeable waveguide thickness

make the design into reality easier. The short formation time also helps to achieve low cost and high throughput objective easily.

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Corresponding Author:
Tsung-Hsun Yang, Ph.D.
Department of Optics and photonics, National Central University, Chungli, Taiwan, ROC
No. 300, Jhongda Rd., Jhongli City, Taoyuan County 32001, Taiwan, ROC
E-mail: thyang@dop.ncu.edu.tw

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Role of Pap smear in early diagnosis of cervical cancer- A Case Study of women in Saudi Arabia

Sheikh Haroon , Manhua Cui

Second Hospital, Jilin University Changchun, China
King Abdul Aziz Hospital and Oncology Centre Jeddah, Saudi Arabia
Cuimanhua@Yahoo.com

Abstract: The fifth most common and deadly cancer amongst women worldwide is Cervical cancer. Cervical cancer mostly affects younger women and during the last two decades the incidence in younger age groups has further increased. The EU established principles for organised population-based cervical screening to control and decrease the incidence of cervical cancer. 1941, Papanicolaou described cervical mass screening for early detection of cervical cancer. The Pap smear has proved valuable for mass screening and enabling lesions detection at an early enough stage for effective treatment and has an incidence of reducing squamous ICC by at least 80%. Organized screening has not been introduced in Saudi Arabia hence the reasons for the Pap smears (n=1475) performed were one or a combination of vaginal discharge, vaginal itching, lower genital tract burning, suspected urinary tract infection by the patient and age of the patient where the doctor performed the pap smear because the patient was in the age range for cervical cancer. For 83% this was their first Pap smear. The total number of abnormal cervical smears was 43. i.e. 2.91% of all screened cytology cases. Our research indicated a high prevalence of CIN 1 and CIN 3. Pap smears play a substantial role in not only detection but also prevention of cervical cancer. Success of organized screening programme is possible when Family physicians at family clinics will be properly trained in performing Pap smears.

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Key words: pap smear, cervical cancer, ICC, cytology, colposcopy.

1. Introduction

Worldwide, cervical cancer is twelfth most common[1] and the fifth most deadly cancer in women.[1] It affects about 16 per 100,000 women per year and kills about 9 per 100,000 per year.[1] Approximately 80% of cervical cancers occur in developing countries. Worldwide, in 2008, it was estimated that there were 473,000 cases of cervical cancer, and 253,500 deaths per year.[1]

Clinical stages and prognosis

The International Federation of Gynecology and Obstetrics staged Cervical cancer as tabulated below.

The stage distribution differ in different parts of the world and this is most likely a reflection of the presence or lack thereof of screening programmes that spot early stage disease. However introduction of effective screening can lead to overestimation of survival estimates as some cases will be diagnosed at an earlier stage (lead time bias).

Treatment

For early stage cervical cancer (I–IIA) the usual treatment is radical hysterectomy and pelvic lymphadenectomy occasionally followed by radiotherapy and chemotherapy but this deprives females of their fertility. Mostly stages IB2 and IIA, are best treated by a combination of radiotherapy and chemotherapy.[2]

Hysterectomy is used to treat the Microinvasive cancer stage IA1 but in order to preserve fertility it is done by one excision of the lower part of the cervix.[3]

For females at stage IA2 or IB1 the following methods may be used; in case of no lymph node metastasis, fertility sparing surgery is opted. The lymph nodes can be taken away laparoscopically and if negative nodes, the cervix is radically removed by a vaginal approach.[4]

The last technique, abdominal approach, requires extensive experience and speciality. Abdominal approach is pelvic lympadenectomy and radical trachelectomy - leaving the uterus.[5]

Etiology

Human papillomavirus

Continual infection with a carcinogenic papillomavirus (HPV), which is a double-stranded circular DNA viruss that infects many species, is a basic cause of squamous cell carcinoma and adenocarcinoma.[6-8]

Risk factors

The following have been identified as co-factors that can raise the risk of precancer and cancer between 3-5 times amongst women already infected by HPV; long term use of oral contraceptives [9-10] high parity, multiple parity [11-12] and smoking.

Since HPV is sexually transmitted hence the sexual behavior, number of sexual partners of the woman and /or her partners both notably impact the risk of ICC [12-13].Some studies show that male

circumcision reduces the risk of ICC in female partners.[14-17]

Protection from condom use is varied as it shows protection in some studies, but not significantly so in several studies. [18-19]

Cervical neoplasia

Carcinogenesis

HPV infections have a high spontaneous clearance, irrespective of the age. Approximately 50% of the infections clear within 6 months, 66.6% within 12 months, and around 80% within two years.[20] The longer the infection lasts, the more it increases the probability that it will continue and cause precancer/cancer. [20] In 20-30% females persistant infections over 12 month period have lead to CIN2+.[20-21]

Cervical screening

Following the World Health Organisation (WHO) definition of screening as "the presumptive identification of unrecognised disease by means of tests or examinations that can be applied rapidly".[18]

Since the precancer time period for cervical cancer is longer as compared to other female cancers hence the mortality rate is comparatively high as well. Also treatment of precancer or early stage ICC usually leads to healthier outcome than late treatment.

Although screening started in Saudi Arabia in 1984 there was and still exists a lack of organized cancer screening programmes that led to lack of trends available hence there was no proper estimation till the study of WHO in September 2010.[23]

Many studies have also shown that the effect of screening programmes has yielded different results across different countries, which may be attributed to differences in the implementation of screening. [18,23-24] Hence the aim of the screening test should be 'high sensitivity' i.e. to permit as few as possible with the disease to get through undetected .

Even though in 1985, the National Board of Welfare recommended screening every third year in ages 20-59 to date there still exists the enormous challenge worldwide to execute organised cervical screening.[25]

Cytologic tests

Conventional cytology

In 1941, Papanicolaou[26] described cervical mass screening for sexually active women for early detection of cervical cancer. For the Pap smear, cells are collected from the surface of the uterine cervix and the cervical canal, smeared on a glass slide, and analysed in a microscope.

According to the American Cancer Society Guideline for the Early Detection of Cervical Neoplasia and Cancer[27] a combination of the extended tip spatula and the endocervical brush provides optimal sampling of the ectocervix, T-zone, and endocervix, and has the lowest false-negative rate.

The Pap smear has proved valuable for mass screening and enabling lesions detection at an early enough stage for effective treatment and has an incidence of reducing squamous ICC by at least 80%.[28] The Pap smear does have its limitations among them the most important being its 47-62% limited sensitivity and the subjective interpretation of the results. [28]

Liquid based cytology

In the Liquid-based cytology known as LBC the cellular material is immersed in a container with a special liquid but it needs to be performed in a special equipment lab.[29] Since the sample is not smeared on a slide the possibility of representative smears and of less obscuring factors (blood, mucus, inflammatory cells) increases. It allows less time for sample interpretation and most importantly the same sample can be used for other analyses (hrHPV, Chlamydia).

Although a meta-analysis of international studies found no evidence of improved accuracy (sensitivity and specificity) with LBC compared to conventional cytology however despite the higher cost, LBC has largely replaced conventional cytology in several countries,[22] and the method is recommended by the Swedish Society for Obstetrics and Gyneacology.[30]

Classification System

In 1988 the Bethesda System (TBS) was introduced in order to standardize cytology reporting. The past decade saw important changes and updates in gynecologic cytology like LBC and (HPV) DNA testing that led to minor amendments in 1991 and a new redefined version of TBS in 2001.

The Bethesda System 2001[31] is as follows:

1) Negative for intraepithelial lesion or malignancy

a) Organisms

Trichomonas vaginalis

Fungal organisms morphologically consistent with *Candida* spp.

Shift in flora suggestive of bacterial vaginosis

Bacteria morphologically consistent with *Actinomyces* spp.

Cellular changes consistent with Herpes simplex virus.

b) Other non-neoplastic findings

Reactive cellular changes

Atrophy

2) Epithelial cell abnormalities

a) Squamous cell

• Atypical squamous cells

Of undetermined significance (ASC-US)

Can not exclude HSIL (ASC-H)

• Low grade squamous intraepithelial lesion (LSIL) encompassing: human papilloma virus (HPV)/mild dysplasia/CIN 1

• High grade squamous intraepithelial lesion (HSIL) encompassing: moderate and severe dysplasia,

CIS/CIN 2 and CIN3 with features suspicious for invasion (if invasion is suspected)

• Squamous cell carcinoma

b) Glandular cell

Atypical

Endocervical adenocarcinoma in situ

Adenocarcinoma

3) Other malignant neoplasms

Specimen adequacy

As per TBS 2001 the minimum requirement for adequacy for a conventional cytology is 8000-12000 visible squamous cells but for LBS only 5000 cells are sufficient. [29,31]

A satisfactory sample means atleast 25% visible squamous cells, but in case atypical cells can be indentified irrespective of the percentage of visible cells then the smear is abnormal'. [31] For the purpose of research the reason for an unsatisfactory sample should always be stated. The unsatisfactory samples can be obscured by blood or inflammatory cells.[29] The sample collected might have very few cells or it could have cells that are poorly fixed.

Visibility of only 25-50% epithelial cells terms the sample as "partially obscured" .[31]

Negative cytology

TBS regroups the category negative for intraepithelial lesion or malignancy for benign or normal alterations.

Abnormal cytology

The two-stage TBS and the three stage system of abnormalities are shown in the tables below.

Although both cytology and histology are fundamentally the same however Cytological interpretation are subjective and they are not always accurate hence the diagnosis should be determined in combination with histopathology.[18,29]

Cytology findings

Saudi Arabia has a population of 6.51 million women ages 15 years and older who are at risk of developing cervical cancer. Current estimates indicate that every year 152 women are diagnosed with cervical cancer and 55 die from the disease.[22]

Cervical cancer ranks as the 11th most frequent cancer among women in Saudi Arabia, and the 8th most frequent cancer among women between 15 and 44 years of age. Data is not yet available on the HPV burden in the general population of Saudi Arabia.[22]

2. Methods and Results

For the purpose of this study data was collected from January 2009 – January 2011 at King Abdul Aziz Hospital & Oncology Centre Jeddah. All Pap smears were performed by the same doctor. This was to ensure that there was no compromise on quality and method of sample collection for Pap smear.

As mentioned earlier organized screening is not prevalent is Saudi Arabia hence the reasons for the Pap smears performed were one or a combination of vaginal discharge, vaginal itching, lower genital tract burning, suspected urinary infection by the patient and age of the patient where the doctor performed the pap smear because the patient was in the age range for cervical cancer. For 83% this was their first Pap smear.

3. Discussion

It has been recognized worldwide, through studies and clinical practices that for early detection of precancerous lesions of cervical cancer the best technique is cytological examination of cervical by Pap smear. This is because an abnormal cervical cytology report show the existence of a precancer lesion which if left untreated mostly progresses to cancer.

There is an international agreement that women with high grade squamous abnormalities and with glandular abnormalities need colposcopy.[18,29] The colposcopy magnifies the cervix 6-40 times and after the application of 3 % or 5% acetic acid solution onto the cervix turns precancer lesions acetowhite. The sensitivity of colposcopy is similar to that reported for Pap smear screening while specificity is lower.[32]

However, because colposcopy relies on subjective visual interpretation, it is crucial to define consistent criteria for suspicious lesions and to train providers to correctly implement these criteria [33] as opposed to Pap smears.

According to one Cochrane review while colposcopy has a reputable role in determining the most suspicious areas for colposcopically directed biopsies and in planning effective treatment, but it is not a diagnostic test and cannot substitute cytological evaluation.

It can be said with great certainty that cytological screening programs play a major role in reducing both the incidence and mortality of ICC. In the US, Canada and Europe widespread introduction of cytological screening decreased the incidence of cancer of the cervix that was paralleled by a reduction in mortality.[32,34-35]

Europe has approximately 55 000 new cases each year and 25 000 deaths. [36]The greater part of these cases are in Eastern Europe where there are no cervical screening programmes.[14]

All abnormal cytology patients were amongst the 83% giving their first Pap smear sample. This indicates that for CIN2 and CIN3, patients earlier Pap smears would have meant earlier detection at the earlier stage leading to an increased rate of recession and hence of mortality.

For patients with CIN1 and early stages of cervical cancer a regular yearly Pap smear would have shown the cellular change and for those with abnormal cytology but non cancerous legions the correlated treatment has begun and their follow-up Pap smear is to be performed after 6 months of the first Pap smear.

4. Conclusion and Recommendations

It is extremely imperative to note that whereas factors like male circumcision and early sexual activity do have a protective affect against HPV transmission many other significant risk factors of cervical cancer are very much prevalent in Saudi Arabia.

The increasing rate of cervical cancer incidence in Saudi Arabia can be greatly reduced, thus increasing the mortality rate, as Pap smears play a substantial role in not only detection but also prevention of cervical cancer. Especially if performed yearly for women after the age of 34 as it is after this age when majority of the incidence cases have been reported in Saudi Arabia.

It is therefore recommended that as part of an organized screening after 34 years of age a yearly Pap smear should be made mandatory as part of the primary health care system in Saudi Arabia and its results recorded in patients' permanent files.

The yearly Pap smear should be performed irrespective of whether any of the before mentioned symptoms exist or not. In case of abnormal but non cancerous cytology a follow-up Pap smear after six months should be performed.

Success of organized screening programme is possible when Family physicians at family clinics will be properly trained in performing Pap smears. It is also essential to educate women over 34 about Pap smear, this can be done by the family physician one 34 years age has been reached and also through pamphlets.

Corresponding author
Prof. Manhua Cui.
Department of Obstetrics & Gynecology &Gynecology Oncology
Second Hospital, Jilin University
Ziqiang Street 218, Changchun 130041
China
Email: drhsheik@hotmail.com

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Abbreviations and Definitions

AGC	Atypical glandular cells
AGUS	Atypical glandular cells of undetermined significance
AIS	Adenocarcinoma in situ
ALTS	ASCUS-LSIL Triage Study
ASCCP	American Society for Colposcopy and Cervical Pathology
ASC-H	Atypical squamous cells, high-grade squamous lesion cannot be excluded

ASCUS Atypical squamous cells of undetermined significance
CGIN Cervical glandular intra-epithelial neoplasia
CIN1-3 Cellular intraepithelial neoplasia grade 1-3
CIS Cancer in situ
DNA Deoxyribonucleic acid
EC Endocervical cells
ECC Endocervical curettage
Effectiveness The extent to which the programme, when deployed in the field in routine circumstances, does what it is intended to do for a specified population
FIGO International Federation of Gynecology and Obstetrics
HC Hybrid Capture
HPV Human papillomavirus
hrHPV High-risk HPV type
HSIL High grade squamous intraepithelial lesion
ICC Invasive cervical cancer
IQR Inter-quartile range
LBC Liquid based cytology
Lead time Period between the detection of a lesion by screening and the time point that it should have progressed, in the absence of screening, to a clinically recognised cancer
LEEP Loop electrosurgical excision procedure
LLETZ Large loop excision of the transformation zone
LSIL Low grade squamous intraepithelial lesion
NETZ Needle excision of the transformation zone
NPV Negative predictive value; i.e. the extent to which subjects are free of the disease in those that give a negative test result
Pap Papanicolaou
PPV Positive predictive value; i.e. the extent to which subjects have the disease in those that give a positive test result
RCT Randomised clinical trial
SAS Statistical analysis system
SNOMED Systematized nomenclature of medicine
Sojourn time Duration of the detectable pre-clinical phase.
Stage IA Microinvasive
Stage IB Localised cancer
Stage II+ Advanced cancer

Table 1. International Federation of Gynecology and Obstetrics Clinical Staging of Cervical Carcinoma and Corresponding MRI Findings

FIGO stage	Description	MRI findings
I	Tumor confined to cervix	
IA IA1 IA2	Microscopic invasive tumor Stromal invasion ≤3 mm; width ≤7 mm Stromal invasion >3 mm but ≤5 mm; width ≤7 mm	No evidence of tumor
IB IB1 IB2	Clinically visible invasive tumor or preclinical tumors > stage IA Tumor ≤4 cm Tumor >4 cm	Intermediate SI mass against low SI of the background cervical stroma
II	Invasion beyond uterus but not to pelvic sidewall or lower third of vagina	
IIA IIA1 IIA2	No parametrial invasion, upper two-thirds of the vagina Tumor ≤4 cm Tumor >4 cm	Loss of the normal low SI of the vaginal fornix or wall, usually contiguous with the primary cervical tumor mass
IIB	Parametrial invasion	Breach of the low SI ring of the cervical stroma
III	Tumor extends to pelvic sidewall and/or involves the lower third of vagina	
IIIA	Tumor extends to lower third of vagina but not to pelvic sidewall	Loss of the normal low SI of the vaginal wall in the lower third of the vagina
IIIB	Extension to pelvic sidewall and/or hydronephrosis or nonfunctioning kidney	Presence of tumor within 3 mm of internal obturator, levator ani and pyriformis muscles or the iliac vessels. Additional signs include increased SI and/or retraction of pelvic muscles, presence of hydronephrosis owing to ureteral obstruction at the level of the primary tumor or nodal metastasis
IV	Tumor extends outside true pelvis or invades bladder or rectal mucosa	
IVA	Invasion of bladder or rectal mucosa	Tumor nodules protruding into the bladder/rectal lumen
IVB	Distant metastasis	Tumor involving organs outside the true pelvis, includes the para-aortic and inguinal nodes

FIGO: International Federation of Gynecology and Obstetrics; SI: Signal intensity.

Medscape Source: Women's Health © 2010 Future Medicine Ltd

Table 2. Risk factors associated with cervical cancer wikipedia

Sexual Activity
Number of sexual partners
Early sexual activity (especially less than 16 years of age)
Sexually transmitted diseases
Human papilloma virus (HPV)
Herpes simplex virus
Early age of first pregnancy
Parity
Cigarette smoking
HIV
Immunosuppression from any cause
Vitamin deficiencies
Interval since last Pap smear
Oral contraceptive use

Table 3

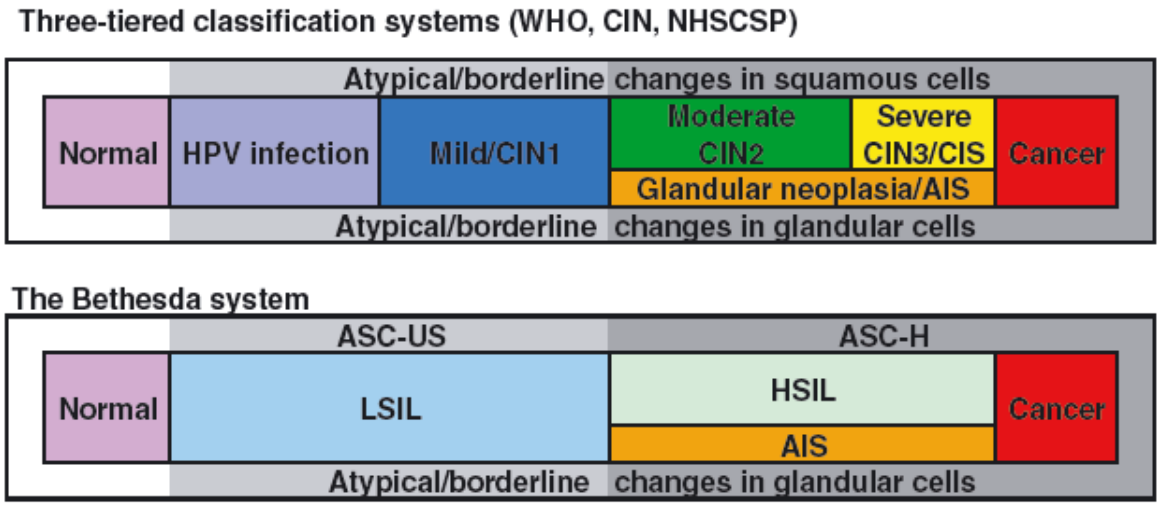


Table 4: Key Statistics on Saudi Arabia (WHO, 2010)

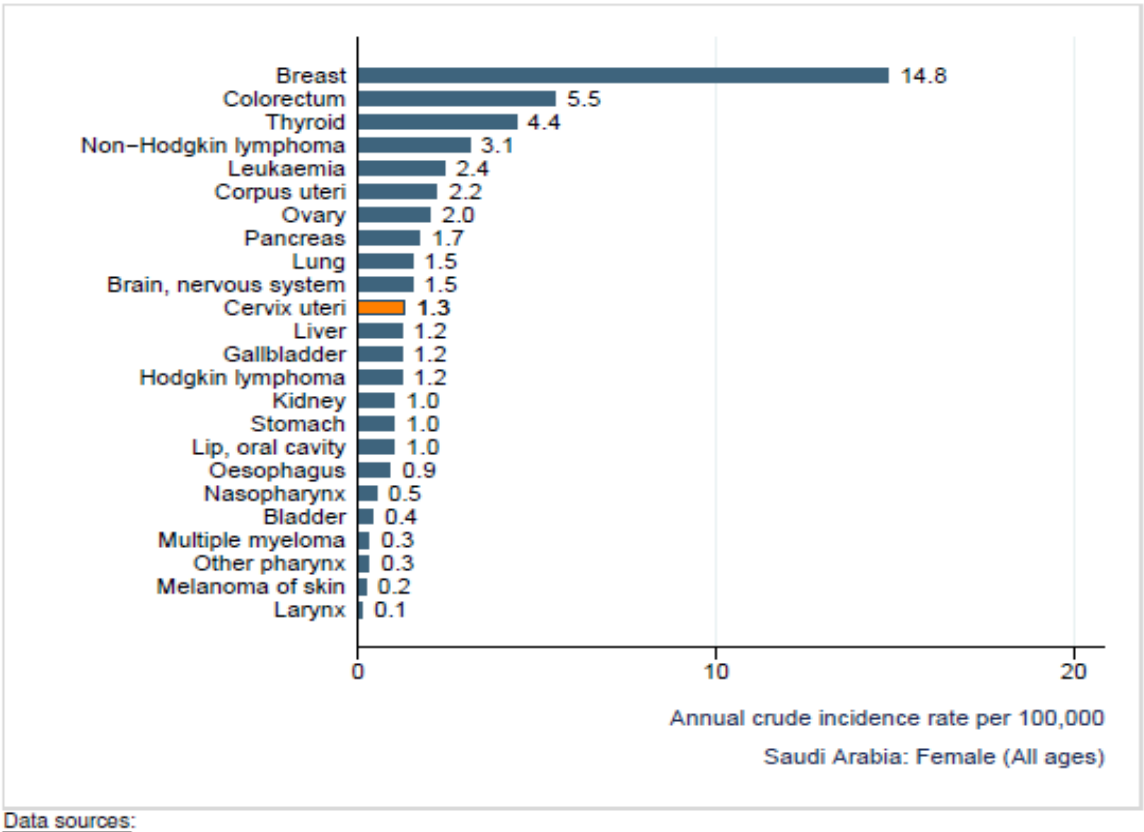
Population	
Women at risk for cervical cancer (Female population aged >=15 yrs)	6.51 millions
Burden of cervical cancer and other HPV-related cancers	
Annual number of cervical cancer cases	152
Annual number of cervical cancer deaths	55
Other factors contributing to cervical cancer	
Smoking prevalence (%), women	3.2
Total fertility rate (live births per women)	3.1
Oral contraceptive use (%)	19.6

Table 5: Incidence of cervical cancer in Saudi Arabia, Western Asia and the World

Indicator	Saudi Arabia	Western Asia[a]	World
Crude incidence rate[1]	1.3	3.6	15.8
Age-standardized incidence rate[1]	2.1	4.5	15.3
Cumulative risk (%). Ages 0-74 years[1]	0.2	0.5	1.6
Annual number of new cancer cases	152	3931	529828

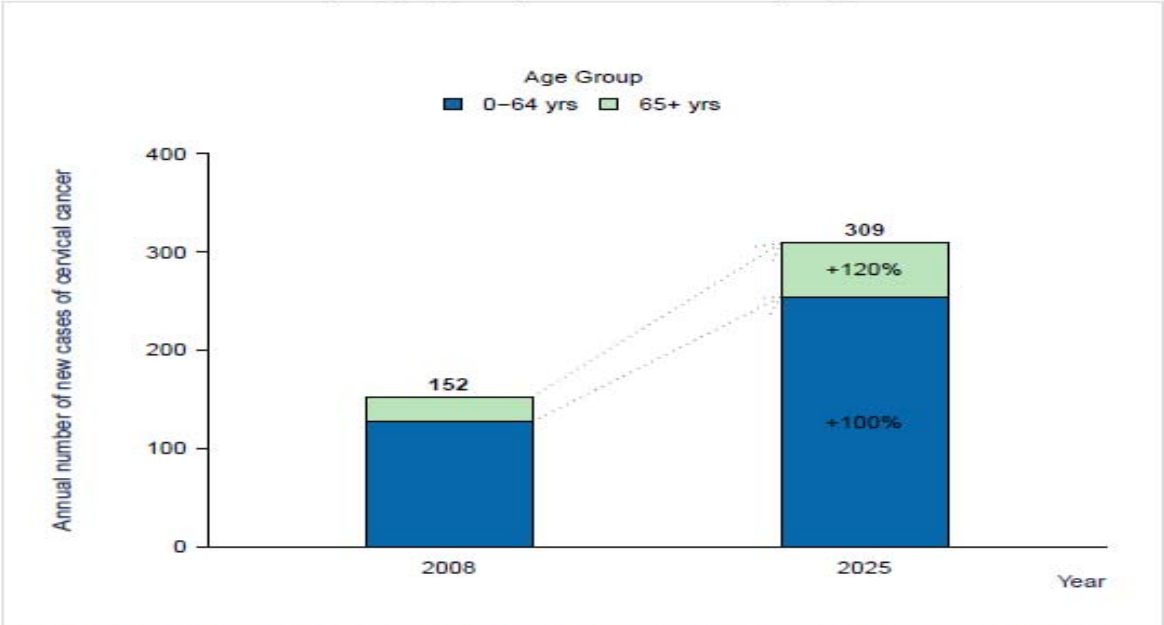
Data sources:
IARC, Globocan 2008. (Specific methodology for Saudi Arabia: National incidence rates (1999-2005, Saudi and non-Saudi populations) were projected to 2008. The rates were 'scaled' using -site, -sex and age-specific percentages of microscopically verified cases, obtained as the mean of the percentages observed in Oman, Omani (1998-2001), Kuwait (1998-2002) and Bahrain, Bahraini (1998-2002) cancer registries. For further details refer to http://globocan.iarc.fr/DataSource_and_methods.asp and http://globocan.iarc.fr/method/method.asp?country=682.)

Table 6: Incidence of cervical cancer compared to other cancers in women of all ages in Saudi Arabia



Data sources:
IARC, Globocan 2008. For specific estimation methodology refer to http://globocan.iarc.fr/DataSource_and_methods.asp.

Table7: Estimated number of new cases of cervical cancer in Saudi Arabia in 2008 and projected in 2025



Projected burden in 2025 is estimated by applying current population forecasts for the country and assuming that current incidence rates of cervical cancer are constant over time.
Data sources:
IARC, Globocan 2008.

Table8: Reasons for performance of Pap smear

Reason/Symptom	% of total (n=1475)
Vaginal discharge	37.6%
Vaginal itching	21.3%
Lower genital tract burning	9.06%
Suspected urinary infection	3.04%
Age	29%

A total of 1475 cases, ages 30-64 years, were available for the study. The total number of abnormal cervical smears was 40. i.e. 2.706% of all screened cytology cases. The median age of the patients was 47 years.

Table 9: Age, size and cytological distribution of sample size

Age range	Number of patients	Number of abnormal cytology
30-34	200	1
35-39	325	5
40-44	378	9
45-49	255	11
50-54	120	3
55-59	147	7
60-64	50	4
Total	**1,475**	**40**

All the abnormal smears were re-examined and then classified according to the Bethesda System of TBS diagnosis Table 1o below.

Table 10: The disease categories listed with their percentages compared to the total number of cervical smears performed during the period of the study (Jan. 2009 – Jan. 2011)

Disease Category	Age range	Number of Patients	Prevalence of disease (n=1475)
Benign Cellular Changes/infection Herpes	30-34	1	0.068%
Benign reactive changes Cervictis/inflammation Atrophy Radiation Repair	45-49	1	0.068%
Epithelial cell abnormalities ASCUS	40-59	4	0.27%
LSIL CIN1+HPV	35-59	15	1.02%
HSIL CIN2+CIN3+CIS	35-64	13	0.88%
Invasive squamous cell	45-50	3	0.20%
AGUS Adenocarcinoma	40-64	3	0.20%
Total		**4o**	**2.706%**

Table 11: Richart (1966)[37] Cervical cancer Conversion chart

Conversion	Time in Years (approximately)
CIN1 to CIN 3	5
CIN 2 to CIN 3	2
CIN 1 and CIN 2 to CIN 3	4

Comparing our percentage of categorized diagnoses indicates that there is a high prevalence of CIN 1 and CIN 3.

The Effect of Aerobic and Anaerobic Exercise Bouts on CD^{34+} Stem Cells and Some Physiological Parameters

Mohammed NaderShalaby[(1)], Jin Yu Liu [(2)], Hussein Heshmat[(3)],Nader M. Shalaby[(4)], Mohammed Salah Zaeid[(5)],Ahmed Ibrahim Shalgham[(4)] ,MagedElazazy[(4)], SamyAkar[(5)], Hossam Elaraby,[(5)] Mohammed AbdelrazikTaha[(4)] and WaelElfiel[(6)]

[(1)]Department of Pathobiology key lab of Ministry of Education, Norman Bethune College of Medicine, Jilin University, China and Department of Sports Science, Faculty of Physical Education, Port Said University, Egypt
[(2)] Department of Pathobiology key lab of Ministry of Education, Norman Bethune College of Medicine, Jilin University, China.
[(3)]Faculty of Medicine, Zagazig University Egypt. [(4)]Faculty of Physical Education, Suez CanalUniversity, Egypt.
[(5)]Faculty of Physical Education, PortsaidUniversity, Egypt.
[(6)]Poultry and Rabbit medicine department, college of Vet medicine, Suez Canal University, Egypt 41522 and college of animal science and Vet medicine, Jilin University, China 130062
E-mail :dr.m.nader@hotmail.com

Abstract: Aerobic exercise draws energy mainly from biochemical processes requiring oxygen, whereas anaerobic exercise draws energy from processes not requiring oxygen.20 healthy male athletes aged (18-24 yrs.) were recuited for this study. Healthy low active males and BMI matched participants (n=10) aged (20-22 yrs.) wererecuited as controls. Aerobic and anaerobic testing was performed on a cycle ergometer. The testing wasa modification of AstrandRhyming protocol forVo2max. Pulserate estimation,Rbcs,Wbcs, HB and hematocrit were estimated using coulter counter. Lactateby accusport, CD34+ stem cells were determined by flow cytometry. Results indicated:VO2 max was increased in case of aerobic exercise bout compared to anaerobic one.Lactate concentration was decreased in case of aerobic exercise bout compared to anaerobicone.Hb, Rbcs,Wbcs and hematocrit were increased after both exercise bouts.CD34+ stem cells were increased in case of anaerobic exercise bout than aerobic one. It is concluded that Vo2 max increased in case of aerobic exercise bout compared to anaerobic one due to the longer period of cycling. Lactate concentration was decreased in case of aerobic exercise bout compared to anaerobic one due to the higher intensity expressed in anaerobic bout leading to decrease oxygen .CD^{34+} HPC counts were increased in peripheral blood of anaerobic exercise bout than aerobic one due to stress induced by anaerobic exercise bout .
[Mohammed Nader Shalaby, Jin Yu Liu , Hussein Heshmat ,Nader M. Shalaby, Mohammed Salah Zaeid, Ahmed Ibrahim Shalgham, Maged Elazazy, Samy Akar, Hossam Elaraby, Mohammed Abdelrazik Taha, Wael Elfiel. **The Effect of Aerobic and Anaerobic Exercise Bouts on CD^{34+} Stem Cells and Some Physiological Parameters.** Life Sci J 2012;9(2):1037-1043] (ISSN:1097-8135). http://www.lifesciencesite.com. 154

Key words: Aerobic and anaerobic exercise bouts, CD34+stem cells, physiological parameters.

1. Introduction

Jogging is an endurance exercise; in contrast, resistance exercise involves short periods of contractile activity against high resistance. Weightlifting is a resistance exercise. Sprint exercise consists of short periods of maximal contractile activity against low resistance. A competitive 50m swim is a sprint exercise (Mougios, 2006). He also added that an alternative way of describing exercise type is by the terms aerobic and anaerobic. Aerobic exercise draws energy mainly from biochemical processes requiring oxygen, whereas anaerobic exercise draws energy from processes not requiring oxygen.

Although exercise is considered a physiological stimulus for cell release by the bone marrow (Brenner et al., 1998), surprisingly few data are available on circulating hematopoietic precursors in athletes. Erythrocyte production was studied relative to athletes anemia (Szygula, 1990) and to assess the effects of intermittent hypoxic exposure on exercise performance (Baily and Davies, 1997). Conversely, little is known of the effects of exercise on myeloid precursors. For many years, it was reported that colony forming cells in peripheral blood increased after a short and intense exercise bout in normal subjects (Harrett et al., 1978), but a detailed characterization of hematopoietic precursors in well trained subjects was never obtained. (Bonsignore et al., 2002).

The rationale to study myeloid precursors in athletes is that intense and prolonged exercise increases white blood cell (WBC) and neutrophil count (Brenner et al., 1998 and Nieman, 1997).

Stem cells are not specialized and incomplete division was no similarity of any specialized cell. But are able to form an adult cell is divided after several divisions in appropriate circumstances, and the importance of these cells comes from being unable to form any kind of specialized cells after grow and develop into cells is required.(Laufs et al,2004).

Thus, the stem cells in turn depends on the so-called «old fetal» of the body. There are stem cells that generate the ability to make anything. Then there are the

stem cells «College Ability», which can make more types of tissue, then there are adult stem cells that proliferate to create a special texture to the body, such as the liver or bone marrow or skin..Etc.. Thus, with each step toward adulthood, the successes achieved by the stem cells are narrower, which means that lead to specialization. In adulthood, does not generate liver cells, but other liver cells, skin cells, generate another. However, the sign of recent research suggests that the amount of cells can be manipulated to return back and enable it to produce various tissues, such as conversion of bone cells to produce muscle tissue. There are stem cells in two forms:Embrionicstem cells, and adult stem cells. (Rehman etal, 2004)Barrett et al, (2010).

In healthy moderately trained subjects an acute bout of moderate to hard intensity endurance exercise has been shown to increase EPC number, EPC migration and colony forming units. (Laufs et al., 2005)

The Aim of this study Is to reveal:

1- The role played by aerobic and anaerobic exercise bouts on CD^{34+}stem cellsdetermination.
2- The role of aerobic and anaerobic exercise bouts on some physiological parameters.

2. Material and Methods

Participants:

20 healthy male athletes aged (18-24 yrs.) with a training history of (4-9yrs) were recuited for this study. Athletes have to participate in low to intense exercise greater than 3 days/week. Healthy low active male and BMI matched participants (n=10) aged (20-22yrs) were recruited as controls. Control subjects could not be participating in or have a recent history of low to intense regular exercise. Participants were screened and asked to fill out healthy history and physical activity history questionnaires.

All participants were nonsmokers, non-diabetic and free of cardiovascular, lung, liver disease. Participants did not take any medications that affect EPCs number or function. These include statins, angiotensin 11 receptor antagonists, ACE inhibitors; peroxisome proliferators activated receptor (PPARα) agonist and EPO.

Testing procedures

Written informed consent was obtained for all participants and the study was approved by the University of Suez Canal institutional reviews board. All participants engaged in a preliminary screening visit to evaluate resting blood pressure and fasting blood chemistry profile, and to rule out the presence of cardiovascular disease and to assess and obtain samples of blood for analyses and BMI testing.

They were given a weight data log and instructed to weight themselves in the morning and evening and record their weight in the log. All participants refrained from caffeine and any medications or vitamins 48 hours prior to the test. Participants were instructed to record their intake of foods for the three days before test on a log supplied to them.

Aerobic and anaerobic testing was performed on a cycle ergometer with physician monitoring. Heart rate and blood pressure were monitored continuously throughout the test. The testing was a modification of Astrand Rhyming protocol, until the subject exhaustion.

Maximal oxygen consumption (VO_{2max}) is the maximal rate at which the body can consume oxygen during exercise (Davis et al., 1976). The test of maximal oxygen consumption is an example of both low and high intensity exercise (50 watt increment, 3 min stage protocol in aerobic exercise 25 watt each as for anaerobic exercise 100 watt increment, 30 second stage protocol by adding 50 watt each). The incremental exercise is used by bicycle ergometer against increasing intensities until volitional fatigue. The Astrand Rhyming nomogram for estimating Vo_{2max} to use the nomogram for cycle ergometry exercise, a line is drawn connecting the gender specific heart rate to the specific workload (kg/min). When this straight line intersects the diagonal Vo_{2max} line represents the Vo_{2max} value.

The predicted VO_{2max} value is obtained by connecting the point on the VO_{2max} scale with the corresponding point, on the pulse rate scale.

Rbcs, Wbcs, Hb and hematocrit value were estimated using coulter counter.

The human erythrocyte is the mature unit of the red blood corpuscle; it is circular, elastic non-nucleated, biconcave disc, whose primary function is the transport of hemoglobin.

Hemoglobin is a protein of 200 to 300 million nearly spherical molecules in each red blood cell, having a molecular weight of 64.458 based on the chemical structures of its alpha and beta chains.

Hematocrit (the packed cell volume) is the percentage of the total volume of whole blood that is occupied by packed red blood cell when a known volume of whole blood is centrifuged at a constant speed for a constant period of time.

White blood corpuscle (leukocyte) includes all white cells of the blood, lymphocyte, monocyte neutrophil and basophil and esonophil.(Guyton and Hall20006).

All blood cells were counted using coulter counter which is easy to read numerical presentation.

Lactate analysis was performed by using accusport before and after the test by venipuncture:

Circulating progenitor cell number:

CD^{34+} (HPc, hematopoietic progenitor cell number was determined by flow cytometry for this assay 0.5 ml of blood was collected into an EDTA-coated tube.

Mononuclear cells were separated via density centrifugation. Cells were washed and counted with a hemocytometer.

Mononuclear cell were immunostained with monoclonal anti-bodies against human CD^{34+} for each group of analyses, one set of control tubes for machine calibration was generated. Flow cytometry was performed in a special laboratory.

The forward side scatter plot was used to identify lymphocyte gate. 100.000 events per sample were acquired. Total cell count was averaged.

The following principle, clinical applications precautions and methodology in the following:

IOTest CD34-PE:

Use this fluorochrome-conjugated antibody permits the identification and numeration of cell populations expressing the CD^{34+} antigen present in human biological samples using flow cytometry.

Principle

This test is based on the ability of specific monoclonal antibodies to bind to the antigenic determinants expressed by leucocytes.

Specific staining of the leucocytes is performed by incubating the sample with the IOTest reagent. The red cells are then removed by lysis and the leucocytes, which are unaffected by this process, are analyzed by flow cytometry.

The flow cytometer measures light diffusion and the fluorescence of cells. It makes possible the delimitation of the population of interest within the electronic window defined on a histogram, which correlates the orthogonal diffusion of light (Side Scatter or SS) and the diffusion of narrow angle light (Forward Scatter or FS). Other histograms combining two of the different parameters available on the cytometer can be used as supports in the gating stage depending on the application chosen by the user.

The fluorescence of the delimited cells is analyzed in order to distinguish the positively stained events from the unstained ones. The results are expressed as a percentage of positive events in relation to all the events acquired by the gating.

Procedure:

Note: The procedure below is valid for standard applications. Sample and/or VersaLyse volumes for certain Beckman Coulter applications may be different. If such is the case, follow the instructions on the application's technical leaflet.

For each sample analyzed, in addition to the test tube, one control tube is required in which the cells are mixed in the presence of the isotopic control (Ref. A07796).

1- Add 20 µL of specific IOTest conjugated antibody to each test tube, and 20 µL of the isotypic control to each control tube.
2- Add 100 µL of the test sample to both tubes. Vortex the tubes gently.
3- Incubate for 15 to 20 minutes at room temperature (18 – 25°C), protected from light.
4- Then perform lysis of the red cells, if necessary, by following the recommendations of the lysis reagent used. As an example, if you wish to use VersaLyse (Ref. A09777), refer to the leaflet and follow preferably the procedure called "with concomitant fixation", which consists of adding 1 Ml of the "Fix-and-Lyse" mixture prepared extemporaneously. Vortex immediately for one second and incubate for 10 minutes at room temperature, protected from light. If the sample does not contain red cells, add 2 mL of PBS.
5- Centrifuge for 5 minutes at 150 x g at room temperature.
6- Remove the supernatant by aspiration.
7- Resuspend the cell pellet using 3 mL of PBS.
8- Repeat step 5.
9- Remove the supernatant by aspiration and resuspend the cell pellet using:
   - 0.5 Ml or 1 Ml of PBS plus 0.1% of formaldehyde if the preparations are to be kept for more than 2 hours and less than 24 hours. (A 0.1% formaldehyde PBS can be obtained by diluting 12.5 µL of the IOTest 3 Fixative Solution (Ref. A07800) at its 10X concentration in 1 Ml of PBS).
   - 0.5 Ml or 1 Ml of PBS without formaldehyde, if the preparations are tobe analyzed within 2 hours.

Note: In all cases, keep the preparations between 2 and 8°C and protected from light.

Height and weight were recorded and body mass index, BMI (kg/m2) was calculated for all subjects. A BMI score less than 20 is considered underweight, 20 to 24,9, is considered desirable, 25 is considered overweight, and greater than 30 is considered obese.

Statistical Analysis

Student's t tests were used to test for differences between athletes and control groups and between aerobic and anaerobic groups where data were found to not meet the assumption of normality, the non-parametric Mann Whitney utest (Wilcoxon rank sum test) was used to compare difference between groups. In these cases, for descriptive data the median (Lowest value-highest value) are displayed. Difference between groups were testing using a measure of analysis of variance (ANOVA). For parameters with non-normal distributions non parametric Spearman correlation coefficients were used. F test was used to test 3 groups. An α level of 0.05 was used to indicate statistical significance.

3. Results

Subjects characteristics:

20athletes and 10 low active control males participated in the study. Groups were matched for age,

weight and height (table 1). Also for BMI non-significant changes in basic characteristics, to compare athletes and control males. Pulse rate and VO_{2max} showed significant changes table (1), as expected athletes had a lower pulse rate compared to control. Physical activity questionnaire data revealed that athletes exercised an average of 5±0.5 days a week for 5±0.2 years.

Control group participants were not engaging in regular exercise, nor did they have a recent history of physical activity.Data for CD^{34+} number. There were significant difference between athletes after anaerobic exercise bout compared to aerobic and control indicated in Table (2).Data for CD^{34+} number. There were significant difference between athletes after aerobic and anaerobic exercise bout as indicated in Table (3).Lactate Revealed a significant increase after anaerobic bout values are means +SE $P<0.05$.) Revealed NS change in case of participants of control and athletes groups at rest in hematological values in Table (4). Revealed a significant change in participants after aerobic and anaerobic bout of exercise in hematological values $P< 0.05$ in Table (5).VO_{2max} (mL/kg/min) results indicated an increased value between the healthy sedemtary participants and after aerobic exercise bout and anaerobic one in Table (6).

4. Discussion:

The data presented indicated that lactate concentration in Table (1) was in the normal range with non-significant changes in both groups (control and athletes).

After aerobic exercise bout and anaerobic one, the concentration of lactate showed a higher value in case of anaerobic exercise compared to aerobic bout Table (3).The increased lactate may be due to higher intensity expressed in anaerobic bout leading to decrease oxygen.

In case of intense exercise, which can be defined as any intensity that exceeds an individual's capacity to maintain a steady state condition, ATP regeneration must be met by creatine phosphate hydrolysis and by glycolysis terminating in the production of lactate and the eventual development of acidosis. Intense exercise can be performed in many ways, such as the intense exercise of sprint, swimming, cycling, or in incremental exercise (Robergs and Roberts, 1997), lactate and protons leave the muscle fiber by a similar mechanism of incremental exercise. Roth and Brooks (1989) have presented the kinetics of a lactate transporter and have shown that it is a saturated transport process. It is believed that protons leave the muscle in combination with the lactate transporter (MCTs) via facilitated transport (Stanley et al., 1985) which accounts for similar changes in blood lactate and acidosis during intense exercise.

During prolonged exercise, muscles and blood lactate concentration peak a few minutes after the start of exercise of moderate to low intensity and drop slightly as exercise continues. After the end of exercise both return to baseline gradually (Fitts, 2004).

Vo_{2max}. values range from those of persons extremely low capacities, such as chronically ill individuals (< 20 ml/ kg/min) to those of well-trained and elite endurance athletes (> 80ml/ Kg/ min) (Robergs and Roberts, 1997). They also added that the factors that combine to influence Vo_{2max} are a high proportion of slow twitch motor units, high central and peripheral cardiovascular capacities, and the quality and duration of training. Having more slow twitch muscle fibers increases the oxidative capacity of the muscle (Jacobs, 1983*)*.He stated that muscle motor unit proportions are genetically determined and therefore a person's abilities to respond to endurance training and increase to Vo_{2max} have important genetic constraints. This opinion is in accordance with the results in table (6), as aerobic exercise bout of participants a higher Vo_{2max} than control and anaerobic participants. As an increased mitochondrial volume would also provide skeletal muscle with the ability to increase maximal oxygen consumption. However, cardiovascular adaptation are also involved in increasing Vo_{2max} after training, and muscle adaptations should not be viewed as the role determinant of Vo_{2max}. As different training strategies influence the values of Vo_{2max}., and it appears that the type and quality of training are also important. The extent of improvement in Vo_{2max} depends on the value of Vo_{2max} before training. (Robergs and Roberts 1997).

The hemoconcentration may be the main cause of the increase blood parameters of Rbcs, Wbcs, Hb and Hematocrit (Table 4,5) after the aerobic and anaerobic exercise bout, and the increased blood parameters could be caused by the stress induced by physical activities (Montain and Coyle 1992).

The results in Table (2,3) indicated that CD^{34+} increased after exercise bouts. The increased haematopoietic stem cells CD^{34+} revealed a positive results specially the anaerobic bout who were subjected to stress more than the athletes subjected to aerobic bout.

Previous studies have shown that an acute bout of exercise increases the number of bone marrow derived endothelial cells in the blood (Shaffer et al., 2006 and Vancraenenbroeck et al., 2008 and Amany and Mohamed, 2011).

This is consistent with our data, as aerobic and anaerobic bout of exercise revealed an increase in CD^{34+}(SC) as shown in table (3), anaerobic bout of exercise was more prominent in increasing CD^{34+} (SC).

Table (1): Basic characteristics

Variable	Athletes n=20			Control n=10			Sig.
Age (yr)	21.6	±	1.83	20.6	±	0.89	NS
Height (cm)	179	±	2.78	178.8	±	1.92	NS
Weight (kg)	75	±	3.16	74	±	1.5	NS
BMI	22	±	1.4	23	±	2.2	NS
Pulse rate (count/m)	68	±	2.3	74	±	2.1	S
VO_{2max}(ml/kg)	52	±	1.8	36	±	1.7	S
Lactate (mmol/L)	1.1	±	0.02	1.2	±	0.03	NS

Values are means ±SE P<0.05
BMI = body mass index

Table (2): CD^{34+} in case of aerobic and anaerobic exercise bout and control

	CD^{34+}						ANOVA	
	Range			Mean	±	SD	F	P-value
GI (anaerobicexercice bout) n=10	227	-	366	284.5	±	51.5		
GII (aerobicexercice bout) n=10	144	-	216	173	±	22.7	26.85	0.001
Control n=10	140	-	210	172	±	24.1		

Tukey's test

GI (anaerobic) VS GII (aerobic)	GI (anaerobic) VS control	GII (aerobic) VS control
0.001	0.001	0.999

Table (2) There were significant change in CD^{34+} for the favor of anaerobic exercise bout.

Hematopoietic stem cells:

Data for CD^{34+} number. There were significant difference between athletes after anaerobic exercise bout compared to aerobic and control indicated in table (2).

Table (3): Revealed data of CD^{34+} (SC) and lactate after exercise bout aerobic and anaerobic

Variable	Aerobic exercise bout			Anaerobic exercise bout		
CD^{34+} (HPc) cells	173	±	22.7	284.5	±	51.5
Lactate (mmol/L)	3.2	±	0.4	5.6	±	0.8

Table (3) Data for CD^{34+} number. There were significant difference between athletes after aerobic and anaerobic exercise bout as indicated in CD^{34+} for the favor of anaerobic exercise bout also Lactate revealed a significant increase after anaerobic bout values are means +SE P<0.05.

Table (4):Haematological values of RBcs, Wbcs, hematorit (PCV) and hemoglobin in control and athletes at rest.

Variable	Control			Athletes			Sig
Rbcs (million/mm3)	4.9	±	0.9	4.1	±	0.6	NS
Wbcs (thousands/ mm3)	4.8	±	0.7	5.9	±	0.8	NS
Hb (g/dL)	12.8	±	0.8	14.2	±	0.9	NS
Hematocrit (%)	42	±	3.2	42.1	±	2.7	NS

Table (4) Revealed NS change in case of participants of control and athletes groups at rest in hematological values. P< 0.05 mean ± SE.

Table (5):Hematological values of Rbcs, Wbcs, Hb, and Hematocrit (PCV) in aerobic and anaerobic exercise bout.

Variable	Aerobic			Anaerobic			Sig
Rbcs (million/mm3)	4.8	±	0.3	5.1	±	0.2	S
Wbcs (thousands/ mm3)	5.9	±	0.5	6.5	±	0.4	S
Hb (g/dL)	13.8	±	0.9	15..2	±	0.8	S
Hematocrit (%)	43	±	1.2	45	±	1.1	S

Table (5): Revealed a significant change in participants after aerobic and anaerobic bout of exercise in hematological values P< 0.05

Table (6): The variation in VO_{2max} for participants healthy sedentary, aerobic and anaerobic exercise bout.

Participants	VO_{2max} (mL/kg/min)		
Healthy sedentary (mL/kg/m)	36	±	1.7
Aerobic exercice (mL/kg/m)	57	±	2.4
Anaerobic exercice (mL/kg/m)	54	±	2.5

The results are expressed as mean ± SE ($P<0.05$).

Table (6) VO_{2max} (mL/kg/min) results indicated an increased value between the healthy sedentary participants and after aerobic exercise bout and anaerobic one.

The number of circulating EPSs likely represents the balance between liberation of EPCs from the bone marrow and incorporation at the level of the vessel or differentiation. Laufs et al.,(2005) demonstrated that CD^{34+}/KDr^{+} increase after 30 minutes of high intensity running in healthy participants, but returned to resting levels by 24 hours following exercise. It can be speculated that in healthy regularly exercising individuals, by 24 hours following exercise.

Also, it was reported that human subjects undergoing exhaustive dynamic exercise revealed an increased EPC counts in the peripheral blood (Laufs et al., 2005 and Rehman et al., 2004).

Giuseffe et al., (2005) reported an increased CD^{34+}stem cells and reticulocytes after supramaximal exercise, they added that this increase was unlikely to depend on changes in blood or plasma volume, since these were much smaller than changes in cell counts. either been incorporated for endothelial repair, neovascularization or have undergone differentiation.

Ewa and Pawet, (2007) reported that a decrease in the blood supply to a bodily organ or tissue, caused by constrictor or obstruction of the blood vessels, is a common cause of ischemia. This process is probably responsible for the use of EPCs in postnatal vascular growth and remodeling. In the study performed by Adams et al., (2004), patients with stable CAD were subjected to the single-exercise stress test to compare peripheral blood EPC counts before and after the experiment. It was found that the peripheral blood EPC count was increased significantly in ischemic patients within 24-48 hours after exercise. They observed that an increase in EPC levels was accompanied by an elevation of VEGF concentration in plasma in these patients. These results confirmed that VEGF is a significant factor responsible for EPC mobilization from bone marrow to peripheral blood (Adams et al., 2004).

Conclusion

It may be concluded that:

- Vo2 max increased in case of aerobic exercise bout compared to anaerobic one due to the longer period of cycling.
- Lactate concentration was decreased in case of aerobic exercise bout compared to anaerobic one due to the higher intensity expressed in anaerobic bout leading to decrease oxygen .
- Hb, Rbcs, Wbcs and hematocrit value were increased after aerobic and anaerobic exercise bout.
- CD^{34+} HPC counts were increased in peripheral blood of anaerobic exercise bout than aerobic one due to stress induced by anaerobic exercise bout.

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Corresponding author

1-Mohammed Nader Mohammed Shalaby

Department of Pathobiology key lab of Ministry of Education, Norman Bethune College of Medicine, Jilin University, China and Department of Sports Science, Faculty of Physical Education, Port Said University, Egypt

Phone number: +201000400900 . +8615044059524

E-mail :dr.m.nader@hotmail.com

2-Prof.Dr.Jin Yu Liu

Department of Pathobiology key lab of Ministry of Education, Norman Bethune College of Medicine, Jilin University, China

Phone number: +8613843056822

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Performance Obstacles Experiences Among Critical Care Nurses in Damanhur Teaching Hospital

Lamiaa Ismail Keshk[1]; Shereen Ahmed Qalawa[2] and Azza Anwar Aly[3]

[1]Department of nursing administration, Faculty of Nursing, Helwan University
[2]Department of Medical-Surgical Nursing, Faculty of Nursing, Port said University
[3]Department of Medical-Surgical Nursing, Faculty of Nursing, Damanhour University
keshk_lamiaa@yahoo.com

Abstract The work environment of intensive care nurses may have substantial impact on both nursing outcomes and patient safety. Performance obstacles are the factors that hinder intensive care nurses' capacity to perform their jobs and that are associated closely with their immediate work environment**. Aim:** To identify the performance obstacles experienced by critical care nurses in their work environment that covers all elements of the work system model. **Subject and methods:** An exploratory, descriptive design was utilized. The sample included all available nurses (n=60). Data was collected by using questionnaire performance obstacles. It was conducted in Damanhur teaching hospital in Damanhur city in 2 critical care units. **Results:** indicated that nurses experience in critical care units a wide variety of performance obstacles that cover all elements of the work system model. **Conclusion:** Performance obstacles represent the following elements of the work system: environment (6 obstacles), organization (7 obstacles), technologies or tools (4 obstacles), and task (4 obstacles).

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Keywords: Critical care, intensive care, nurses, performance obstacles, work environment

1. Introduction

A critical care unit is a dynamic and highly technological environment. Professional nurses who have been working in the critical care unit for a period of time are passionate about the environment in which they work. They find their on duty time challenging and stimulating. The critical care environment is slowly changing due to the fact that there are fewer professional nurses with an additional qualification in critical care available to work in the critical care units **(Dunsdon, 2011).** The work environment of intensive care nurses may have substantial impact on both nursing outcomes and patient safety. Performance obstacles are the factors that hinder intensive care nurses' capacity to perform their jobs and that are associated closely with their immediate work environment**. (Gurses and Carayon, 2007)**

Intensive care nurses play a key role in patients' recovery. They must respond continuously and quickly to the needs of patients and families, carry out procedures accurately, and interact with the most intense emotional aspects of life. They work in demanding and stressful work environments to help patients in critical conditions. In addition, patient safety and quality of care are major problems in intensive care units. Characteristics of the ICU work environment can create obstacles for nurses in performing patient care tasks, therefore threatening the quality and safety of care provided by nurses. Factors such as interruptions, overwork, fatigue, illegible physician writing, lack of information about the patient, and problems with equipment can increase the likelihood of medication administration errors by nurses **(Institute of Medicine, 2004).**

Performance obstacles have significant impact on nursing workload, perceived quality and safety of care, and quality of working life(QWL). Workload mediates the effects of performance obstacles on perceived quality and safety of care and QWL. Factors that affect QWL of ICU nurses include high workload; task complexity; high patient mortality and morbidity; unnecessary prolongation of life; emergencies, admissions, and transfers; communication problems with coworkers; high noise level; low autonomy; insufficient or malfunctioning equipment; and frequent use of sophisticated technology. Studies have found that inadequate staffing, admissions and transfers, and a high number of severely sick patients can increase ICU nursing workload. (Re)designing ICU work systems by reducing performance obstacles may be an effective strategy for reducing workload and improving quality and safety of care as well as QWL among nurses, thereby complementing the efforts on optimizing the nurse/patient ratio. **(Gurses *et al.*, 2009)**

Performance obstacles concept can also be used to identify problems in other health care settings, for other types of care providers and patients. In addition to , Performance errors were classified as skill based errors (failure to carry out intended plans of action, including unintended acts and lapses or omitted acts), rule-based mistakes (such as using an incorrect treatment protocol), and knowledge-based mistakes. **(Gurses and Carayon, 2007)**

The heavy workload of hospital nurses is a major problem for health care system. Nurses are experiencing higher workloads than ever before due to four main reasons: increased demand for nurses, inadequate supply of nurses, reduced staffing , overtime, and reduction in patient length of stay**.(Lang *et al.,* 2004).** Furthermore, performance obstacle and workload are negatively affects nursing job satisfaction and, as a result, contributes to high turnover and the nursing shortage. In addition to the higher patient acuity, work system factors and expectations also contribute to the nurses' workload: nurses are expected to perform nonprofessional tasks such as delivering and retrieving food trays; housekeeping duties; transporting patients; and ordering, coordinating, or performing ancillary services**.** The five elements of the work system are task; organizational factors; environment; equipment and technology; and individual. Performance obstacles can arise from any element of the work system or from interactions between the elements of the work system. **(Gurses and Carayon, 2007)**

Aim:

This study aim to identify the performance obstacles experienced by critical care nurses in their work environment that covers all elements of the work system model.

2. Subjects and Methods

Research design

An exploratory, descriptive design was utilized to accomplish the study.

Sample:

The sample included all available nurses (n=60)were assigned to work in the critical care units in Damanhur teaching hospital in Damanhur city, data were collected between July to September (2011). The nurses are working in the following critical care units:

Intensive care unit

Cardiac care unit

Setting

The study was conducted in Damanhur teaching hospital in Damanhur city in the critical care units that mentioned above.

Tools for Data Collection:

Data was collected by using A questionnaire was developed, validated from Gurses and Carayon (2007)

Tool I- Socio demographic data:

It was contain information related to demographic characteristics of the studied nurses as their sex, age, social status, and educational degree, total experience in nursing field, hospital work and intensive care units.

Tool II: critical care units background variables

It was contain number of hours worked in critical care units, preferable shift for work, total numbers of patients responsible for, number of patients admitted to critical care units, number of isolated patients, number of daily discharge patient, number of assistant nurses; clerk, and work environment.

Tool III: Performance Obstacles assessment Questionnaire:

The questionnaire included 37questions about the performance obstacles experienced by critical care nurses during a particular shift. Twenty-four items had a nominal scale (yes or no) and 13 items had a semantic differential response format with a 5-point rating scale and bipolar adjective pairs such as organized-disorganized and noisy-quiet. Combinations of positively and negatively worded items were used in the questionnaire. For example, for the item ''I spent much time searching for patients' charts,'' the response category of yes indicated that the nurse experienced that obstacle, whereas for the item ''the isolation rooms that I worked in were well-stocked,'' the response category of no meant that the nurse experienced an obstacle. Questions on demographic and were also included in the questionnaire.

Methods of Data collection:

Ethical Consideration:

Human rights and ethical permission were obtained to conduct the study. An official permission was obtained from Damanhur faculty of nursing dean and then the official permission was obtained from Damanhur teaching hospital director. Nurses were fully informed of the study. The voluntary nature of participation was stressed as well as confidentiality. Consent was obtained from each nurse.

Pilot Study:

A pilot study of the questionnaire was conducted to: (a) estimate the time necessary for nurses to fill out the entire questionnaire; (b) ensure that all the important obstacles and facilitators were covered in the questionnaire, all the questions were relevant, and nothing was missing (content validity); (c) test the clarity of the questions (whether any question was unclear or ambiguous); (d) identify the most appropriate response categories for specific questions; and (e) Test whether there was any question that might frustrate nurses.

Data collection procedures:

The interview sheet was filled out individualized with the nurse in the intensive care unit and cardiac care unit. Data was collected from the selecting settings by the researchers using the pre constructed tools. 1) Each nurse was individually filling

questionnaire ; the questionnaire was collected from 3 shifts by all the nurses while they are on duty, purpose of the study was explained prior to get the questionnaire sheet, and it distributed to be answered within (30 -45 minutes) then collected. 2) The questionnaire was filling from 1-2 nurses per day started from July to September 2011, over a period of 3 months starting according to nurses' schedule for attendance to the hospital and availability of time for both nurses and their units.

Data Analysis Plan:

Descriptive statistics were used to summarize demographic characteristics of the critical care nurses to give an overview results for the instruments. Data were revised, coded, analyzed and tabulated using the number and percentage distribution and carried out using SPSS version 16. The statistical tests used are chi square test. A value of $p<0.05$ was considered to be statistically significant.

Scoring system:

The performance obstacle sheet is contains 3 main parts the first one concerned with 24 questions ranged from yes, No, I cannot rule and positive it's scores ranged from 1 to yes answer, 2 for No and zero for I can't rule after organization were done for all statements and negative it's scores ranged from 2 to yes answer, 1 for No and zero for I can't rule. While the second part is contains 4 main parts contains questions regarding assistant nurse if available. The possible choice for each item was; Very disciplined, Disciplined, Disciplined fairly, Undisciplined and Late each one scores for each questions ranged from 4 to zero respectively. The final part in satisfaction assessment tool was questions regarding place of work especially in the morning shift. It contains 4 questions and it's scores for first questions ranged from zero to 4 for very noisy , noisy , noisy some times , Somewhat quiet, very quiet respectively , second questions scores was ranged from zero to 4 for very crowded , crowded , crowded sometimes, wide respectively ,third questions ranged from zero to 4 for very difficult Critical , Sometimes difficult , Somewhat difficult and Calm . The last question was ranged from zero to 4 for Very confused, muddled, confused at times, somewhat confusingly, the organized respectively.

Limitation of study

One limitation of the study was the use of a single data collection method (a self-administered questionnaire) which may have biased the results. Nurses who filled out the questionnaire during busy shifts (high patient acuity and load) may have no time to mention their obstacles in details. The questionnaire was designed to ask about performance obstacles as objectively as possible, therefore trying to minimize biases due to individual or emotional variables.

3. Results

Table (1) describes that the half of the nurses were ranged age (26-34) years. The majority (98.3%) of nurses were married and females. Also, it reflect (68.3%) of them had nursing diploma. As regard nursing experience (88.3%) of nurses had an experience more than 6 years.

Table (2): Shows that (33.3%) of the nurses had ICU experience ranged between (4-6) years. The majority of the studied nurses were working 8 hours daily in ICU. As regard favorable work time (61.7%) of the nurses choose the morning shift in ICU. Also, it reflect that (65%) of nurses were responsible to give nursing care for more than 5 patients in ICU. While, (48.3%) of them had only one assistant nurse to help them in nursing care.

Figure (1): shows that (45%) of the nurses selected the number of patients' admissions by each nurse over the shift in ICU was more than 5 patients.

Figure (2): illustrates that (48.3%) of the nurses' experience with (2-4) patients daily transfer out of the ICU

Figure (3): illustrates that (75%) of the nurses' experience with (5) patients daily were isolated in the ICU

Table (3): demonstrates the performance obstacles related to environment in work system that included insufficient work place for completing paper work (40%), patients' room full with visitors(36.7%),receiving many phone calls from patients (33.4%), distractions from family members (31.7%) receiving many phones calls from family members(25%)

Table (4) shows the performance obstacles related to organization in work system that included change of shift report(s) took longer than they should ; inadequate information given to me by the previous shift's nurse(s) during the shift change (25%); getting adequate information from physicians about my patient(s) (21.7%) and delay in seeing new medical orders for my patient(s) (20%).

Table (5) demonstrates the performance obstacles related to Technology or Tools in work system that included the isolation rooms were not well stocked (60%), the central stock area was not well-stocked (25%), having to use equipment that was in poor condition (21.7%). waiting to use a piece of equipment because someone else was using it. (20%)

Table (6) illustrates the performance obstacles related to Tasks in work system that included Responsible for orienting a nurse (100%), Spending a considerable amount of time teaching my patient(s) or family members(66.6%), Spending time dealing with

family needs(50%), Accompanying a patient during intra-hospital transport today(33.4%).

Table (7) demonstrates performance obstacles related to organization and work environment that included Help from Nursing Assistants, Help from Other Nurses, Help from Unit clerk, work environment (56.7%, 76.7%, 55%, and 28.3%) respectively with statistically significant difference ($p \leq 0.05$).

Table (1): Number and percent distribution of the nurses according to socio demographic characteristics

Items	No	Percent
Age		
Less than 25	5	8.3
26-34	30	50.0
35-44	21	35.0
45-54	3	5.0
More than 55	1	1.7
Total	60	100.0
Sex		
Male	1	1.7
Female	59	98.3
Total	60	100.0
Social status		
Married	59	98.3
Single	1	1.7
Total	60	100.0
Education		
Diploma	41	68.3
Associate nurse	3	5.0
Bachelor	16	26.7
Total	60	100.0
Nursing experience		
Less than year	1	1.7
1-5	2	3.3
4-6	4	6.7
More than 6	53	88.3
Total	60	100.0

Table (2): Number and percent distribution of the nurses regarding critical care units background variables

Items	No	percent
ICU experience		
Less than year	10	16.7
1-3	13	21.7
4-6	20	33.3
More than 6	17	28.3
Total	60	100.0
Daily Work hours in ICU		
4hours	3	5.0
8hours	57	95.0
Total	60	100.0
Favorable work time		
Morning shift	37	61.7
Afternoon shift	14	23.3
Night shift	3	5.0
I do not like work time	6	10.0
Total	60	100.0
Daily patients numbers who give them nursing care		
One patient	4	6.7
Two patient	4	6.7
2-4 patients	13	21.7
More than 5 patients	39	65.0
Total	60	100.0
Numbers Assistant nurses		
1	29	48.3
2	12	20.0
3	4	6.7
4	5	8.3
5	10	16.7
Total	60	100.0

Table (3): Number and percent of critical care nurses' experience about performance obstacles according elements of the work system model regarding Environment obstacles

Performance Obstacles related Environment*	No	%
Insufficient place to sit down and do my paperwork in the unit	24	40
Pts' room full with visitors	22	36.7
Patients' rooms were organized.	11	18.3
Receiving many phone calls from family members.	15	25
Distractions from family members.	19	31.7
Receiving many phone calls from pts.	20	33.4

*Carayon&Smith,2000

Fig (1): Pie charts of the nurses' opinion regarding daily numbers of Patients Admission in critical care units

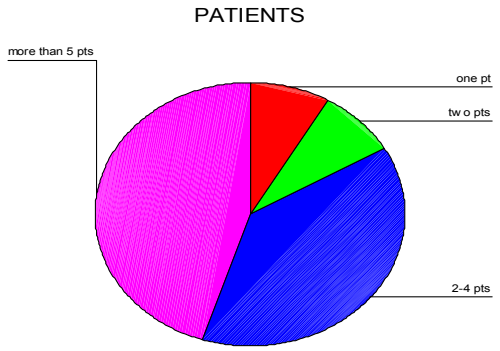


Figure (2): Pie charts of the nurses' opinion regarding numbers of transferred Patients from critical care units

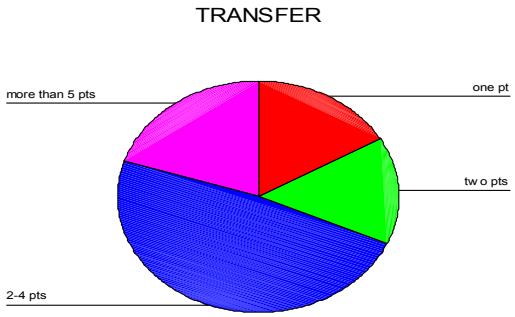


Figure (3): Bar charts of the nurses' opinion regarding numbers of isolated patients incritical care units

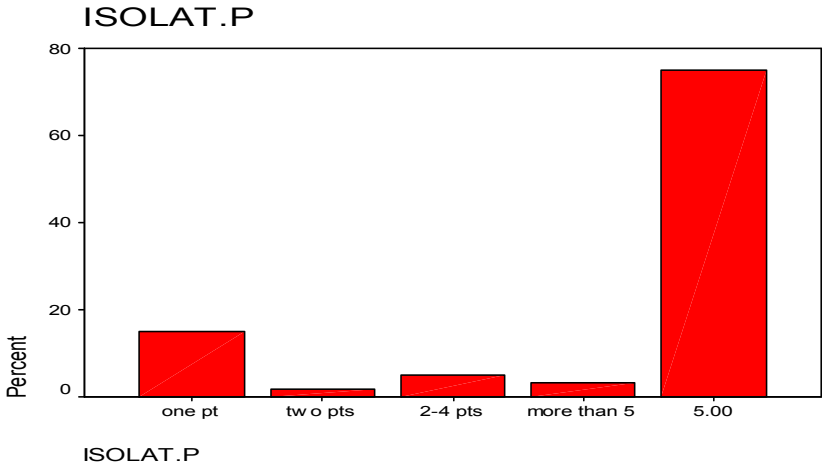


Table (4): Number and percent of critical care nurses' experience about performance obstacles according elements of the work system model regarding Organization obstacles

Performance Obstacles Organization*	No	%
Delay in getting medications for my patient(s) from pharmacy.	11	18.3
Getting adequate information from physicians about my patient(s).	13	21.7
Change of shift report(s) took longer than they should.	15	25
Delay in seeing new medical orders for my patient(s).	12	20
Spending time searching for my patients' charts.	6	10
Inadequate information given to me by the previous shift's nurse(s) during the shift change	15	25
Unnecessarily information given to me by the previous shift's nurse(s) during the shift change report	6	10

*Carayon&Smith,2000

Table (5): Number and percent of critical care nurses' experience about performance obstacles according elements of the work system model regarding Technology or tools obstacles

Performance Technology or Tools Obstacles*	No	%
Having to use equipment that was in poor condition.	13	21.7
Spending time looking for equipment because it was not located where it was supposed to be.	10	16.7
Waiting to use a piece of equipment because someone else was using it.	12	20
Spending time seeking for supplies in the central stock area.	6	10
The central stock area was well-stocked.	15	25
The isolation rooms that I worked in were well-stocked.	36	60
The patient rooms that I worked in were well-stocked.	6	10

*Carayon&Smith,2000

Table (6): Number and percent of critical care nurses' experience about performance obstacles according elements of the work system model regarding Tasks obstacles

Performance Tasks obstacles*	No	%
Responsible for orienting a nurse.	60	100
Spending time dealing with family needs	30	50
Spending a considerable amount of time teaching my patient(s) or family members.	40	66.6
Accompanying a patient during intra-hospital transport today.	20	33.4

*Carayon&Smith, 2000

Table (7): Number and percent of critical care nurses' experience about organization and Environment performance obstacles related to assistance nurses, secretary and work environment

Items	No Obstacles		Obstacles		x^2
	No	%	No	%	
Help from Nursing Assistants (Organization)	26	43.3	34	56.7	.000*
Help from Other Nurses(Organization)	14	23.3	46	76.7	.000*
Help from Unit clerk(Organization)	27	45.0	33	55.0	.000*
work environment (Environment)	43	71.7	17	28.3	.000*

*Carayon&Smith,2000

4. Discussion

The aim of this study was to identify the performance obstacles experienced by critical care nurses in their work environment that cover all elements of the work system model. The work system model **(Carayon & Smith, 2000)** provides a macro ergonomic conceptual framework to identify performance obstacles in ICU work environments. The five elements of the work system are task; organizational factors; environment; equipment and technology; and individual**.** Performance obstacles can arise from any element of the work system or from interactions between the elements of the work system.

This study revealed some important findings about impact of various performance obstacles on nursing workload, nursing quality of working life, and quality and safety of care, as well as the impact of interventions aimed at redesigning the work system of ICU nurses to remove performance obstacles. The study revealed that half of the studied sample age ranged between (26–34) years, (Table1). This result was agreed by **Kotzer *et al.,* (2006)** stated that respondents primarily ranged in age from 20 to 35 years, **Abd El-Latif (2004),** who found that more than two fifth of nurses aged 25 years, while **Kim *et al.,* (2008),** found that most of nurses ages ranged between 35 up to 53years.

Regarding nurses qualification and marital status, the current study found that 68.3% of nurses had nursing diploma and (98.3%) were married and female (Table 1). These results were agreed with **Todd *et al.,* (2007).** In relation to working condition, the results revealed that (88.3%) of nurses had nursing experience more than 6 years. While ICU experience (33.3%) of the nurses were ranged between (4-6) years. These results agreed with **Kotzer *et al.,* (2006),** they stated that nurses worked on their unit less than 6 years. **Clawson & Haskins (2006),** commented that there is evidence that the more experienced expert nurse is able to grasp the intricacies of clinical situation rapidly and can sort out relevant from irrelevant information.

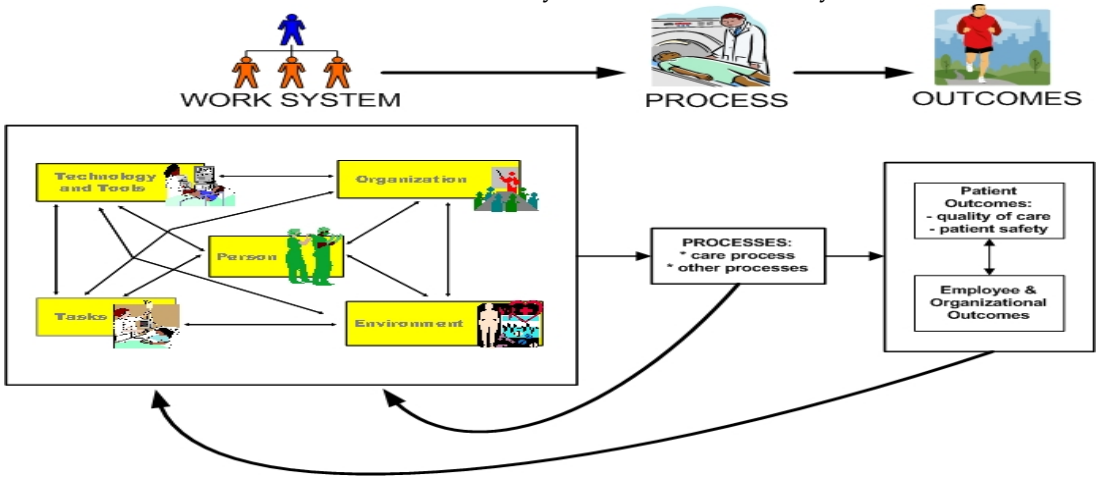
The study showed that the majority of the nurses were working 8 hours in ICU. This result agreed with **Bellebaum (2008)** who stated that 38% of the nurses recalled making at least one error during the two month study period. When nurses worked greater than 12.5 consecutive hours, the risk of that nurse making an error almost doubled compared to nurses who worked 8.5 consecutive hours or less. Among healthcare professionals, fatigue has been found to increase over the duration of a shift, regardless of the length of it. In nurses specifically, an extended workday of nine hours led to greater fatigue and a greater number of health complaints in nurses compared to an 8-hour shift. Nurses also reported that the quality of their work

suffered with the introduction of 9-hours shifts to their workday **(Josten *et al.,* 2003).** As regard favorable work time (61.7%) of the nurses choose the morning shift in ICU. This result congruent with **Costa *et al.,* (2004); Costa *et al.,* (2005)**, they found that shift workers to report lower work ability compared to day workers, with increased discrepancies occurring with age. **Camerino *et al.,* (2008)** that described the nursing staff may suffer from a lack of voluntary choice of the type of shift scheme they can work, but it may be also that they are not offered adequate incentives for their night duties. **Dorrian *et al.,* (2006)** added that the nurses on night shifts have reported high levels of stress, physical exhaustion, and mental exhaustion. Fatigue has deleterious effects on all types of performance. **Ellis (2008)** added that shift work can result fatigue has negative effects on alertness, vigilance, concentration, judgment, mood, and performance. Additionally, **Rogers *et al.,* (2004)** added that scheduled shifts for nurses studied were designated as 8, 12, and sometimes 16 hours long. Although, in their study they referred to these as 8.5, 12.5, and 16.5 hours shifts because each required a "hand-over" time. **JNA (2010)** illustrated that working hours that reach a dangerous level does not only mean working in rotating shifts but also working more than 60 hours overtime. Such a workload level was pointed out by the court as harsh working conditions leading to karoshi (death from overwork). The introduction of flexible working styles aims to allow nursing professionals to combine work with life and continue their career. Examples: One can choose the working hours. ;One can choose the time zone. ; One can choose whether to work in rotational shifts or not. So, **Admi *et al.,*(2008)** recommended that design of the shift work system, such as length of shift (8-12 hours); principles of rotation (day, night, evening); scheduling (clockwise, number of shifts); and adjustment to individual needs ("morning people" vs. "night people")

The study reflect that (65%) of the nurses were responsible to give nursing care for more than 5 patients in ICU. While, (48.3%) of them had only one assistant nurse to help them in nursing care. **Aiken (2003)** reported that three major sources of job dissatisfaction that intuitively have an adverse affect on perceived work pressure: increased patient assignments, too few registered nurses for quality care, and inadequate support services. Further investigation into these factors may lead to interventions that offer an improvement in this dimension of the work environment. Also, the study revealed that (45%) of the nurses' experience with numbers of patients' admissions daily in ICU was more than 5 patients. (48.3%) of them experience with numbers daily patients transfer out of the ICU was (2-4)pts. These results agreed by **Gurses and Carayon (2007**) showed that intra hospital transport for ICU patients need to be transferred to other units of the hospital for tests and procedures and are at high risk en route. Furthermore, accompanying a patient for intra hospital transportation increases the workload of nurses considerably and takes them away from other patients staying in the ICU.

The results of this study indicated that critical care nurses experience in a wide variety of performance obstacles that *cover all elements of the work system model*. It Focusing on performance obstacles represent the following elements of the work system: environment (6 obstacles), organization (7 obstacles), technologies or tools (4 obstacles), and task (4 obstacles). These results congruent by **Gurses and Carayon (2007**).

SEIPS Model of Work System and Patient Safety



Carayon et al., 2006

Family-Related Issues as Performance Obstacles

Several family-related issues were identified as performance obstacles by critical care nurses. Depending the family-related issues were either categorized in the task element of the work system (e.g., spending time dealing with family needs or teaching family) or in the environment element (e.g., distractions from family members and many phone calls from family).The results illustrated that nurses were receiving many phone calls from patients (33.4%), distractions from family members (31.7%) receiving many phones calls from family members(25%),Work environment, (28.3%) Spending a considerable amount of time teaching my patient(s) or family members(66.6%), Spending time dealing with family needs(50%) (Environment).These results agreed with **Gurses and Carayon (2007**) reported that noisy work environment (46%), distractions from families (42%), receiving many phone calls from families (23%). It has been reported previously that nurses may view getting involved in some of the family-related issues as problems or barriers to their job. ICU nurses reported high workload and their limited training in dealing with some of the family situations as factors that affect nurse-family relations adversely**.** They suggested that ICU nurses may view families as obstacles due to inadequate staffing in the unit as evidenced by a quote from an ICU nurse. **Soderstrom *et al.*, (2006)** revealed that some nurses consider medical and technical tasks as their main focus and express not having enough time for families**. Gross (2006)** added that dealing with angry and distraught family members, continually calling nurses for an update on patient's status, and no social workers to help with communication with families were identified as obstacles experienced by ICU nurses providing end-of-life care. Also, She reported here provides additional support for the strong need to improve nurse-family relations in ICUs and to make families an integrated part of patient care in ICUs.

Communication between critical care Nurses and Other Providers

Two different issues related to nurse-physician communication were identified as performance obstacles by critical care nurses inadequate information from physicians (21.7%) and delay in seeing new medical orders for my patient(s) (20%). These results agreed with **Gurses and Carayon (2007)** described that all the ICUs involved in the study were using, at least partially, paper based patient charts. When a physician writes an order for a patient, nurses often learn about the order by looking at the chart. In an ICU, there are different care providers who need the patient chart from time to time to provide appropriate care; therefore, getting the chart in a timely manner can become a challenge for nurses. It could take 2 to 3 hours before the nurse could get the chart and become aware of a new order written for the patient. A delay of 3 hours in starting a new treatment may have a significant effect on ICU patients. According, **Tammelleo (2001)** revealed that nurses may not get the information they need from physicians during both the day and night shifts. During the day shift, physicians may not be available immediately to respond to nurses' questions because they face other demands such as being in surgery or attending meetings. Even if they are available, they may forget or unintentionally omit to communicate important information to nurses due to their high workload. During the night shift, the cross-covering physician may not have adequate knowledge of the patient to be able to answer nurses' questions. Ineffective communication between nurses and physicians has been linked to medication errors, patient injuries, and patient deaths. **Narasimhan *et al.*, (2006); Gurses & Xiao (2006)** suggested that methods should be explored for improving nurse-physician communication such as developing and using new information tools for multidisciplinary rounds such as a daily goals worksheet by ensuring clarity of what is discussed and by preventing physicians from forgetting to discuss an issue important for patient care.

The results of the study showed that performance obstacles reported by critical care nurses was related to organization element such as change of shift report(s) took longer than they should and inadequate information given to me by the previous shift's nurse(s) during the shift change (25%). These results congruent by **Currie (2002)** conducted in non-ICU environments, missing information (omission of critical information regarding a patient or the omission of an entire report of a patient) was identified as one of the major problems with shift change report. Furthermore, if designed well, information technology has a great potential to improve inter provider communication, including shift change report **(Gurses & Xiao, 2006).**

The Physical Environment

The results of the study revealed performance obstacles reported by critical care nurses was related to environment element such as experience with insufficient work place for completing paper work (40%), patients' room full with visitors (36.7%). These results agreed with **Gurses and Carayon (2007)** emphasized that importance of the physical environment in ICU nurses' work. **Tyson *et al.*, (2002)** added that nurses viewed the increased private space in the new units as positive for patients. However, the increased space led to feelings of isolation among nurses and increased nurse burnout, as observing patients and interacting with coworkers became more difficult**. Smith *et al.*,(2005)** stated that the redesign of

a neonatal ICU from an open bay design to a private room design led to significant improvements in the quality of the physical environment as reported by neonatal ICU nurses, but to a deterioration in the quality of patient care team interaction. Specific improvements in the physical environment were related to increased staff privacy, parental privacy and thermal comfort, and decreased noise. **(Patterson et al., 2004)** added that the work environment, or organizational climate, is generally understood to influence the behavior of employees**.** Social exchange theory suggests that when an organization values and supports its employees, the employees feel obligated to reciprocate **(Dawley et al., 2008).**Thus, employees who perceive a positive work environment feel positive toward the organization. These feelings can lead to outcomes such as increased commitment and performance, and extra-role behaviors **Aryee *et al.,* (2002).**

Regarding performance obstacles reported by critical care nurses was related to technologies or tools element such as the isolation rooms were not well stocked (60%), the central stock area was not well-stocked (25%), having to use equipment that was in poor condition (21.7%). waiting to use a piece of equipment because someone else was using it. (20%). These results congruent by **Janakiraman *et al.,* (2011)** focused on three aspects of the physical environment that are of particular importance to nurses: quality of patient areas, safety, and quality of work spaces. Quality of patient areas refers to the comfort and privacy afforded patients and families due to the physical design of the area in which they spend time. Given the hospital setting, the authors focused on the patient rooms. Safety is a basic need that takes on added prominence in work roles that are inherently dangerous. Safety is defined as the degree of hazard for staff and patients related to facility design. Quality of work spaces refers to convenient access to needed supplies, storage, parking, meeting space, and equipment, and a workstation with the features needed for the job. They found that the perceived safety of the physical environment is associated with perceived service quality. **Kotzer *et al.,* (2006)** added that physical features that afford comfort and privacy can benefit both the patient and the caregiver. Nurses may benefit not only because they spend considerable time in these spaces but also because it is easier to serve patients and families who are pleased with the facility. The authors asked the nurses about a wide range of features related to their work space (for example, storage for supplies) and found that these design elements significantly impact their perceptions of service quality and their commitment. Nurses benefit from well-designed work areas that meet their needs and enhance their ability to accomplish their work..

The ICU work system needs to be redesigned to reduce nursing workload and improve QWL as well as patient safety and quality of care **(Institute of Medicine, 2004).** The first step in redesigning a healthcare work system is to determine where to focus efforts, which can be accomplished by identifying the performance obstacles in the work system **(Carayon *et al.,* 2005). Miller (2006)** stated that organizational or work-role competencies that include co-ordination, prioritization and management of multiple responsibilities. A supportive work environment may also contribute to quality of care, morale among staff, and successful recruitment and retention.

Regarding performance obstacles reported by critical care nurses were related to task element for responsibility about orientation for new nurses was (100%). According this result, **Donna (2002)** illustrated that many companies feel that orientation is a function of their Human Resources or Administrative department that involves completing the paperwork for payroll and benefits packages. Other times the new employee is hurriedly introduced and passed on to a fellow employee who has been assigned to "show you the job". Because they usually remain accountable for their primary responsibilities during this time, they often end up demonstrating the "quick version" and leave the new employee with the remark "Let me know if you have any questions or problems." **Roberts *et al,* (2004)** found that new nurses were more likely to stay in their current position if they were satisfied with aspects of the work environment, including co-workers, interaction, professional opportunities and recognition. **O' neil (2008)** added that people work better and achieve more when they are clear on the organization's mission, strategic directions and are aligned around shared values.

Another performance obstacle reported by critical care nurses was related to intra hospital patient transport (33.4%). This result agreed by **Gurses & Carayon (2007)** stated that ICU patients need to be transferred to other units of the hospital for tests and procedures and are at high risk en route. Furthermore, accompanying a patient for intra hospital transportation increases the workload of nurses considerably and takes them away from other patients staying in the ICU.

Regarding performance obstacles reported by critical care nurses was related to help from Nursing Assistants, Help from Other Nurses, Help from Unit clerk, work environment (56.7%, 76.7%, 55%, and 28.3%) respectively with statistically significant difference. **Roberts *et al.,* (2004)** stated that entering professional nurses described the difficulties they encountered in being able to offer the type of care they believe patients deserve. They cited the nurse shortage, demands on time, conflicting values and lack of autonomy as major obstacles to good work in nursing.

Demands on time involved the need to perform multiple tasks with fewer resources, such as supplies or assisting staff. **Wolfe (2012)** added that a good ward clerk should be able to work efficiently amidst a very fast-paced working environment. They should be able to effectively follow the hospital's record-keeping systems and procedures. They should also have the presence of mind to give assistance when necessary. Customer service in hospitals can vary greatly in quality. While some hospitals provide low wait times, friendly staff and professional, unhurried physicians, other hospitals do not have the means or will to provide such attentive care. The reasons a health care facility would deliver poor customer service can vary widely. **Gurses (2005)** stated that the distance between the patients' rooms assigned to a nurse affects physical workload; the condition of the work environment (noisy versus quiet, hectic versus calm) affects the overall effort spent by the nurse to perform her job**.**

Conclusion

Performance obstacles represent the following elements of the work system: environment (6 obstacles), organization (7 obstacles), technologies or tools (4 obstacles), and task (4 obstacles).The results of this study indicate that critical care nurses' experience a wide variety of performance obstacles that cover all elements of the work system model

Performance obstacle reported by critical care nurses was related to Help from Nursing Assistants, Help from Other Nurses, Help from Unit clerk, work environment were statistically significant difference.

Recommendation

- The proposed strategy is focused on a few obstacles that are relatively easy to change, and do not require a large amount of resources. For example, performance obstacles related to misplacement of equipment, supplies, and patient charts may be easier to eliminate than problems related to inadequate workspace. Whereas performance obstacles related to misplacement of equipment, supplies, and patient charts may be eliminated by creating and reinforcing a protocol or by establishing a tracking system, the performance obstacle of inadequate workspace may require a major redesign of the physical layout of the ICU.
- Future research should investigate the impact of various performance obstacles on nursing workload, nursing quality of working life, and quality and safety of care, as well as the impact of interventions aimed at redesigning the work system of critical care nurses to remove performance obstacles.
- Organizations can use the findings as a blueprint to improve work environments and increase retention of critical care nurses.

Corresponding author
Lamiaa Ismail Keshk
Department of nursing administration, Faculty of Nursing, Helwan University
keshk_lamiaa@yahoo.com

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PID Power System Stabilizer Design based on Shuffled Frog Leaping Algorithm

Mojtaba Shirvani [1], Pejman Shakeri [2], Elahe Behzadipour [3], Iman Baghbani[4]

[1,2,3,4]Department of Electrical Engineering, Boroujen Branch, Islamic Azad University, Boroujen, Iran
mo_shirvani@yahoo.com

Abstract: Power System Stabilizers (PSS) are used to generate supplementary damping control signals for the excitation system in order to damp the Low Frequency Oscillations (LFO) of the electric power system. The PSS is usually designed based on classical control approaches but this Conventional PSS (CPSS) has some problems. To overcome the drawbacks of CPSS, numerous techniques have been proposed in literatures. In this paper a PID type PSS (PID-PSS) is considered. The parameters of this PID type PSS (PID-PSS) are tuned based on Shuffled Frog Leaping algorithm. The proposed PID-PSS is evaluated against the conventional power system stabilizer (CPSS) at a single machine infinite bus power system considering system parametric uncertainties. The simulation results clearly indicate the effectiveness and validity of the proposed method.
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Keywords: Power System Stabilizer, Dynamic Stability, Shuffled Frog Leaping algorithm, PID Controller

1. Introduction

Large electric power systems are complex nonlinear systems and often exhibit low frequency electromechanical oscillations due to insufficient damping caused by adverse operating. These oscillations with small magnitude and low frequency often persist for long periods of time and in some cases they even present limitations on power transfer capability (Liu et al., 2005). In analyzing and controlling the power system's stability, two distinct types of system oscillations are recognized. One is associated with generators at a generating station swinging with respect to the rest of the power system. Such oscillations are referred to as "intra-area mode" oscillations. The second type is associated with swinging of many machines in an area of the system against machines in other areas. This is referred to as "inter-area mode" oscillations. Power System Stabilizers (PSS) are used to generate supplementary control signals for the excitation system in order to damp both types of oscillations (Liu et al. 2005). The widely used Conventional Power System Stabilizers (CPSS) are designed using the theory of phase compensation in the frequency domain and are introduced as a lead-lag compensator. The parameters of CPSS are determined based on the linearized model of the power system. Providing good damping over a wide operating range, the CPSS parameters should be fine tuned in response to both types of oscillations. Since power systems are highly nonlinear systems, with configurations and parameters which alter through time, the CPSS design based on the linearized model of the power system cannot guarantee its performance in a practical operating environment. Therefore, an adaptive PSS which considers the nonlinear nature of the plant and adapts to the changes in the environment is required for the power system (Liu et al. 2005). In order to improve the performance of CPSSs, numerous techniques have been proposed for designing them, such as intelligent optimization methods (Linda and Nair 2010; Yassami et al. 2010; Sumathi et al. 2007; Jiang et al. 2008; Sudha et al. 2009) and Fuzzy logic method (Hwanga et al. 2008; Dubey 2007). Also many other different techniques have been reported by Chatterjee et al. (2009) and Nambu and Ohsawa (1996) and the application of robust control methods for designing PSS has been presented by Gupta et al. (2005), Mocwane and Folly (2007), Sil et al. (2009) and Bouhamida et al. (2005). This paper deals with a design method for the stability enhancement of a single machine infinite bus power system using PID-PSS which its parameters are tuned by Shuffled Frog Leaping algorithm Optimization method. To show effectiveness of the new optimal control method, this method is compared with the CPSS. Simulation results show that the proposed method guarantees robust performance under a wide range of operating conditions.

Apart from this introductory section, this paper is structured as follows. The system under study is presented in section 2. Section 3 describes about the system modeling and system analysis is presented in section 4. The power system stabilizers are briefly explained in section 5. Section 6 is devoted to explaining the proposed methods. The design methodology is developed in section 7 and eventually the simulation results are presented in section 8.

2. System under Study

Figure 1 shows a single machine infinite bus power system (Kundur 1993). The static excitation system has been considered as model type *IEEE – ST1A*.

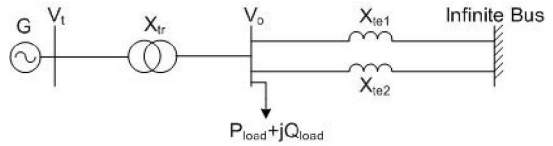


Figure 1. A single machine infinite bus power system

3. Dynamic Model of the System

A non-linear dynamic model of the system is derived by disregarding the resistances and the transients of generator, transformers and transmission lines (Kundur 1993). A linear dynamic model of the system is obtained by linearizing the non-linear dynamic model around the nominal operating condition. The linearized model of the system is obtained as (1) (Kundur 1993).

$$\begin{cases} \Delta\dot{\delta} = \omega_0 \Delta\omega \\ \Delta\dot{\omega} = \dfrac{-\Delta P_e - D\Delta\omega}{M} \\ \Delta\dot{E}'_q = (-\Delta E_q + \Delta E_{fd})/T'_{do} \\ \Delta\dot{E}_{fd} = -\left(1/T_A\right)\Delta E_{fd} - \left(K_A/T_A\right)\Delta V \end{cases} \quad (1)$$

Figure 2 shows the block diagram model of the system. This model is known as Heffron-Phillips model (Kundur 1993). The model has some constants denoted by K_i. These constants are functions of the system parameters and the nominal operating condition. The nominal operating condition is given in the appendix.

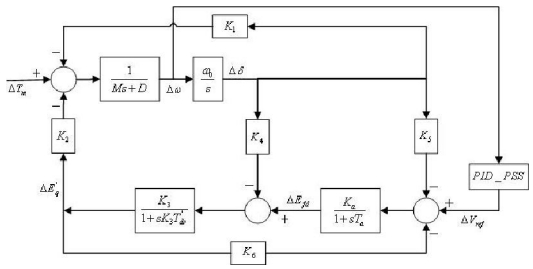


Figure 2. Heffron-Phillips model of the power system

3.1. Dynamic model of the system in the state-space form

The dynamic model of the system in the state-space form is obtained as (2) (Kundur 1993).

4. Analysis

In the nominal operating condition, the eigen values of the system are obtained using analysis of the state-space model of the system presented in (2) and these eigen values are shown in Table 1. It is clearly seen that the system has two unstable poles at the right half plane and therefore the system is unstable and needs the Power System Stabilizer (PSS) for stability.

$$\begin{bmatrix} \Delta\dot{\delta} \\ \Delta\dot{\omega} \\ \Delta\dot{E}'_q \\ \Delta\dot{E}_{fd} \end{bmatrix} = \begin{bmatrix} 0 & \omega_0 & 0 & 0 \\ -\frac{K_1}{M} & 0 & -\frac{K_2}{M} & 0 \\ -\frac{K_4}{T'_{do}} & 0 & -\frac{K_3}{T'_{do}} & \frac{1}{T'_{do}} \\ -\frac{K_A K_5}{T_A} & 0 & -\frac{K_A K_6}{T_A} & -\frac{1}{T_A} \end{bmatrix} \times \begin{bmatrix} \Delta\delta \\ \Delta\omega \\ \Delta E'_q \\ \Delta E_{fd} \end{bmatrix} + \begin{bmatrix} 0 & 0 \\ \frac{1}{M} & 0 \\ 0 & 0 \\ 0 & \frac{K_A}{T_A} \end{bmatrix} \times \begin{bmatrix} \Delta T_m \\ \Delta V_{ref} \end{bmatrix} \quad (2)$$

Table 1. The eigen values of the closed loop system

-4.2797
-46.366
+0.1009 + j4.758
+0.1009 - j4.758

5. Power System Stabilizer

A Power System Stabilizer (PSS) is provided to improve the damping of power system oscillations. Power system stabilizer provides an electrical damping torque (ΔT_m) in phase with the speed deviation ($\Delta\omega$) in order to improve damping of power system oscillations (Kundur 1993). As referred before, many different methods have been applied to design power system stabilizers so far. In this paper a new optimal method based on the SFL algorithm is considered to tuning parameters of the PID-PSS. In the next section, the proposed method is briefly introduced and then designing the PID-PSS, based on the proposed methods, is done.

6. The proposed method

In this paper the SFL algorithm optimization method is considered to adjustment PID-PSS. For more introductions, the proposed methods are briefly introduced in the following subsections.

6.1. SFLA Overview

Over the last decades there has been a growing concern in algorithms inspired by the observation of natural phenomenon. It has been shown by many researches that these algorithms are good alternative tools to solve complex computational problems.

The SFLA is a meta-heuristic optimization method inspired from the memetic evolution of a group of frogs when searching for food (Huynh

2008). SFLA, originally developed in determining the optimal discrete pipe sizes for new pipe networks and for existing network expansions. Due to the advantages of the SFLA, it is being researched and utilized in different subjects by researchers around the world, since 2003 (Elbeltagi 2007; Ebrahimi et al. 2011).

The SFL algorithm is a memetic meta-heuristic method that is derived from a virtual population of frogs in which individual frogs represent a set of possible solutions. Each frog is distributed to a different subset of the whole population described as memeplexes. The different memeplexes are considered as different culture of frogs that are located at different places in the solution space (i.e. global search). Each culture of frogs performs simultaneously an independent deep local search using a particle swarm optimization like method. To ensure global exploration, after a defined number of memeplex evolution steps (i.e. local search iterations), information is passed between memeplexes in a shuffling process. Shuffling improves frog ideas quality after being infected by the frogs from different memeplexes, ensure that the cultural evolution towards any particular interest is free from bias. In addition, to improved information, random virtual frogs are generated and substituted in the population if the local search cannot find better solutions. After this, local search and shuffling processes (global relocation) continue until defined convergence criteria are satisfied. The flowchart of the SFLA is illustrated in Figure 3.

The SFLA begins with an initial population of "P" frogs $F=\{X_1,X_2,...,X_n\}$ created randomly within the feasible space Ω. For S-dimensional problems (S variables), the position of the i^{th} frog is represented as $X_i=[x_{i1},x_{i2},...,x_{is}]^T$. A fitness function is defined to evaluate the frog's position. Afterward the performance of each frog is computed based on its position. The frogs are sorted in a descending order according to their fitness. Then, the entire population is divided into m memeplexes, each of which consisting of n frogs (i.e. $P=n\times m$). The division is done with the first frog goes to the first memeplex, the second frog goes to the second memeplex, frog m goes to the m^{th} memeplex, and the $(m+1)^{th}$ frog back to the first memeplex, and so on. The local search block of Figure 3 is shown in Figure 4.

According to Figure 4, during memeplex evolution, the position of frog i^{th} (D_i) is adjusted according to the different between the frog with the worst fitness (X_w) and the frog with the best fitness (X_b) as shown in (3). Then, the worst frog X_w leaps toward the best frog X_b and the position of the worst frog is updated based on the leaping rule, as shown in (4).

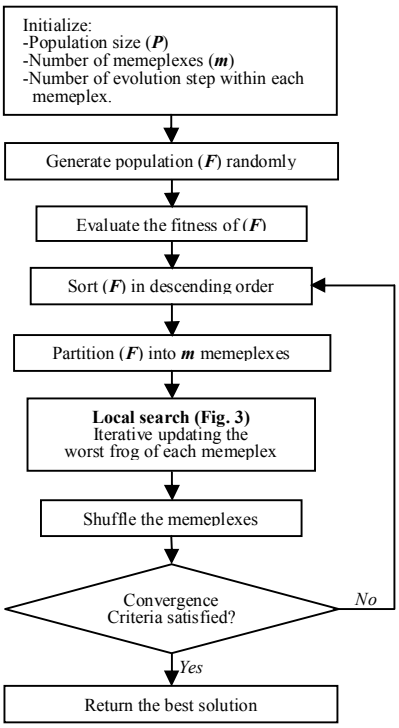


Figure 3.General principle of SFLA (Ebrahimi et al., 2011)

$$\text{Position change}(D_i) = rand() \times (X_b - X_w) \quad (3)$$

$$X_w(new) = X_w + D, (\|D\| < D_{\max}) \quad (4)$$

where *rand ()* is a random number in the rang [0,1] and D_{max} is the maximum allowed change of frog's position in one jump. If this repositioning process produces a frog with better fitness, it replaces the worst frog, otherwise, the calculation in (3) and (4) are repeated with respect to the global best frog (X_g), (i.e. X_g replaces X_b). If no improvement becomes possible in this case, then a new frog within the feasible space is randomly generated to replace the worst frog. Based on Figure 3, the evolution process is continued until the termination criterion is met. The termination criterion could be the number of iterations or when a frog of optimum fitness is found (Huynh 2008).

To compute the fitness value for each frog, firstly, the values of the I_{pi} variables are extracted by decoding the frog information. In this study the fitness index is considered as (5). In fact, the performance index is the Integral of the Time multiplied Absolute value of the Error (*ITAE*).

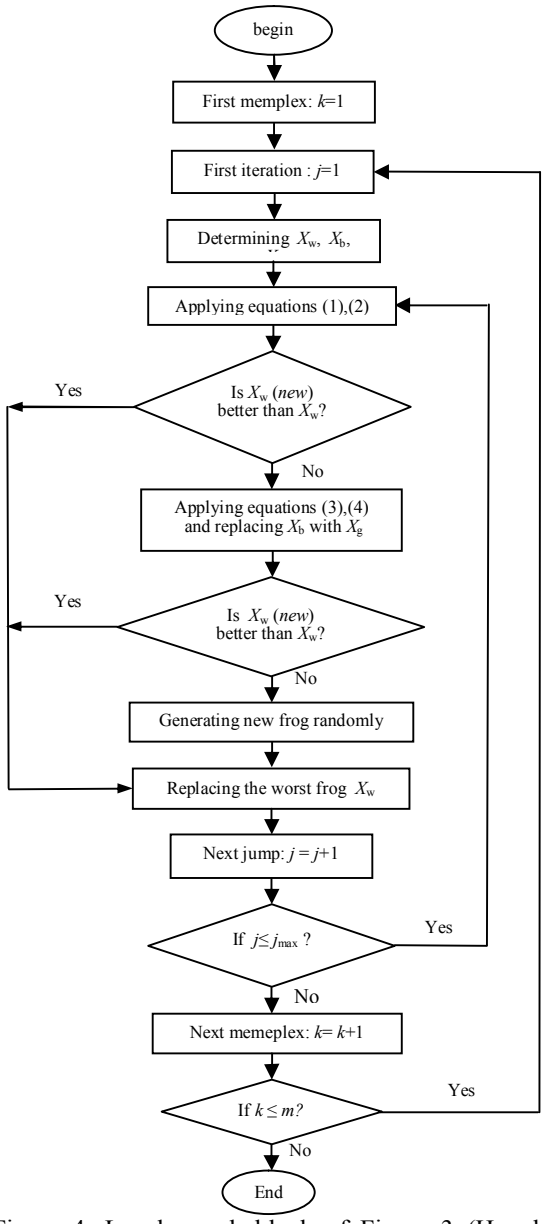


Figure 4. Local search block of Figure 3 (Huynh, 2008).

$$ITAE = \int_0^t t|\Delta\omega|dt \qquad (5)$$

Based on Figure 3 the local search and shuffling processes (global relocation) continue until the last iteration is met. In this paper, the number of iteration is set to be 50.

7. Design methodology

In this section the PID-PSS parameters tuning based on the Shuffled Frog Leaping algorithm is presented. The PID-PSS configuration is as (6).

$$PID-PSS = K_P + \frac{K_I}{S} + K_D S \qquad (6)$$

The parameter ΔE_{ref} is modulated to output of PID-PSS and speed deviation $\Delta\omega$ is considered as input to PID-PSS. The optimum values of K_P, K_I and K_D which minimize an array of different fitness indexes are computed using the SFLA. It is clear that the controller with lower fitness is better than the other controllers. To compute the optimum parameter values, a 0.1 step change in reference mechanical torque (ΔTm) is assumed and the performance index is minimized using SFLA. The first step to implement the SFL is generating the initial population (*N* frogs) where *N* is considered to be 20. The number of memeplex is considered to be 3 and the number of evaluation for local search is set to 3. Also D_{max} is chosen as *inf*. To find the best value for the solution, the algorithms are run for 10 independent runs under different random seeds. The optimum values of the parameters K_P, K_I and K_D are obtained using SFLA and summarized in the Table 2.

Table 2. Obtained parameters of PID-PSS using Shuffled Frog Leaping algorithm

PID Parameters	KP	KI	KD
Obtained Value	54.860	9.248	16.237

8. Simulation results

In this section, the proposed optimal PID-PSS is applied to the under study system (single machine infinite bus power system). To show effectiveness of the proposed optimal PID-PSS, A classical lead-lag PSS based on phase compensation technique (CPSS) is considered for comparing purposes.

The detailed step-by-step procedure for computing the parameters of the classical lead-lag PSS (CPSS) using phase compensation technique is presented in (Kundur 1993). Here, the CPSS has been designed and obtained as (7).

$$CPSS = \frac{35(0.3S+1)}{(0.1S+1)} \qquad (7)$$

In order to study the PSS performance under system uncertainties (controller robustness), three operating conditions are considered as follow:

i : Nominal operating condition

ii: Heavy operating condition (20% changing parameters from their typical values)

iii: Very heavy operating condition (50% changing parameters from their typical values)

Also to demonstrate the robustness performance of the proposed method, the *ITAE* is calculated following a 10% step change in the reference mechanical torque (ΔT_m) at all operating conditions (Nominal, heavy and Very heavy) and results are shown at Table 3. Following step change at ΔT_m, the optimal PID-PSS has better performance

than the CPSS at all operating conditions. Where, the optimal PID-PSS has lower *ITAE* index in comparison with CPSS, therefore the optimal PID-PSS can damp power system oscillations more successfully.

To demonstrate the robustness and safe performance of the proposed method, speed deviations of the machine following a 10% step change in the reference mechanical torque (ΔT_m) at all operating conditions (Nominal, heavy and Very heavy) is shown in figure 5.

Table 3. The calculated ITAE

	Optimal PID-PSS	CPSS
Nominal operating condition	4.3231×10-4	5.5686×10-4
Heavy operating condition	3.4428×10-4	7.2451×10-4
Very heavy operating condition	2.9189×10-4	8.9021×10-4

9. Conclusions

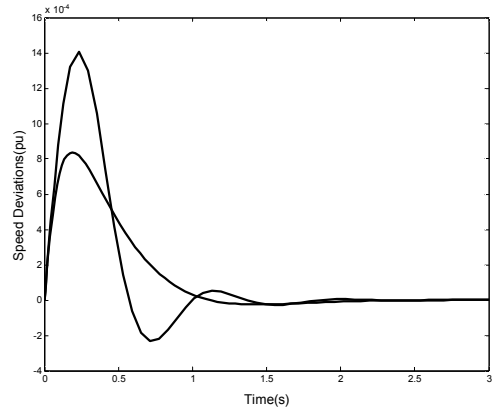
In this paper a new optimal PID-PSS based on SFLA has been successfully proposed. The design strategy includes enough flexibility to set the desired level of stability and performance, and to consider the practical constraints by introducing appropriate uncertainties. Also the final designed optimal PID-PSS is low order and its implementation is easy and cheap. The proposed method was applied to a typical single machine infinite bus power system containing system parametric uncertainties and various loads conditions. The simulation results demonstrated that the designed optimal PID-PSS is capable of guaranteeing the robust stability and robust performance of the power system under a wide range of system uncertainties.

10. Appendix

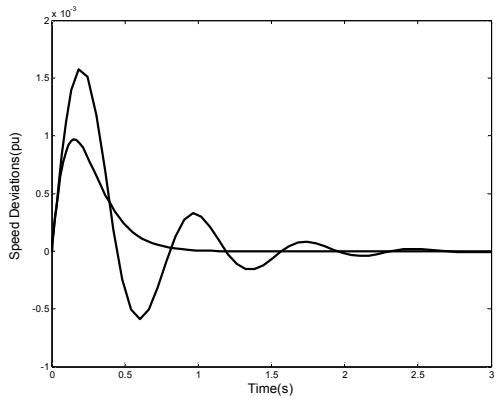
The nominal parameters and operating conditions of the system are listed in Table 4.

Table 4. The nominal system parameters

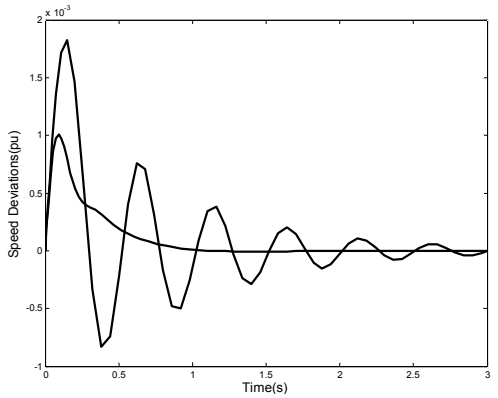
Generator	M=10Mj/MVA Xq=1.6 p.u.	T´do= 7.5 s X´d=0.3 p.u.	Xd=1.68p.u. D = 0
Excitation system		Ka=50	Ta = 0.02 s
Transformer		Xtr=0.1p.u.	
Transmission lines	Xte1=0.5p.u.	Xte2=0.9p.u.	
Operating condition	Vt=1.05p.u.	P=1 p.u.	Q=0.2 p.u.



a



b



c

Figure 5. Dynamic responses Δω following 0.1 step in the reference mechanical torque (ΔTm)
a: Nominal operating condition
b: Heavy operating condition
c: Very heavy operating condition
solid line (HS-PSS), dashed line (CPSS)

Corresponding Author:
Mojtaba Shirvani
Department of Electrical Engineering,
Boroujen Branch, Islamic Azad University,
Boroujen, Iran.
E-mail: mo_shirvani@yahoo.com

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