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Role of Voriconazole in the Management of Fungal Corneal Ulcers

Ali M. Ismail

Ophthalmology Department, Faculty of Medicine, Sohag University Hospital
aliismail30002000@yahoo.com

Abstract: Purpose: To evaluate the role of Voriconazole in the management of fungal corneal ulcers. **Design:** prospective interventional comparative study. **Patients & Methods:** Eighty eyes of 80 patients who suffered from uncomplicated fungal corneal ulcer were enrolled in this study, they were subjected to the following: Full history taking, visual acuity (VA), slit lamp biomicroscopy, ultrasonography (to assess the posterior segment), corneal scraping for potassium hydroxide (KOH) test and culture to confirm fungal infection and identify species (if possible). Patients were divided into four groups: Group 1: 20 eyes received one intrastromal injection with Voriconazole (50 microgram/0.1 ml) at the junction of clear cornea and infiltrates, using a 30-gauge needle in five sites to form a barrage around the ulcer. Group 2: 20 eyes received topical antifungal (Voriconazole eye drops (E.D) 1%). Group 3: 20 eyes received intracameral Voriconazole 100 microgram Voriconazole in 0.1 mL. Group 4: 20 received topical, intrastromal and intracameral Voriconazole (combined therapy). The four groups received topical Moxifloxacin hydrochloride 0.5% (E.D) four times a day and 1% Isopto-atropine E.D twice a day. Treatment has been started as soon as the (KOH) test is positive and followed for at least 2 months. **Results:** The four groups were matched regarding the mean baseline BCVA. In four groups, the BCVA after treatment with Voriconazole improved significantly than the pre-treatment one. When the mean post-treatment BCVA was compared between four groups, there was a significantly higher BCVA in group 4 than in groups 1,2 and 3 ($p=0.038$). In group 1, complete healing was obtained in 17 eyes (85%) versus 6 eyes only in group 2 (30%), 18 eyes in group 3 (90%) and 19 eyes in group 4 (95%) which also was a statistically significant difference between four groups ($P=0.041$). The mean resolution time varied significantly between four groups, it was 20.4 ± 3 days for group (1), 26.4 ± 3.50 days for group (2), 18 ± 2 days for group (3), 14 ± 2 days for group (4) ($P=0.0213$). Complication rate was higher for group (2) of topical treatment (25%) than for group (1) (10%), no complications were recorded in groups (3) and (4). **Conclusion:** Voriconazole is effective in the management of fungal corneal ulcer either topical antifungal (Voriconazole eye drops 1%), intrastromal injection with Voriconazole (50 microgram/0.1 ml) or intracameral Voriconazole (100 microgram Voriconazole in 0.1 mL). but intracameral and Intraströmål are more effective, they have a high success rate.

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Key words: Voriconazole, fungal corneal ulcer.

1. Introduction

Fungal keratitis accounts for nearly 50% of all cases of infectious keratitis in developing countries and has a poor prognosis compared with bacterial keratitis.^{1, 2} Currently available topical antifungal drugs have limitations such as poor penetration into the eye, limited spectrum of activity and surface toxicity.³⁻⁵ Surgical intervention in the form of therapeutic Keratoplasty is required more often in cases of fungal keratitis, compared with bacterial keratitis, indicating a poor response to treatment with antifungal agents.^{2,6}

A less invasive surgical modality of use of intrastromal Amphotericin B and Voriconazole for cases of deep-seated fungal keratitis, non-responsive to topical and oral antifungal agents have been described in anecdotal reports.⁷⁻⁹

Voriconazole, a more recent azole antifungal drug, is available commercially for systemic administration in the form of oral and intravenous

formulations. It has an excellent broad spectrum antifungal activity and is active against species that are known to be resistant to the other antifungal agents commonly used in fungal keratitis.¹⁰

Voriconazole is increasingly being used topically as eye drops. Topical Voriconazole has demonstrated good penetration into the different parts of the eye,^{11,12} with sufficient concentrations achieved to cover a wide range of keratitis-causative fungi.¹⁰ Few recent papers showed that Voriconazole is more effective when injected intrastromally. In experimental studies, Voriconazole has been shown to be less toxic to the retina than amphotericin B and to exhibit exponential decay with a half-life of 2.5 hours in rabbit vitreous and a very low aqueous concentration, below the therapeutic levels of fungal species. Therefore, intracameral Voriconazole injection is indicated to achieve a higher aqueous concentration and is considered to be an alternative in the treatment of fungal endophthalmitis spreading as

a result of keratitis^{13, 14}. In our study we aim to know the best method of using Voriconazole in the management of fungal corneal ulcers.

Herein, we compared the different modalities of Voriconazole in the management of fungal corneal ulcers.

2. Patients and Methods:

Eighty eyes of 80 patients with microbiologically proven fungal corneal ulcer were enrolled in this prospective comparative study from the outpatient ophthalmology clinic at Sohag University Hospital from January 1st to December 31st 2011. The study was conducted in accordance with the rules of the scientific ethical committee of the faculty. Informed consent was obtained from all subjects. The diagnosis of fungal infection was made on the basis of clinical evaluation, positive smear and/or cultures of the fungus.

Inclusion criteria

Patients with uncomplicated microbial keratitis and proven presence of fungal organism on smear and/or culture.

Exclusion criteria

Cases that had some involvement of adjacent sclera, impending or frank corneal perforation, presence of descemetocele and concomitant endophthalmitis were excluded from the study.

At the initial presentation, each patient underwent a detailed evaluation that included clinical history, recording of visual acuity and slit-lamp biomicroscopy. Corneal scrapings were obtained under topical anaesthesia (0.4% Benoxinate hydrochloride) and were sent for microbiological investigation including a potassium hydroxide (KOH) wet-mount preparation, Gram smear and cultures on Sabouraud dextrose agar to confirm diagnosis (Figure 1). The diagnosis was made on the basis of clinical evaluation, positive smear and/or cultures. Antifungal therapy was started as soon as fungus was identified by KOH wet-mount preparation with Moxifloxacin hydrochloride eye-drops (0.5%) four times a day and topical atropine two times a day. According to the type of therapy started with; the patients were divided into four groups:

Group 1: Randomly 20 eyes received intrastromal Voriconazole solution 50 micrograms/0.1 ml circumferentially around the fungal ulcer in a single dose.

Group 2: 20 eyes received topical antifungal (Voriconazole eye drops 1% every 2 hours) for four weeks.

Group 3: 20 eyes received intracameral Voriconazole (100 microgram Voriconazole in 0.1 mL).

Group 4: 20 eyes received combined therapy in the form of topical, intrastromal and intracameral Voriconazole.

Follow-up:

Follow-up continued for two months, the first visit was 2 days after start of treatment and repeated each other day for 2 weeks then weakly regarding to :

-Size of the ulcer.

-Scar formation.

-Development of complications (perforation, endophthalmitis...etc.)

The ulcer was defined as not improved if there was no change in the size of the ulcer or the infiltrates, and defined as worsened if there was an increase in size or depth of ulcer/infiltrate by 20% or perforation. All patients had deep-seated corneal infiltrates with or without hypopyon. The depth of corneal involvement in all these cases extended up to or deeper than midstromal level.



Figure 1: positive fungal culture, Aspergillus.

Method of Intrastromal Injection:

Voriconazole (VFEND) is available as 200 mg of white lyophilized powder in a glass vial (Figure 2). The powder was reconstituted with 19 ml of lactated Ringer solution (LR) to obtain 20 ml of clear concentrate containing 10 mg/ml of Voriconazole. A 1-ml of this solution was further diluted with 20 ml of LR to a concentration of 0.5 mg/ml (50 microgram/0.1 ml). The reconstituted solution was loaded in a 1-ml tuberculin syringe with a 30-gauge needle (Fig.3).

After administration of peribulbar anesthesia, the patient was shifted to the operating table. Under full aseptic conditions, the preloaded drug was administered under Operating microscope. With the bevel down, the needle was inserted obliquely from the uninvolved, clear cornea to reach just flush to the ulcer at the mid-stromal level (as the intended level for drug deposit) in each case. The drug then was injected and the amount of hydration of the cornea was used as a guide to assess the area covered. Once the desired amount of hydration was achieved, the plunger was withdrawn slightly to ensure discontinuation of the capillary column and thus prevent back-leakage of the drug. Five divided doses

were given around the ulcer to form a deposit of the drug around the circumference of the lesion. The total



(Figure 2)

However, the topical 1% Voriconazole solution was stored under aseptic conditions at a temperature of 2-8°C under refrigeration.¹⁰ As per the Voriconazole package insert, the powder is reconstituted with 19 mL of water for injection to produce a 20 mL aqueous Voriconazole solution with a concentration of 10 mg/mL (1%). This Voriconazole solution is what is typically being used as eye drops.

Topical therapy was continued until 2 weeks after the resolution of the infection.

Method of intracameral Voriconazole:

The procedure for intracameral Voriconazole injections was as follows. Voriconazole (VFEND; Pfizer, Inc, New York, New York, USA) was obtained in pure powder form and was reconstituted in sterile water to obtain a concentration of 100 g/100/L. Intracameral Voriconazole injection was administered under aseptic conditions using an operating microscope. After instillation of topical proparacaine, a speculum was inserted. If necessary, an aqueous aspiration was performed for diagnosis before Voriconazole injection was administered. A volume of 100 g Voriconazole in 0.1 mL was injected into the anterior chamber using a 30-gauge needle attached to a 1.0-mL regular insulin syringe as shown in (Figure 3). Intracameral Voriconazole injection was given once daily, and the treatment was discontinued while the eyes showed resolution of the anterior chamber fungal infiltrate. If anterior chamber fungal infiltrate or an enlargement of the endothelial plaque subsequently developed in these patients, intracameral Voriconazole injection was added to the treatment regimen again.

3. Results:

The mean age of the patients in four groups was 56.5±15.13 years. There were 59 males and 21 females. The risk factors identified in these cases were trauma with vegetable matter (n=70), drugs

amount of drug injected intrastromally ranged from 0.06 ml to 0.10 ml. (Figure 4).



(Figure 3)

abuse (topical corticosteroid drops or chronic topical antibiotics (n=6) and systemic disease (n=4), two cases were renal failure and two cases were uncontrolled diabetes (Table 1).

Table 1: showing characters of patients at presentation

No of patients	80 patients	Percentage%
<u>Age</u>	43 – 70 years	
<u>Sex</u>		
Male	59	73.75%
Female	21	26.25%
<u>Risk factors</u>		
plant trauma	70	87.50%
drugs abuse	6	7.50%
systemic disease	4	5%

Four groups were matched regarding the mean baseline BCVA. In four groups, the mean BCVA after Voriconazole treatment improved significantly than the pre-treatment vision but improvement in group 4 was highly significant ($P=0.038$).

When compared between four groups, the post-treatment BCVA was significantly better in group (4) than groups (1-3) (Table 2).

Table 2: Comparison between pre-and post-treatment BCVA in four groups

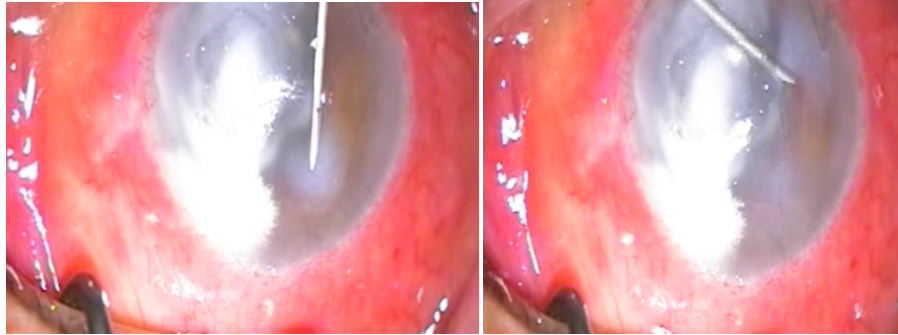
Group	Pre-treatment mean BCVA	Post-treatment mean BCVA	P-value
Group 1	0.048±0.057	0.351±0.15	0.000*
Group 2	0.12±0.111	0.194±0.20	0.05*
Group 3	0.16±0.127	0.207±0.23	0.06*
Group 4	0.052±0.060	0.367±0.20	0.000*
P-value	0.115	0.038*	

*P-value is statistically significant

In group(1), complete healing was obtained in 17 eyes (85%) , 6 eyes only in group 2 (30%) ,18

eyes in group 3 (90%) (Figure 5) and 19 eyes in group 4 (95%) (Figure 6) which also was a statistically significant difference between four groups, The mean resolution time was 20.4 ± 3 days in group (1) compared to 26.4 ± 3.50 for group (2), 18

± 2 days for group(3) and 14 ± 2 days for group (4). Complication rate was higher for group (2) of topical treatment (25%) than for group (1)(10%), no complications were recorded in groups (3 and 4)..



(Figure4):Showing method of intrastromal injection of voriconazole

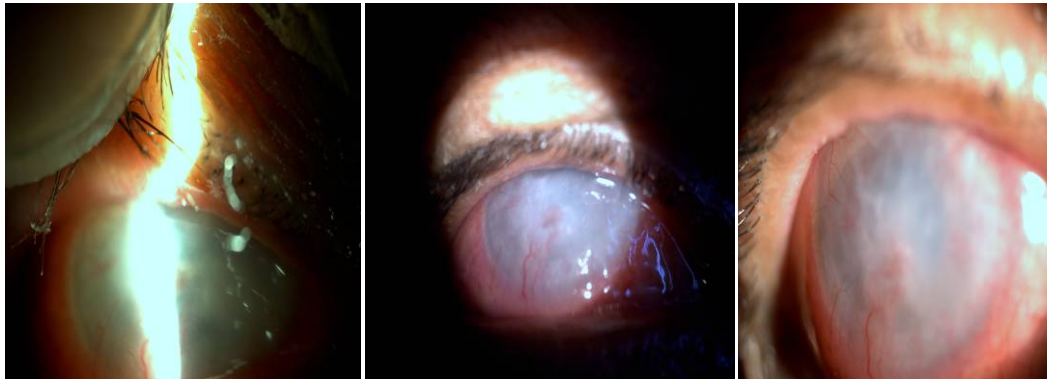


Figure 5: A case of intracameral Voriconazole before (left), one week (middle) and two weeks (right) after intracameral injection.

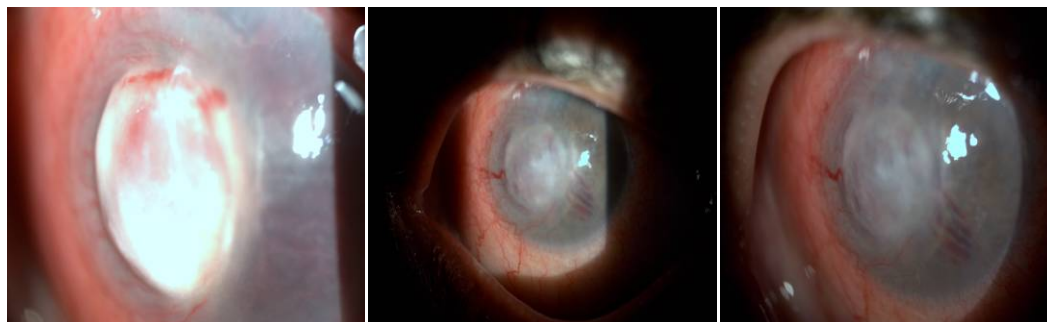


Figure 6: A case of combined Voriconazole therapy before (left), one week (middle) and two weeks (right) after combined therapy.

Complication rate was higher for group 2 of topical treatment (25%) than for group 1 (10%) but did not reach a statistically significant level ($P=0.106$). no complications were recorded in groups 3 and 4.. In group 1, two patients developed complications (one endophthalmitis and the other

perforated ulcer). One patient was lost during the follow up. In group 2 of topical therapy, 5 eyes developed complications (3 perforated ulcers, 2 endophthalmitis), 3 eyes were lost during the follow up, 6 eyes were shifted to intrastromal injection after no improvement after 4 weeks. (Table 3).

Table3: Comparison between intrastromal and topical Voriconazole.

No recurrence of keratitis were noted in either groups

	(Group1) with intrastromal injection of Voriconazole	(Group 2) with topical Voriconazole	(Group3) Intracameral Voriconazole	(Group4) Intrastromal, Intracameral and Topical Voriconazole	P-value
No. Of eyes	20	20	20	20	
Success rate	85%	30%	18	19	0.000*
Complications	2 cases	5 cases	0 cases	0 cases	0.106

4. Discussion

Fungal keratitis can present as superficial keratitis, corneal abscess, and may be associated with hypopyon.¹⁵ The commonly available antifungal agents are Amphotericin B, Natamycin, Fluconazole, Ketoconazole, 5-flucytosine, Itraconazole and voriconazole.¹⁵ The fungistatic activity of Amphotericin B is limited against filamentous fungi, and its systemic use is associated with various side effects.¹⁶ Natamycin has poor corneal penetration and precipitates on the corneal surface.¹⁷ *In vitro* susceptibility data show that Voriconazole has the best efficacy against pathogenic fungi compared with other agents.¹⁸⁻²⁰ Voriconazole is a triazole antifungal agent and is a second-generation synthetic derivative of Fluconazole; it is effective against yeast and filamentous fungi. The primary mode of action of Voriconazole is the inhibition of cytochrome P-450-mediated 14- α -lanosterol demethylation, an essential step in fungal Ergosterol biosynthesis and the resulting Ergosterol depletion causes fungal cell wall destruction. It is well tolerated after oral administration; therapeutic aqueous and vitreous levels are achieved after administration of up to 200 mg twice a day.^{8,12} It is evident from previous studies that oral and topical antifungal agents have poor ocular penetration, thereby achieving suboptimal drug levels at the site of infection.^{3-5, 17} hence, targeted drug delivery is required to achieve adequate drug levels at the site of infection.²¹⁻²⁴ In order to achieve adequate intracorneal concentration of antifungals, intrastromal injections of antifungals have been tried.⁷⁻⁹ Intracameral Voriconazole injection is indicated to achieve a higher aqueous Concentration and is considered to be an alternative in the treatment of fungal endophthalmitis spreading as a result of keratitis.^(13,14)

In group (1) of this study, the drug was injected around the ulcer to form a drug deposit around the circumference of the lesion. This was done in such a manner that a centripetally directed progressive wave of fluid appeared to encompass the ulcer along each

meridian. Circumferential injection ensured the formation of a barrage of intrastromal Voriconazole around the entire ulcer.

A statistically significant higher success rate with complete resolution of the ulcer was obtained in group (4) of combined intracameral and intrastromal injection of Voriconazole associated with topical Voriconazole (95%) than in other groups.

In this study, a significantly better vision was obtained with combined injection of Voriconazole than other groups which means that it is more effective.

Also, we did not report any complications related to combined Voriconazole injection which is also in agreement to that obtained by Prakash *et al.*⁸

This study has the largest series of patients with fungal keratitis treated primarily with combined injection of Voriconazole with a success rate of 95% at the end of 2 months follow-up. None of our patients developed any toxic effects with the drug after the injection. The results of this study regarding the significantly improved vision, higher success rate and lower complications with combined Voriconazole injection may favorite this line of therapy as an initial line for management of fungal corneal ulcer. This will decrease the rate of complications during the treatment period than if other modality of therapy is used and also will decrease the rate of penetrating Keratoplasty as we will start the combined injection early and will not wait until the ulcer become resistant with the resultant larger area of ulcer and infiltration which will heal with a large scar, but with early intervention scar may be smaller and may not necessitate Keratoplasty. Further studies and randomized controlled trials are recommended before this choice of treatment assumes a standard approach. The major advantage of this treatment modality is that it delivers the drug at the site of infection, achieving a high intracorneal and intracameral concentration, which may not be possible with topical and systemic antifungal therapy.

In this study, we can conclude that, the safety of combined injection is comparable to topical

Voriconazole but with a more superior efficacy and less complications for the treatment of fungal ulcers which may favorite its use as an initial line of therapy.

Corresponding author

Ali M. Ismail

Ophthalmology Department, Faculty of Medicine,
Sohag University Hospital
aliismail30002000@yahoo.com

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Assessment of DNA Damage in Testes from Young Wistar Male Rat Treated with Monosodium Glutamate

Ismail, N. H.

Zoology Dept., Faculty of Girls for Art Sciences and Education, Ain Shams University
nadia-hussein@hotmail.com

Abstract: Monosodium glutamate (MSG), the sodium salt of glutamate, is a flavor enhancing food additive that may be present in packaged food without appearing on the label. This could lead to inadvertent consumption of monosodium glutamate in high concentrations. The study investigated the effects of MSG on testes of young male Wistar rats, by daily oral exposure to 8g /kg b.wt. of monosodium glutamate for 90 days. Wistar rats (n=24) of average weight of 65- 80g were randomly assigned into two groups A, and B in each group (n=12). The control group (A) received distilled water. The treatment group (B) were given oral doses each was 8g/kg body weight of monosodium glutamate by gavage on daily doses for 90 days. Rats were sacrificed on day 90 of the experiment. The testes were carefully dissected out and quickly fixed in Bouin's for routine histological procedures. Results :The histological changes in the testes of rats due to the administrated MSG were mainly alterations of the seminiferous tubules which included atrophied malformed profiles ,appearance of hyaline material in the lumina of the seminiferous tubules and interstitial connective tissues .In addition sloughing and exfoliation of spermatocytes , spermatids, and immature germ cells appeared into the lumen of the seminiferous tubules .Many cells of the different types of spermatogenesis appeared with pyknotic nuclei and necrotic cells ,and dilated congested inter tubular blood vessels. Vacuolations were also observed between the inner cells of seminiferous tubules in the treated group. Increase in sperm shape abnormality was recorded . Furthermore, the alkaline Comet Assay showed significantly increased tail moment in testes cells of animals treated with MSG compared to control group. Conclusion: MSG may have some deleterious effects on the testes of Wister rats and by extension may contribute to the causes of male infertility. Thus, it is important to reconsider the usage of MSG as a flavor enhancer.

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Key words: DNA Damage; Monosodium Glutamate; Male Rat.

1. Introduction

Monosodium glutamate, MSG, is a widely used flavor enhancing food additive that may be present in packaged foods without appearing on the label .This flavour enhancer, not very long ago, was isolated in the laboratory, and identified Monosodium Glutamate (MSG). At a later stage this flavour gained immense popularity worldwide. Since then it has been in use, widely in restaurants (particularly mixed in noodles, soups etc.), packaged food industries (e.g. instant meals) and household kitchens. Modern commercial MSG is produced by fermentation of starch, sugar, beet sugarcane or molasses (Walker and, Lupien , 2000). This is particularly disturbing given the reported cases of MSG-induced adverse effect in animals (Belluardo *et al.*,1990, Gonzalez-Burgos *et al.*,2004, Mozes *et al.*,2004), even at a relatively lower concentration (Egbonu *et al.*,2009). Although, MSG could improve the palatability of foods by exerting a positive influence on the appetite centre, it increased body weight (Rogers and Blundell, 1990, Egbonu *et al.*,2010). Fallarino *et al.* (2010) demonstrated that Glutamate might affect neuroinflammation via effects on immune cells. However, in humans, adverse effects of MSG appear to manifest in

MSG-sensitive individuals suggesting that some people may have an MSG intolerance that causes MSG symptom complex, with symptoms such as headaches, or migraine in some individuals. In addition, MSG gives rise to a characteristic taste called umami (Yamaguchi and Ninomiya, 1998), which is one of the five taste qualities detected by mammals. Umami is not palatable in itself; nevertheless, it makes a variety of foods delectable (Yamaguchi, 1998).

Previous scientific investigations aimed at determining the effect of MSG on testes (Das and Ghosh, 2010; Igwebuikwe, *et al.*,2011;Ihab, 2012) .MSG caused a reduction in the sperm count (Onakewhor *et al.*,1998; Giovambattista *et al.*, 2003;Nayanatara *et al.*, 2008) . However, it couldn't get any literature regarding the histological studies of the testes and DNA damage in testes treatment of animals with MSG. So the present study was designed to investigate the effects of monosodium glutamate on the testes and cauda epididymal sperm reserves of young male rats. and DNA damage in testes.

2. Material And Methods

(a) Experimental animals

This study was performed on 24 young male Wistar rats, weighing about 65-80 g b.wt.. The animals were bred and maintained under standardized conditions away from any stressful conditions with 12/12 light and dark cycle with free access to food and water in the animal house. They were acclimatized for one week prior to the experiment and caged six per cage in a fully ventilated room at room temperature. All experimental procedures and animal maintenance were conducted in accordance with the accepted standards of animal care.

(b) Tested compound

The chemical used monosodium glutamate (MSG) (C₅H₉NO₄.Na) from Al-Dawlya Chemicals Co., Egypt with Purity > 98% NT., it was dissolved in distilled water, 1g of MSG in 1 ml of distilled water (**Nayanatara et al.,2008**). A stock solution was prepared by dissolving 8g of MSG crystals in 100 ml of distilled water (D.W.). The dose schedule was so adjusted that the amount of MSG administration per animal as per their respective weight.

(c) Experimental design

The animals were divided randomly into 2 groups; each included 12 rats.

Group 1(Control group): The rats were orally given distilled water /day for 90 days.

Group 2(Treated group): The rats were orally given 8g /kg b.wt /day for 90 days.

(d) Histological procedures

At the end of the experimental period, the animals were sacrificed by ether overdose. The testes were excised, rinsed in physiological saline and fixed by immersion in Bouin's fluid for 24 hours. Later, they were dehydrated in graded concentrations of ethanol, cleared in xylene, and embedded in paraffin wax. The sections were cut at 5 µm thick, mounted on glass slides, and stained with hematoxylin and eosin and mounted with (DPX). Sections of testes were examined by light microscope.

(e) Sperm shape abnormality assay

Caudal epididymis was collected from the rats, then sperm-shape abnormality was made according to the technique described by **Wyrobek and Bruce (1978)**. The extracted caudal epididymis from rats were placed in a Petri-plate containing 1 ml of saline solution (0.9%NaCl) at room temperature. The epididymis was cut into small portions to allow the sperms to swim out. After that, smears were prepared using 3-4 drops of the solution, air dried overnight, fixed with absolute methanol for 15 min and stained with

haematoxylin and eosin. 5 hundred sperm per animal were examined to determine the morphological abnormalities under oil immersion.

(f) Comet Assay:

The comet assay was performed as described by **Singh et al. (1988)** with minor modifications. Regular agarose (RA) and low melting point agarose (LMPA) were prepared at 0.75% and 0.5% respectively in Ca⁺⁺ and Mg⁺⁺ free PBS, 110 µL of RA were added to fully frosted microscope slides. 75 µL of LMPA containing 105 cells were added. Finally, a top layer of 75 µL LMPA was added. Slides were immersed in lysis solution for at least 1 h at 4°C, and were then left in alkaline buffer for 15 minutes to allow the expression of alkali-labile sites and DNA unwinding. The slides were electrophoresed at 25V and 300 mA for 20 minutes, washed with neutralizing buffer and stained with ethidium bromide (2g/ml in distilled water).

Observations were made at 400X magnification using a fluorescent microscope (Olympus) equipped with an excitation filter of 515-560 nm and a barrier filter of 590 nm. When possible, fifty cells per animal were analyzed for DNA migration. The tail length was measured from the trailing edge of the nucleus to the leading edge of the tail, using a calibrated scale in the ocular. The severity of DNA damage was measured comparing comet tail lengths (µm) with the diameter of the nucleus of undamaged cells observed in the same field. To determine the effect of malnutrition on the extent of DNA damage, two parameters were evaluated: the mean tail length of DNA migration, (compared by student t test) and the proportion of damaged cells, compared by the nonparametric Mann Whitney two samples test.

Statistical analysis

Data from control and treated animals were analyzed statistically to assess the significant differences using student's t-test. (**Fowler et al., 1998**) was used for comparing the level of significance in the results between the MSG treated group and the untreated control.

3. Results

Histological examination of the testes:

Sections of testes of rats given a daily dose of MSG 8g/kg body weight for 90 days depicted severely damaged of seminiferous tubules. The following microscopical changes were considered as characteristic signs for the severely damaged tubules. The majority of somniferous tubules exhibited, severely atrophied, malformed somniferous tubules, and hyaline material in intertubular connective tissues. At the same time exfoliated spermatids appeared in the lumina due to cellular debris in some tubules and sloughing in

other tubules (Fig.2).Some seminiferous tubules manifested grade damage that included disorganization of spermatogenesis cells, hyaline material involved in between somniferous tubules, and the damaged germ cells lifting off the basal lamina(Fig.3). Abnormality shaped seminiferous tubules ,with hyalinized in intertubular connective tissues were also manifested(Fig.4). Also some seminiferous tubules appeared with maturation arrest. Other tubules displayed marked elongated forms with hyaline material involved in their lumena. Many spematocyte appeared with pyknotic nuclei ,which acquired deeply basophilic stain ability. Also destruction of most spermatogenesis' layers with absence of spermatozoa was clearly recognized in other seminiferous tubule (Fig.5).Daily doses of MSG for 90 days revealed degenerative alteration in seminiferous tubules, maturation arrest in early and late stages of spermatids and numerous vacuoles of variable sizes in both the seminiferous tubules and interstitial connective tissues. Additionally, degenerated spermatogonia and spematocyte were common features (Fig.6--7).At the same time the nuclei of spermatogonia and spermatocytes exhibited signs of pyknosis and necrosis(Fig.6).Dilated congested blood vessels were also detected(Fig.7).

Sperm shape abnormality assay:

From the results obtained, it was clear that MSG caused highly significant ($P < 0.0001$) increase in the average of total sperm abnormalities,head,tail

and head&tail as compared with control as shown in (Table1).Figs.(8&9,a-1) showed various morphological sperm-shape abnormalities in control and treated group. Tail abnormalities were included as disconnected tail (Fig.8). Head abnormalities were represented as banana, hookless, triangular, amorphous and double head (Fig. 9).

Comet Assay:

Comet assay revealed that, MSG induced statistically significant ($P < 0.05$) increase in the average of the tail moment from (6.66 ± 0.56) in control group to (13.01 ± 3.9) in treated group, (Table 2).

Figure 10& table 2 show the distribution pattern of nuclear DNA expressed in the present cells as five comet classes from type 1 (undamaged) to type 5 (maximally damaged) in testes of rats treated with MSG. The results in Table 2 indicate that the administration of MSG decreased the level of undamaged cells when compared to control, average reached 30 ± 1.58 & 50 ± 2.52 respectively, and increased levels in type 2 and 3 (55.2 ± 1.3 & 8.8 ± 1.92) compared with control (45.8 ± 1.92 & 4.2 ± 1.7).Some cells nuclei showed high levels of DNA damaged (Category 4) the average reach 4 ± 0.7 and the other show maximally damaged (Category 5) recorded 2 ± 0.7 .

Table (1): Showing sperm abnormalities in rats administered MSG for 90 .

Groups	Total abnormalities (M ± SD)	Average of abnormally spems/500		
		Head (M ± SD) %	Tail (M ± SD) %	H&T (M ± SD) %
Control	7.4 ± 1	3.6 ± 0.5	2.4 ± 0.5	1.4 ± 0.6
Treated	21.8 ± 2.6	9.2 ± 0.8	9.2 ± 0.9	3.4 ± 0.6
Percentage	208.1	155.6	323.8	142.9

Values are mean (±SD) of 5 rat.

Table (2): Showing tail moment and types of damaged DNA in rats administered MSG for 90 days.

Groups	Tail moment (M±SD)	Average of types of damaged DNA				
		Type 1	Type 2	Type 3	Type 4	Type 5
Control	6.66 ± 0.6	50 ± 2.5	45.8 ± 1.9	4.2 ± 1.7	non	non
Treated	13.01 ± 3.9***	30 ± 1.6***	55.2 ± 81.3***	8.8 ± 1.9***	4 ± 0.7***	2 ± 0.7***
Percentage	95.43	- 40	20.5	11		

*** Significant at P value < 0.001

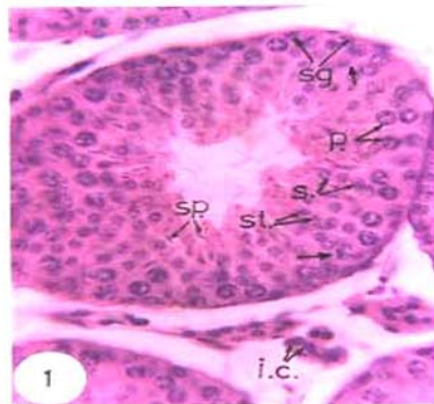


Fig (1) photomicrograph of testes section of control rat showing normal spermatogenesis progression, and interstitial cells. H.E X 200

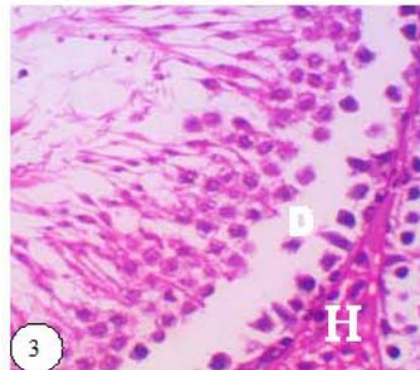
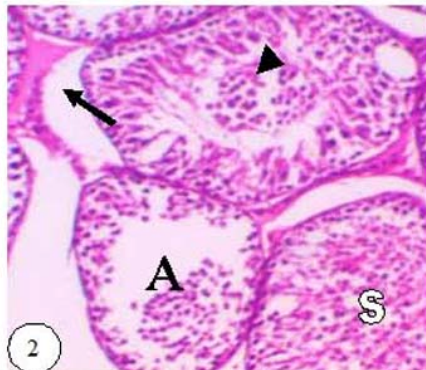


Fig (2) photomicrograph of testes section of treated showing severe atrophied seminiferous tubule (A) hyalinization with loss of interstitial connective tissue cells (arrow).Also accumulation of cellular debris within the lumina of some seminiferous tubules (head) another appeared sloughed(s) (X200)
 Fig(3) photomicrograph of testes section of treated showing disorganization of spermatogenic cells and between the seminiferous tubules(H)damaged germ cells are lifting off the basal lamina (D) (X400)

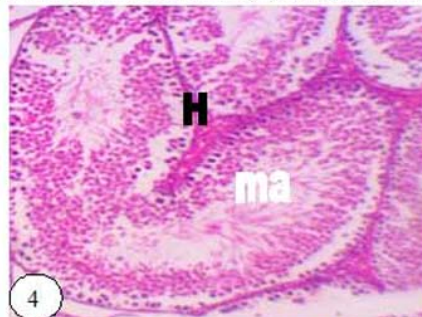


Fig (4) photomicrograph of testes section of treated showing abnormal shape of S.T. and damage Interstitial cells with hyalinization (H).and S.T. showed maturation arrest (ma) (X100)
 (5) Showing elongated, with hyalinization in lumina and marked reduction of spermatocytes (head) and Pyknosis of nuclei (arrow), destruction of most spermatogenesis layers and absence of spermatozoa oth (Short arrow). X400

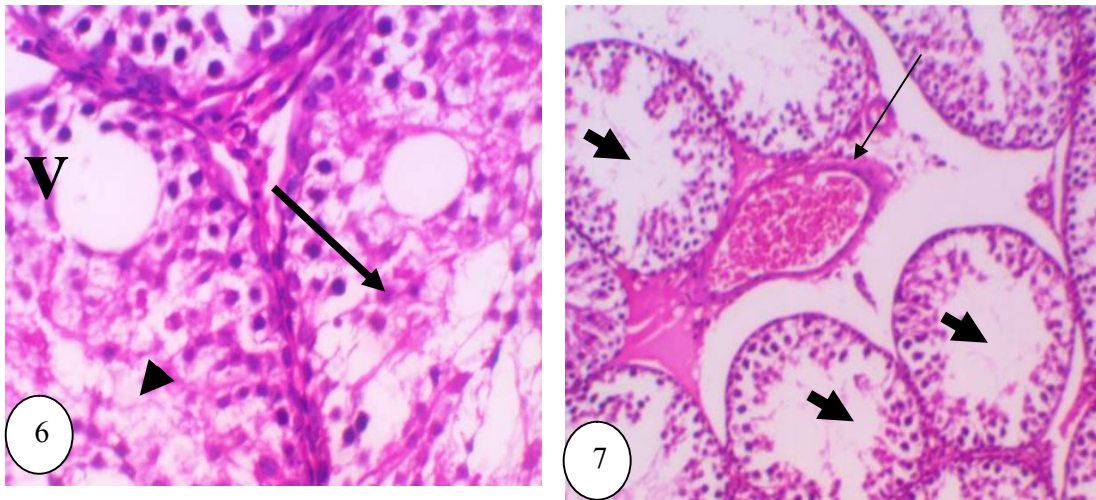


Fig (6): Photomicrograph of testes section of treated showing vacuolation (v) in between the spermatogenic cells, reduction of spermatocytes with pyknotic nuclei (arrow) also absence of sperms (head). (x400)
 Fig.(7):Photomicrograph of testes of treated showing dilated congested blood vessel. (Long arrow) loss of spermatids (x200)

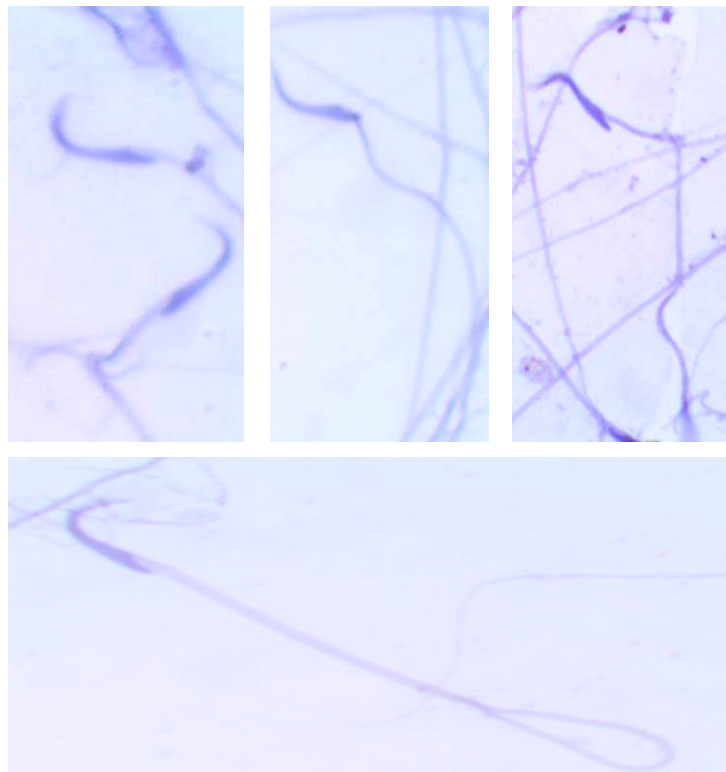


Fig. (8): Showing abnormal tail of sperms. X100

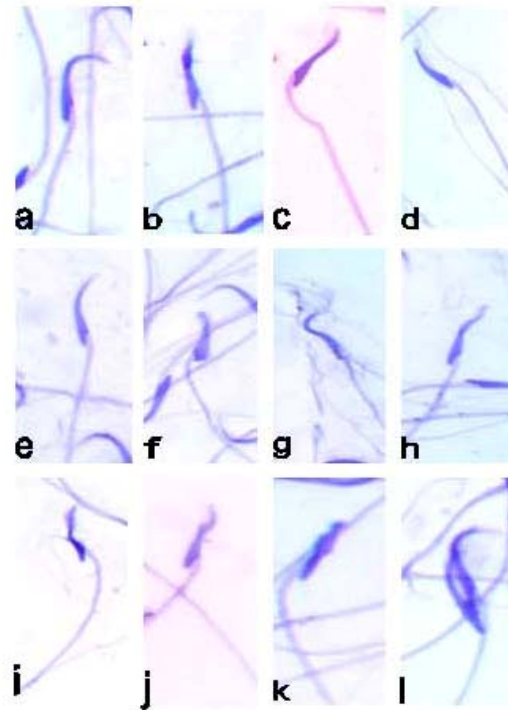


Fig. (9):Showing normal and abnormal head of sperms.(a) normal,(b,c &d) banana shape, (e) hook less, (f)triangular, (g,h,i,j&k)amorphous and (l) double headed . X1000

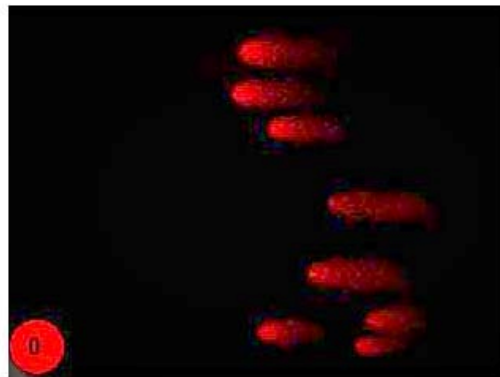


Fig. (10) Photograph comet assay of testes showing D.N.A damage induced after treatment of MSG for 90 days.

4. Discussion

Monosodium glutamate (MSG) is a widely used flavor enhancing food additive that may be present in packaged foods without appearing on the label. This could lead to inadvertent consumption of MSG above the average daily intake of 1.0 g in enlightened societies (Marshall, 1994). However,

inadvertent abuse of this food additive may occur because of its abundance, mostly without labeling, in many food ingredients (Egbonu *et al.*, 2009).

The histological changes evoked in the testes by the administered MSG were mainly alterations of the seminiferous tubules which included atrophied seminiferous tubules, appearance of

hyaline material in the lumina of seminiferous tubules and interstitial connective tissues. In addition sloughing and exfoliation of spermatocytes, spermatids, and immature germ cells appeared into the lumen of the seminiferous tubules. Many nuclei of different types of spermatogenesis appeared pyknotic and necrotic, and dilated congested inter tubular blood vessels, and vacuolations were also observed between the inner cells of seminiferous tubules in the treated group, accompanied by maturation arrest in others.

A great deal of changes recorded in the current investigation are in accordance to the histological studies that were carried out on the testes of defirent animals treated with MSG, **Das and Ghosh (2010)** found that the MSG induced histological changes in the testes of neonatal mice showed that both the germinal epithelium and Leydig cells were affected. **Mohamed (2012)** reported that the treatment with MSG at short-term exhibited slight to moderate damaged seminiferous tubules, included vacuoles were found inside the cytoplasm of spermatogonia and loss of late spermatids, shrinkage, widening of the spaces between the tubules. Long-term treatment caused severe damage of germ cells and large masses of necrotic cells were present in many tubules. Focusing on exfoliated, sloughing early spermatids and vacuolation in some damage of seminiferous tubules proved the presence of many signs of deterioration of these cells of tubules.

Present results were more or less in accordance with those encountered in the testicular tissue of rats after treatment with a toxic substance nitroso-dimethylamine (NDMA). **Attalla(1966)** suggested that these histological changes may be due to either local effect of the chemical or indirectly caused by imbalance in gonadotrophic hormones. **Balasubramanian et al. (1980)** explained the congestion of blood vessels as being due to the inhibition of prostaglandins synthesis, since these compounds are known to be involved in regulation of testicular blood flow. **El-Deeb et al.(2000)** stated that vacuolation and exfoliation might be a sign of testicular toxicity and cell degeneration. The maturation arrest observed in the present study was explained by (**El-Zayat, 1988**, **El-Wesemy,2007**) who correlated this arrest to the testosterone inhibition which caused stopping of spermatogenesis.

Previous research's have explained the mechanisms by which MSG inhibited the spermatogenesis in the current experiment. Glutamate receptors are present in different tissues: hypothalamus, spleen, thymus, liver, kidneys, endocrine system, ovaries, etc. (**Gill and Pulido, 2005; Gill et al., 2008**). Earlier studies proved the presence of functional glutamate transporters and receptors in testes of rat (**Gill et al., 2000. Takarada et al., 2004**) and (**Hu et al., 2004**) in

mice. Therefore, Testes are considered as target organ for MSG. So, one of the mechanisms may be a direct effect of MSG via glutamate receptors and transporters of the epithelial cells of the seminiferous tubules. The second mechanism was proved by other researchers (**Gong et al., 1995; Giovabattisa et al., 2003**); that stipulates that there are neurotoxin effects of MSG on function of hypothalamus-pituitary-gonadal system. The effects of such toxicants on male reproduction may be anatomical or only functional, depending on whether they produce structural changes in the reproductive system, or merely affect the functions of the reproductive organs (**Witorsch, 1995**). **Franc et al. (2006)** reported that the central nervous system of MSG-treated animals showed neurogenic function in the levels of FSH, LH and testosterone. These hormones are essential for normal testes function and healthy spermatogenesis. In mammals, spermatogenesis is totally dependent upon testosterone (**Pakarainen et al., 2005; Wang et al., 2009**). Glutamate is a predominant excitatory neurotransmitter in the mammals central nervous system (**Schlett, 2006; Greenwood and Connolly, 2007; Liguz-Leczna and Skangiel-Kramaska, 2007**). Such excessive activation of glutamate receptors and overloading with intracellular calcium can induce neural death (**Gil-Loyzaga et al., 1993**). Therefore, the present study suggested that spermatogenesis was affected indirectly via the hypothalamic lesions. The ability of monosodium glutamate to damage nerve cells of the hypothalamus is a pointer to the fact that it may alter the neural control of reproductive hormone secretion via the hypothalamic-pituitary-gonadal regulatory axis. Such alterations in reproductive hormone secretion may adversely affect the reproductive capacity of the affected animals.

The third mechanism reported that exposure to MSG resulted in a decrease in the testicular

ascorbic acid level that could lead to oxidative damage of rat testes (**Nayanatara et al., 2008; Vinodini et al., 2010**), and oxidative damage in different organ (**Moreno et al., 2005; Farmobi and Onyemia, 2006; Pavlovic et al., 2007**)

There was a significant reduction in the caudal epididymal sperm reserves ($P < 0.05$) of the rats that received monosodium glutamate relative to the control rats. Present results agree with, previous studies found that animals treated with MSG revealed a reduction in sperm count and increased incidences of abnormal sperm (**Giovambattista et al., 2003; Nayanatara et al., 2008**). **Igwebuikie et al.(2011)** showed that the indication is that the reduced caudal epididymal sperm counts observed in the MSG-treatment rats may be the end result of a considerable decline in the influence of testosterone on spermatogenesis in these rats.

Egbuonu et al. (2011) reported that exposure to high dose of monosodium glutamate (15 g/kg), such as through its inadvertent abuse, may alter lipid status in animals by damaging high metabolic organs, such as the liver.

From the above mentioned results, it was clear that the sperm shape abnormality assay and comet assay effected of treatment with MSG on rat testes the adverse effects of MSG could have been due to oxidative damage induced by MSG on this tissue. previous studies showed that administration of MSG induces oxidative damage and changes in the level of lipid peroxide (**Moreno et al., 2005**; **Farmobi and Onyemia, 2006**; **Pavlovic et al., 2007**; **Nayanatara et al., 2008**; **Vinodini et al., 2010**). Lipid peroxidation (LP) is one of the main manifestations of oxidative damage and has been found to play an important role in the toxicity and carcinogenesis of many carcinogens. Free radicals are known to attack the highly unsaturated fatty acids of the cell membrane to induce LP which considered a key process in many pathological events and is one of the reactions induced by oxidative stress (**Schinella et al., 2002**).

Damage of DNA of the present study are similar to that obtained by some investigators using different treatments were recorded. **Dobrzynska (2007)** found that X-rays enhanced DNA damage in somatic and germ cells caused by acrylamide. Such damage in somatic cells, if not repaired or if repaired incorrectly can lead to mutation, cancer transformation or cell death in bone marrow, spleen, liver, kidney, lungs and testes. Damage in germ cells can affect the process of fertilization and lead to spontaneous abortion, congenital malformations and in heritable diseases, including cancer in the offspring (**Olsham et al., 1993**, **Wyrobck et al., 1997**). In particular, the integrity of germ cell DNA plays an important part in the transmission of genetic information to the offspring. DNA damage measured using the comet assay in human spermatozoa has been shown to be associated with infertility (Irvine et al., 2000). MSG has a toxic effect on many body organs by altering ionic permeability of neural membrane and induces persistent depolarization (**Robinson, 2006**).

The current study has been the first to the best of researchers' knowledge to describe the histological, sperm shape abnormality assay and comet assay of the rat testes that resulted from daily oral treatment with MSG for 90 days. However, treatment may lead into infertility problem in rats. Accordingly, using MSG as flavor enhancer should be reconsidered and it is the time to stop the slow poisoning of mankind via such flavors enhancers.

Corresponding author
Ismail, N. H.

Zoology Dept., Faculty of Girls for Art Sciences and Education, Ain Shams University
nadia-hussein@hotmail.com

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Mandibuloacral Dysplasia Mutation Detection in Three Egyptian Families: A Report of a Novel Mutation

Khalda Amr¹, Mostafa Mostafa² and Ghada El-Kamah³

¹Dept. of Molecular Genetics, Human Genetics & Genome Research Division, National Research Center (NRC), Cairo, Egypt.

² Dept. of Oro-Dental Genetics, Division of Oro-Dental Research, NRC, Cairo, Egypt.

³Dept. of Clinical Genetics, Human Genetics & Genome Research Division, NRC, Cairo, Egypt.

Khalda_nrc@yahoo.com

Abstract: Mandibuloacral dysplasia (MAD) is a rare autosomal recessive disorder characterized by mandibular and clavicular hypoplasia, acroosteolysis, delayed closure of the cranial suture and joint contractures. Mutations in *Lamin A/C* have been reported in patients with MAD. Laminopathies refer to many disorders caused by defects in the nuclear lamina associated proteins. Lamins are integral structural components of the nuclear lamina hypothesized to be involved in numerous cellular processes. LMNA gene maps to chromosome 1q21.2 and encodes lamin A and lamin C through alternative splicing. We investigated three consanguineous Egyptian families having severe MAD disorder. Subsequently, direct sequencing of the coding region of the LMNA gene in patients and their parents revealed the identification of two homozygous missense mutations that replace a conserved residue: Arginine 527, a novel R527L mutation in one patient and R527C mutation in the other two patients.

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Key word: Mandibuloacral dysplasia MAD, LMNA gene, mutation.

1. Introduction

A-type lamins (lamins A and C), encoded by the *LMNA* gene, are major protein constituents of the mammalian nuclear lamina, a complex structure that acts as a scaffold for protein complexes that regulate nuclear structure and functions (Andrés and González, 2009). LMNA, which maps to chromosome 1q21.2 and encodes lamin A and lamin C through alternative splicing of LMNA is responsible for many unrelated diseases with different affected organ systems attributed to lamin A/C mutations. These include Charcot-Marie-Tooth disease type 2B (De Sandre-Giovannoli *et al.*, 2002), forms of dilated cardiomyopathy (Fatkin *et al.*, 1999), both autosomal dominant and autosomal recessive forms of Emery-Dreifuss muscular dystrophy (Raffaele Di Barletta *et al.*, 2000), limb girdle muscular dystrophy type 1B (Muchir *et al.*, 2000), Dunnigan-type familial partial lipodystrophy (Speckman *et al.*, 2000) and Hutchinson-Gilford progeria (Prabhavathi *et al.*, 2011).

Patients with MAD type A (partial) lipodystrophy have mutations in *lamin A/C* gene. Mutation in the zinc metalloproteinase (*ZMPSTE24*), also involved in the proteolytic cleavage of pre lamin A to form mature lamin A, have been noted in patients with MAD and type B (generalized) lipodystrophy (Agarwal *et al.*, 2003).

Mandibuloacral dysplasia (MAD; MIM 248370) is a rare autosomal recessive disorder combining a characteristic facial appearance with acro-osteolysis and lipodystrophy. In the majority of MAD patients,

Symptoms appear around the age of four years, mostly in the form of mandibular hypoplasia with subsequent teeth crowding, delayed cranial suture closure, dysplastic clavicles, acroosteolysis, and typical facial changes. Patients may also suffer; lipodystrophy and clinical features of metabolic syndromes as insulin resistance diabetes mellitus and hypertriglyceridemia (Agarwal *et al.*, 2008). MAD is considered a progeroid disorder, so far one mutation in LMNA has been associated with the disease, R527H (Simha *et al.*, 2003). A homozygous missense mutation (R527H) in *LMNA* gene was first reported in nine Italian MAD patients with type A pattern of lipodystrophy belonging to five consanguineous pedigrees (Novelli *et al.*, 2002). The R527 amino acid is located in the C-terminal domain common to lamin A and lamin C, which has an immunoglobulin-like three-dimensional structure. R527 is localized at the external surface of the domain, and thus R527H substitution would disrupt the surface structure of the protein, altering binding fundamental sites (Dhe-Paganon *et al.*, 2002). However, we report a fully expressed phenotype of MAD Egyptian patient with a novel homozygous mutation R527L in *LMNA* gene in addition to the previously reported R257C.

2. Patients and Methods

Four Egyptian patients descending from three families were included in our study. A written informed consent was obtained from the patients' parents. Genomic DNA was extracted from peripheral mononuclear cells of patients and their parents by QIAamp blood kits (Qiagen, Hilden, Germany). Direct

sequencing of the entire coding region and the surrounding intron-exon boundaries of the LMNA gene was conducted for the probands and their parents from each pedigree. Primers of all twelve coding exons were amplified according to the published sequence information (De Sandre-Giovannoli *et al.*, 2002). PCR reaction was carried out in a total volume of 50ml containing 150ng DNA using the following condition 95°C for 5 min followed by 35 cycles of 95°C for 50 sec, annealing for 50 sec, 72°C for 50 sec and extension of 72°C for 7 min. The PCR products were purified with a QIA quick PCR purification Kit (Qiagen). Cycle sequencing reactions were performed

on column-purified PCR product using Big Dye terminator cycle sequencing Kit (Applied Biosystem, Foster City, CA). Both strands of each exon were sequenced and analyzed on ABI Prism 310 automated sequencer (Applied Biosystem).

3. Results:

Four Egyptian patients descending from three unrelated families, fulfilling the diagnostic criteria of MAD through full clinical, dental examination as well as molecular findings (Table1), were included in our study. Patients No. 3 & 4 are two sibs.

Table1: Molecular findings with full clinical and dental examination.

Patient No.	1	2	3	4
Sex	Male	Female	Female	Male
Age of onset in months	9	18	7	8
Consanguinity	+ve	+ve	+ve	
Progeroid facies. Fig.1	+	+	+	+
Hypoplastic mandible	+	+	+	+
Hypoplastic clavicle	+	+	+	+
Acroosteolysis Fig. 2	+	+	+	+
Stature	-3.5 SD	-3.66 SD	-2.42 SD	-2.7 SD
Oro-Dental findings	Both mandibular and maxillary hypoplasia with subsequent teeth crowding, microstomia, limitation of mouth opening and gingivitis. Radiographic examination showed hypoplastic condyle and accentuated antgonial angle of the mandible (Fig. 1&3)			
Metabolic disorders	-	-	-	-
Molecular diagnosis	Arginine527leucine	Arginine527cysteine		

Direct sequencing of the LMNA gene of the first patient revealed a novel missense mutation R257L resulting in substitution of Arginine by leucine (Fig. 4). A transition of G to T at the second base of codon 527 resulted in a missense mutation. While sequencing of the other three patients revealed a substitution of the

first base (C > T) at codon 527 leading to the transversion of Arginine to cysteine R257C. All detected mutations were present in a homozygous state in our studied patients and in a heterozygous state in their parents (Fig.4).



Fig. (1): Progeroid facies



Fig. (2): Acro-osteolysis



Fig.(3): Panoramic radiograph showing teeth crowding, hypoplastic condyle and accentuated antegonial angle

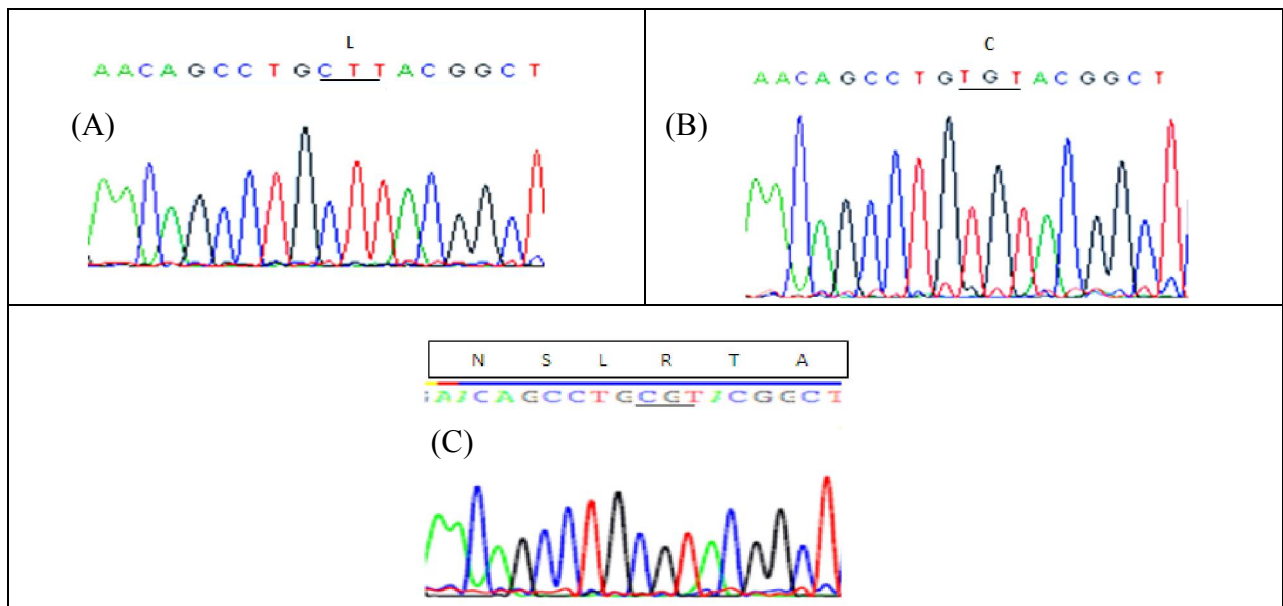


Fig.4 DNA sequence chromatograms of LMNA gene in 3 patients with MAD.

Figure (A) showing patient 1 with transition of G>T (R 527 L).

- **Figure (B) showing patient 2 and 3 with transition of C>T (R 527 C).**
- **Figure(C) showing the normal sequence.**

4. Discussion:

Laminopathies refers to different disorders with phenotypic variability caused by defects in the nuclear lamina associated proteins. We report the first study for four Egyptian patients with MAD syndrome. A novel homozygous mutation in codon 527, in which the Arginine is converted to Lucien in one patient—in addition to the previously reported, Arg 527 cys, in three patients (Agarwal *et al.*, 2008). It is highly likely that the Arg257leu missense mutation causes inactivation of LMNA protein; since the other previously reported mutation R257H is affecting the same amino acid in many MAD patients (Novelli *et al.*, 2002), suggesting that this amino acid plays a key role in the structure and function of the LMNA protein. Presumably, the substitution of a basic amino acid

(Arginine) with a non- polar amino acid (cysteine) at codon 527 may alter the polarity of the protein, leading to conformational modifications of the protein.

So far, approximately 28 MAD patients have been reported to harbor homozygous or compound heterozygous missense mutations in the C-terminal of lamin A/C (Novelli *et al.*, 2002; Cao and Hegele, 2003; Simha *et al.*, 2003; Plasilova *et al.*, 2004; Garg *et al.*, 2005; Van Esch *et al.*, 2006; Kosho *et al.*, 2007; Lombardi *et al.*, 2007; Agarwal *et al.*, 2008; Zirn *et al.*, 2008; Garavelli *et al.*, 2009; Madej-Pilarczyk *et al.*, 2009). Nearly all of them have type A pattern of partial lipodystrophy. Our studied patients have a homozygous mutation in codon 527 and they all suffer manifestations of partial lipodystrophy together with all other clinical manifestations of MAD.

However, the age of onset among our studied patients was considerably younger than previous reports (Agarwal *et al.*, 2008). Only 6 MAD patients have been reported with either compound heterozygous or homozygous mutations in *ZMPSTE24* (Agarwal *et al.*, 2003; Shackleton *et al.*, 2005; Agarwal *et al.*, 2006; Denecke *et al.*, 2006; Miyoshi *et al.*, 2008).

Mutations in *LMNA* gene have been reported in 23 patients with MAD, which include homozygous Arg527His mutation in six Italian and two Hispanic pedigrees, homozygous Ala529Val mutation in two Turkish pedigrees, homozygous Ala529Thr mutation in a Japanese woman, homozygous Lys542Asn mutation in an Indian pedigree, and compound heterozygous Arg471Cys/Arg527Cys and Arg527His/Val440Met mutations in two Caucasian pedigrees (Fatkin *et al.*, 1999; Muchir *et al.*, 2000; Raffaele Di Barletta *et al.*, 2000; Speckman *et al.*, 2000; De Sandre-Giovannoli *et al.*, 2002; Agarwal *et al.*, 2003; Cao and Hegele, 2003; Agarwal *et al.*, 2008; Andrés and González, 2009; Prabhavathi *et al.*, 2011).

Our finding of a homozygous missense mutation, Arg527Cys LMNA mutation was firstly reported in a 7-yr-old girl German-Irish MAD patient descending from a consanguineous pedigree (Agarwal *et al.*, 2008). Subsequent subjects have been described with homozygous LMNA mutations causing R527C or A529V amino-acid substitutions (Garg *et al.*, 2005; Agarwal *et al.*, 2008). Lombardi *et al.*, 2007, also reported a compound heterozygous subject for the LMNA R527H and a V440M mutation with some features of mandibuloacral dysplasia, lack of muscle strength, and decreased muscle tone.

Interestingly, Lloyd *et al.*, 2002, have identified a binding site of lamin A for the adipocyte differentiation factor sterol-response element-binding protein 1 (SREBP1) between residues 227 and 487. This confirms the possibility that fat loss observed in FPLD and MAD, may be caused by reduced binding of the adipocyte-differentiation factor SREB to lamina (Novelli *et al.*, 2002). The polypeptide amino acids from 470 to 545 has been crystallized and shown to assume an Ig domain. The substitution of Arg to His at position 527 showed disruption of the salt bridge between Arg 527 and Glu at position 537. Similarly, the substitution of Arg 527 to Cys causes salt bridge disruption (Agarwal *et al.*, 2008). Simha *et al.*, 2003, have confirmed that only type A MAD is caused by the R527H mutation in LMNA and no mutations were detected in four families with type B MAD.

Conclusion:

In a first study of Egyptian MAD patients, we concluded that Egyptian mutation spectrum would belong to the LMNA gene (527 locus type) & not to *ZMPSTE24*. We also report a novel mutation (not previously reported according to our date search). We

also concluded an earlier age of onset of the disease among Egyptian patients.

Corresponding author

Khalda Amr

Dept. of Molecular Genetics, Human Genetics & Genome Research Division, National Research Center (NRC), Cairo, Egypt.

Khalda_nrc@yahoo.com

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Measures of Performance in the $M/M/1/N$ Queue via the Methods of Order Statistics

Yousry H. Abdelkader¹ and A. I. Shawky²

¹Math. Dept., Faculty of Science, Alexandria University, Egypt

²Dept. of Statistics, Faculty of Science, King Abdulaziz University, P.O.Box 80203, Jeddah 21589, Saudi Arabia
yabdelkader@yahoo.com

Abstract: This paper computes new measures of performance in Markovian queueing model with single-server and finite system capacity. The expected value and the variance of the minimum (maximum) number of customers in the system (queue) as well as the r th moments of the minimum (maximum) waiting time in the queue are derived. The computations of the proposed measures are depending on the methods of order statistics. The regular performance measures of the $M/M/1/N$ model are considered as special cases of our results.

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Key Words: Queueing; Performance measures, Order statistics, Busy period, Waiting time, $M/M/1/N$ queue.

1. Introduction

The study of queueing systems has often been concerned on the busy period and the waiting time, because they play a very significant role in the understanding of various queueing systems and their management. A busy period in a queueing system normally starts with the arrival of a customer who finds the system empty, and ends with the first time at which the system becomes empty again. Takagi and Tarabia (2009) provided an explicit probability density function of the length of a busy period starting with i customers for more general model $M/M/1/N$, where N is the capacity of the system, see also Tarabia (2001). Al Hanbali and Boxma (2010) studied the transient behaviour of a state dependent $M/M/1/N$ queue during the busy period. Virtual waiting time at time t is defined to be the time that an imaginary customer would have to wait before service if they arrived in a queueing system at instant t . Gross and Harris (2003, Chapter 2) gave a derivation of the steady-state virtual waiting time distribution for an $M/M/c$ model. Berger and Whitt (1995) provided various approximation and simulation techniques for several different queueing processes (waiting time, virtual waiting time, and the queue length). For more general queue models in waiting time, see Brandt and Brandt (2008). Limit theorems are proved by investigating the extreme values of the maximum queue length, the waiting time and virtual waiting time for different queue models. Serfozo (1987) discussed the asymptotic behavior of the maximum value of birth-death processes over large time intervals. Serfozo's results concerned on the transient and recurrent birth and death processes and related $M/M/c$ queues. Asmussen (1998) introduced a survey of the present state of extreme value theory for queues and focused on the regenerative properties of queueing systems, which reduce the problem to study the tail of the maximum $\bar{X}(\tau)$ of the queueing process $\{X(t)\}$ during a regenerative cycle τ , where $\{X(t)\}$ is

in discrete or continuous time. Artalejo *et al.* (2007) presented an efficient algorithm for computing the distribution for the maximum number of customers in orbit (and in the system) during a busy period for the $M/M/c$ retrial queue. The main idea of their algorithm is to reduce the computation of the distribution of the maximum number of customers in orbit by computing certain absorption probabilities. Details about the extreme values in queues can be found in Park (1994) and Minkevičius (2009) and the references cited herein.

Our motivation is to obtain some complementary measures of performance of an $M/M/1/N$ queue. The expected value and the variance of the minimum (maximum) number of customers in system (queue) as well as the r th moments of the minimum (maximum) waiting time will be discussed. This paper generalized the work of Abdelkader and Al-Wohaibi (2011). Their results can be obtained as special case of the results presented in this paper when $N \rightarrow \infty$.

Let us divide the number of arrival customers into k intervals and let X_j be the number of customers in each interval, the corresponding order statistics is defined by $X_{i:k}$. Three special cases are introduced:

(a) $i=k$, defines the maximum number of customers presented in the system, (b) $i=1$, defines the minimum number of customers in the system, (c) $i=k=1$, defines the regular performance measures. So, our interest is to compute

$$\mu_{1:k} = E(X^{Min}), \quad \sigma_{1:k}^2 = Var(X^{Min}),$$

$$\mu_{k:k} = E(X^{Max}) \quad \text{and} \quad \sigma_{k:k}^2 = Var(X^{Max}) \quad \text{where}$$

$$X^{Min} = \text{Min}_{1 \leq j \leq k} \{X_j\} \quad \text{and} \quad X^{Max} = \text{Max}_{1 \leq j \leq k} \{X_j\}.$$

T_i be the waiting time in the interval i . The expected value and the variance of the minimum (maximum) waiting times are computed respectively, as

$$\nu_{1:k} = E(T^{Min}), \quad \alpha_{1:k}^2 = Var(T^{Min}), \quad \nu_{k:k} = E(T^{Max})$$

and $\alpha_{k:k}^2 = \text{Var}(T^{Max})$, where $T^{Min} = \text{Min}\{T_j\}_{1 \leq j \leq k}$ and $T^{Max} = \text{Max}\{T_j\}_{1 \leq j \leq k}$, for $1 \leq i \leq k$.

2. Model and Description

Consider an M/M/1/N queue with arrival rate λ and service rate μ , where N denotes the capacity of the system with single server. Let $Q(t)$ be the number of customers in the system at time t . We define

$$p_n(t) = \text{prob}\{Q(t) = n | Q(0) = i\}.$$

Then the governing differential - difference equations for $p_N(t)$, the probability of having $n=N$ customers in the system during t , for $h > 0$, is given by

$$p_N(t+h) = p_N(t)(1-\mu h) + p_{N-1}(t)(\lambda h)(1-\mu h), n = N. \quad (1)$$

In steady-state equation for M/M/1/N queue representing this

$$\begin{aligned} -\rho p_0 + p_1 &= 0, & n = 0, \\ -(1+\rho)p_n + p_{n+1} + \rho p_{n-1} &= 0, & 0 < n < N, \\ -p_N + \rho p_{N-1} &= 0, & n = N. \end{aligned}$$

The solution of the above equations is given by

$$p_n = \begin{cases} \left(\frac{1-\rho}{1-\rho^{N+1}}\right)\rho^n, & \rho \neq 1 \\ \frac{1}{N+1}, & \rho = 1 \end{cases} \quad n = 0, 1, 2, \dots, N. \quad (2)$$

Note that $\rho = \frac{\lambda}{\mu}$ need not be less than one because the number allowed in the system is controlled by the queue length (N-1), not by the rate of arrival and departure, λ and μ , respectively. It is easy to see that the effective rate $\lambda_{eff} = \lambda(1-p_N)$.

Let Q be the number of customers in the system. Define the cumulative distribution function (cdf) of Q as:

$$\begin{aligned} F(x) &= \text{Pr}\{Q \leq x\} \\ &= \sum_{n=0}^x p_n, \end{aligned}$$

Where x assumed to be integer.

For M/M/1/N queue, we have

$$F(x) = \begin{cases} \frac{1-\rho^{x+1}}{1-\rho^{N+1}}, & \rho \neq 1 \\ \frac{x+1}{N+1}, & \rho = 1. \end{cases} \quad (3)$$

In the following, we state two of the needed theorems. These two theorems can be found in Arnold *et al.* (1992) and Barakat and Abdelkader (2004), respectively. the first theorem gives expressions for the first two moments of the i th order statistics, $X_{i:k}$, in a sample of size k in discrete case and the second theorem deals with the r th moments of $X_{i:k}$ in a continuous case.

Theorem 1. Let S , the support of the distribution, be a subset of non-negative integers. Then

$$E(X_{i:k}) = \mu_{i:k} = \sum_{x=0}^{\infty} [1 - F_{i:k}(x)]$$

and

$$E(X_{i:k}^2) = \mu_{i:k}^{(2)} = 2 \sum_{x=0}^{\infty} x [1 - F_{i:k}(x)] + \mu_{i:k}$$

whenever the moment on the left-hand side is assumed to exist.

Hence, the variance is given by

$$\sigma_{k:k}^2 = \mu_{k:k}^{(2)} - \mu_{k:k}^2.$$

In the case of independent identically distributed (*iid*) random variables, we have

$$\mu_{k:k} = \sum_{x=0}^{\infty} [1 - (F(x))^k], \quad (4)$$

$$\mu_{1:k} = \sum_{x=0}^{\infty} [1 - F(x)]^k, \quad (5)$$

$$\mu_{k:k}^{(2)} = 2 \sum_{x=0}^{\infty} x [1 - (F(x))^k] + \mu_{k:k}, \quad (6)$$

$$\mu_{1:k}^{(2)} = 2 \sum_{x=0}^{\infty} x [1 - F(x)]^k + \mu_{1:k}, \quad (7)$$

$$\sigma_{k:k}^2 = \mu_{k:k}^{(2)} - \mu_{k:k}^2, \quad \sigma_{1:k}^2 = \mu_{1:k}^{(2)} - \mu_{1:k}^2, \quad (8)$$

where $F_{k:k}(x) = [F(x)]^k$ and $F_{1:k}(x) = 1 - [1 - F(x)]^k$.

Theorem 2. Let $X_i, 1 \leq i \leq k$, be a non-negative r.v.'s with d.f.'s $F_i(x)$. Then, the r th moment of the i th order statistics in a sample of size k is given by

$$\mu_{i:k}^{(r)} = r \int_0^{\infty} x^{r-1} (1 - F_{i:k}(x)) dx.$$

In case of *iid* random variables, when $i=k$ and $i=1$, we get respectively

$$\mu_{k:k}^{(r)} = r \int_0^{\infty} x^{r-1} (1 - [F(x)]^k) dx, \quad (9)$$

$$\mu_{1:k}^{(r)} = r \int_0^{\infty} x^{r-1} [1 - F(x)]^k dx. \quad (10)$$

3. Performance measures

This section is devoted to introduce the proposed performance measures which are often useful for investigating the behavior of a queueing system. The mean and the variance of the minimum (maximum) number of customers in system as well as the mean and the variance of the minimum (maximum) waiting times are derived. The following lemma and consequence theorems give a procedure for the computations of these measures.

Lemma 1. Let X_i be *iid* r.v.'s, the cdf for the maximum, $F_{k:k}$, and the minimum, $F_{1:k}$, are given by

$$\begin{aligned} i) \quad F_{k:k}(x) &= \frac{(1-\rho^{x+1})^k}{(1-\rho^{N+1})^k}, & \rho \neq 1 \\ &= \frac{(x+1)^k}{(N+1)^k}, & \rho = 1, \end{aligned} \quad (11)$$

$$\begin{aligned} ii) \quad F_{1:k}(x) &= 1 - \frac{\rho^{(x+1)k}}{(1-\rho^{N+1})^k} \sum_{i=0}^k (-1)^i \binom{k}{i} \rho^{i(N-x)}, & \rho \neq 1 \\ &= 1 - \left(\frac{N-x}{N+1}\right)^k, & \rho = 1. \end{aligned} \quad (12)$$

Proof. From the definition of the *cdf* of $X_{k:k}$ and $X_{1:k}$, we have

$$F_{k:k}(x) = [F(x)]^k, \\ F_{1:k}(x) = 1 - [1 - F(x)]^k.$$

Plugging the value of $F(x)$ from (3) into the last two equations, we get (11) and (12). This completes the proof. ■

3.1 The mean and the variance of the minimum and the maximum number of customers in the system

We are now ready to formulate our first result.

Theorem 3. The expected value and the variance of the maximum number of customers in the system are given by

$$\mu_{k:k} = N - \frac{1}{(1-\rho^{N+1})^k} \sum_{i=0}^k (-1)^i \binom{k}{i} \frac{\rho^i (1-\rho^{iN})}{1-\rho^i}, \quad \rho \neq 1 \\ = N - \frac{S(N,k)}{(N+1)^k}, \quad \rho = 1, \quad (13)$$

where,

$$S(n,k) = \sum_{x=1}^n x^k, \quad (14)$$

see, Calik *et al.* (2010) and Abramowitz and Stegun (1972).

The variance is then given by

$$\sigma_{k:k}^2 = \mu_{k:k}^{(2)} - \mu_{k:k}^2, \\ \text{where, at } \rho \neq 1$$

$$\mu_{k:k}^{(2)} = N^2 - \frac{1}{(1-\rho^{N+1})^k} \sum_{i=0}^k (-1)^i \binom{k}{i} \frac{\rho^i}{(1-\rho^i)^2} \{1 + \rho^i - (2N+1)\rho^{iN} + (2N-1)\rho^{i(N+1)}\},$$

and $\rho = 1$

$$\mu_{k:k}^{(2)} = N(N-1) - \frac{2[S(N,k+1) - S(N,k)]}{(N+1)^k} + N - \frac{S(N,k)}{(N+1)^k} \\ = N^2 - \frac{2S(N,k+1) - S(N,k)}{(N+1)^k}.$$

Proof. Applying the definition

$$\mu_{k:k} = \sum_{x=0}^N (1 - F_{k:k}(x)) = \sum_{x=0}^N [1 - (F(x))^k] \\ = \sum_{x=0}^{N-1} [1 - \frac{(1-\rho^{x+1})^k}{(1-\rho^{N+1})^k}] \\ = N - \frac{1}{(1-\rho^{N+1})^k} \sum_{x=0}^{N-1} (1 - \rho^{x+1})^k \\ = N - \frac{1}{(1-\rho^{N+1})^k} \sum_{i=0}^k (-1)^i \binom{k}{i} \rho^i \sum_{x=0}^{N-1} (\rho^i)^x.$$

Expanding binomially and summing $\sum_{x=0}^{N-1} (\rho^i)^x = \frac{1-(\rho^i)^N}{1-\rho^i}$ we get the first part of (13).

For $\rho = 1$, using the definition of $\mu_{k:k}$ and the second part of equation (11), we get the second part of (13).

At $\rho \neq 1$:

$$\mu_{k:k}^{(2)} = 2 \sum_{x=0}^N x(1 - F_{k:k}) + \mu_{k:k} \\ = 2 \sum_{x=0}^N x [1 - (F(x))^k] + \mu_{k:k} \\ = 2 \sum_{x=1}^{N-1} x [1 - (\frac{1-\rho^{x+1}}{1-\rho^{N+1}})^k] + \mu_{k:k} \\ = N(N-1) - \frac{2}{(1-\rho^{N+1})^k} \sum_{i=0}^k (-1)^i \binom{k}{i} \sum_{x=1}^{N-1} x (\rho^{x+1})^i + \mu_{k:k}$$

After some calculations we get

$$\mu_{k:k}^{(2)} = N^2 - \frac{1}{(1-\rho^{N+1})^k} \sum_{i=0}^k (-1)^i \binom{k}{i} \frac{\rho^i}{(1-\rho^i)^2} \{1 + \rho^i - (2N+1)\rho^{iN} + (2N-1)\rho^{i(N+1)}\} \\ \text{At } \rho = 1:$$

$$\mu_{k:k}^{(2)} = 2 \sum_{x=1}^{N-1} x [1 - (\frac{x+1}{N+1})^k] + \mu_{k:k} \\ = N(N-1) - \frac{2}{(N+1)^k} \sum_{x=1}^{N-1} x(x+1)^k + \mu_{k:k} \\ = N(N-1) - \frac{2[S(N,k+1) - S(N,k)]}{(N+1)^k} + N - \frac{S(N,k)}{(N+1)^k} = N^2 - \frac{2S(N,k+1) - S(N,k)}{(N+1)^k}. \quad \blacksquare$$

Corollary 1. The expected value of the number of customers in the system is given by

$$L_s = \frac{\rho(1-(N+1)\rho^N + N\rho^{N+1})}{(1-\rho)(1-\rho^{N+1})}, \quad \rho \neq 1 \\ = \frac{N}{2}, \quad \rho = 1, \quad (15)$$

which studied by Gross and Harris (2003) and Bhat (2008), and many text books in queueing theory.

Proof. Set $k=1$ in (13), we get

$$\mu_{1:1} = L_s = N - \frac{1}{1-\rho^{N+1}} \sum_{x=0}^{N-1} 1 - \rho^{x+1} \\ = N - \frac{1}{1-\rho^{N+1}} \left\{ N - \frac{\rho(1-\rho^N)}{1-\rho} \right\}$$

After simple calculations we get the first part of equation (15). When $\rho = 1$, we have

$$\mu_{1:1} = L_s = N - \frac{S(N,1)}{N+1} = N - \frac{\sum_{x=1}^N x}{N+1} = \frac{N}{2}. \quad \blacksquare$$

Theorem 4. The expected value of the minimum number of customers in the system are given by

$$\mu_{1:k} = \frac{\rho^{k(N+1)}}{(1-\rho^{N+1})^k} \sum_{i=0}^k (-1)^{k-i} \binom{k}{i} \frac{1-\rho^{iN}}{\rho^{iN}(1-\rho^i)}, \quad \rho \neq 1,$$

$$\mu_{1:k} = \frac{S(N,k)}{(N+1)^k}, \quad \rho = 1, \quad (16)$$

where $k = 1, 2, \dots$ represent the busy period and $S(N, k)$ be defined in (14). To get the variance, it is enough to obtain the second moment and applying the definition in Theorem 1. Thus,

$$\begin{aligned} \mu_{1:k}^{(2)} &= \frac{\rho^{k(N+1)}}{(1-\rho^{N+1})^k} \sum_{i=0}^k (-1)^{k-i} \binom{k}{i} \frac{1+\rho^i - (2N+1)\rho^{iN} + (2N-1)\rho^{i(N+1)}}{\rho^{iN}(1-\rho^i)^2}, \quad \rho \neq 1 \\ \mu_{1:k}^{(2)} &= \frac{2[N S(N-1, k) - S(N-1, k+1)] + S(N, k)}{(N+1)^k}, \quad \rho = 1. \end{aligned}$$

Proof. Applying the definition, at $\rho \neq 1$,

$$\begin{aligned} \mu_{1:k} &= \sum_{x=0}^{N-1} \left(1 - \frac{1-\rho^{x+1}}{1-\rho^{N+1}}\right)^k \\ &= \frac{1}{(1-\rho^{N+1})^k} \sum_{x=0}^{N-1} (\rho^{x+1} - \rho^{N+1})^k \\ &= \frac{\rho^{k(N+1)}}{(1-\rho^{N+1})^k} \sum_{i=0}^k (-1)^{k-i} \binom{k}{i} \frac{1-\rho^{iN}}{\rho^{iN}(1-\rho^i)}. \end{aligned}$$

At $\rho = 1$, we have

$$\begin{aligned} \mu_{1:k} &= \sum_{x=0}^{N-1} \left(1 - \frac{x+1}{N+1}\right)^k \\ &= \frac{1}{(N+1)^k} \sum_{x=1}^N x^k = \frac{S(N, k)}{(N+1)^k}. \end{aligned}$$

The second moment is given by, at $\rho \neq 1$,

$$\begin{aligned} \mu_{1:k}^{(2)} &= 2 \sum_{x=0}^N x [1 - F_{1:k}(x)] + \mu_{1:k} \\ &= 2 \sum_{x=0}^N x [1 - F(x)]^k + \mu_{1:k} \\ &= 2 \sum_{x=1}^{N-1} x \left[1 - \frac{1-\rho^{x+1}}{1-\rho^{N+1}}\right]^k + \mu_{1:k} \\ &= \frac{2}{(1-\rho^{N+1})^k} \sum_{x=1}^{N-1} x (\rho^{x+1} - \rho^{N+1})^k + \mu_{1:k} \\ &= \frac{2\rho^{k(N+1)}}{(1-\rho^{N+1})^k} \sum_{i=0}^k (-1)^{k-i} \binom{k}{i} \frac{\rho^i [1 - N\rho^{i(N-1)} + (N-1)\rho^{iN}]}{\rho^{iN}(1-\rho^i)^2} \\ &+ \mu_{1:k} \\ &= \frac{\rho^{k(N+1)}}{(1-\rho^{N+1})^k} \sum_{i=0}^k (-1)^{k-i} \binom{k}{i} \frac{1+\rho^i - (2N+1)\rho^{iN} + (2N-1)\rho^{i(N+1)}}{\rho^{iN}(1-\rho^i)^2}. \end{aligned}$$

At $\rho = 1$, we have

$$\begin{aligned} \mu_{1:k}^{(2)} &= 2 \sum_{x=1}^{N-1} x \left(1 - \frac{x+1}{N+1}\right)^k + \mu_{1:k} \\ &= \frac{2}{(N+1)^k} \sum_{x=1}^{N-1} x(N-x)^k + \mu_{1:k} \\ &= \frac{2[N S(N-1, k) - S(N-1, k+1)] + S(N, k)}{(N+1)^k}. \blacksquare \end{aligned}$$

3.2 The mean and the variance of the minimum and the maximum number of customers in the queue

Theorem 5. The expected value of the maximum number of customers in the queue are given by

$$\begin{aligned} \mu_{k:k} &= N-1 - \frac{1}{(1-\rho^{N+1})^k} \sum_{i=0}^k (-1)^i \binom{k}{i} \frac{\rho^{2i(1-\rho^{i(N-1)})}}{1-\rho^i}, \quad \rho \neq 1, \\ \mu_{k:k} &= N-1 - \frac{S(N, k)-1}{(N+1)^k}, \quad \rho = 1, \end{aligned} \tag{17}$$

where $k=1, 2, \dots$ represent the busy period and

$$S(N, k) = \sum_{x=1}^N x^k.$$

To get the variance, it is enough to obtain the second moment and applying the definition in Theorem 1. Thus,

$$\begin{aligned} \mu_{k:k}^{(2)} &= N^2 - 1 - \frac{1}{(1-\rho^{N+1})^k} \sum_{i=0}^k (-1)^i \binom{k}{i} \frac{\rho^{2i}}{(1-\rho^i)^2} \{3 - \rho^i + (2N+1)\rho^{i(N-1)} + (2N-1)\rho^{iN}\}, \quad \rho \neq 1 \\ \mu_{k:k}^{(2)} &= N^2 - 1 + \frac{1 + S(N, k) - 2S(N, k+1)}{(N+1)^k}, \quad \rho = 1. \end{aligned}$$

Proof. Applying the definition

$$\begin{aligned} \mu_{k:k} &= \sum_{x=1}^N (1 - F_{k:k}(x)) = \sum_{x=1}^N [1 - (F(x))^k] \\ &= \sum_{x=1}^{N-1} \left[1 - \frac{(1-\rho^{x+1})^k}{(1-\rho^{N+1})^k}\right] \\ &= N-1 - \frac{1}{(1-\rho^{N+1})^k} \sum_{x=1}^{N-1} (1-\rho^{x+1})^k \\ &= N-1 - \frac{1}{(1-\rho^{N+1})^k} \sum_{i=0}^k (-1)^i \rho^i \sum_{x=1}^{N-1} (\rho^i)^x \end{aligned}$$

Since $\sum_{x=1}^{N-1} (\rho^i)^x = \frac{\rho^i [1 - (\rho^i)^{N-1}]}{1 - \rho^i}$, we get the first part of (17).

For $\rho = 1$, using the definition of $\mu'_{k:k}$ and the second part of equation (11), we get the second part of (17). The second moment is given by

at $\rho \neq 1$,

$$\begin{aligned} \mu_{k:k}^{(2)} &= 2 \sum_{x=1}^N x(1 - F_{k:k}) + \mu'_{k:k} \\ &= 2 \sum_{x=1}^N x [1 - (F(x))^k] + \mu'_{k:k} \\ &= 2 \sum_{x=1}^{N-1} x \left[1 - \left(\frac{1 - \rho^{x+1}}{1 - \rho^{N+1}} \right)^k \right] + \mu'_{k:k} \\ &= N(N-1) \\ &\quad - \frac{2}{(1 - \rho^{N+1})^k} \sum_{i=0}^k (-1)^i \binom{k}{i} \sum_{x=1}^{N-1} x (\rho^{x+1})^i + \mu'_{k:k} \end{aligned}$$

After some algebra, we obtain

$$\begin{aligned} \mu_{k:k}^{(2)} &= N(N-1) - \frac{2}{(1 - \rho^{N+1})^k} \sum_{i=0}^k (-1)^i \binom{k}{i} \frac{\rho^{2i}}{(1 - \rho^i)^2} \\ &\quad \{1 - N\rho^{i(N-1)} + (N-1)\rho^{iN}\} + \mu'_{k:k} \\ \mu_{k:k}^{(2)} &= N^2 - 1 - \frac{1}{(1 - \rho^{N+1})^k} \sum_{i=0}^k (-1)^i \binom{k}{i} \frac{\rho^{2i}}{(1 - \rho^i)^2} \\ &\quad \{1 - \rho^i + (2N+1)\rho^{i(N-1)} + (2N-1)\rho^{iN}\}. \end{aligned}$$

At $\rho = 1$:

$$\begin{aligned} \mu_{k:k}^{(2)} &= 2 \sum_{x=1}^{N-1} x \left[1 - \frac{(x+1)^k}{(N+1)^k} \right] + \mu'_{k:k} \\ &= N(N-1) \\ &\quad - \frac{2}{(N+1)^k} \sum_{x=1}^{N-1} x(x+1)^k + \mu'_{k:k} \\ &= N(N-1) - \frac{2[S(N, k+1) - S(N, k)]}{(N+1)^k} + N-1 \\ &\quad - \frac{S(N, k) - 1}{(N+1)^k} \\ &= N^2 - 1 + \frac{1+S(N, k) - 2S(N, k+1)}{(N+1)^k}. \end{aligned}$$

Corollary 2. The expected value of the number of customers in the queue is given by

$$\begin{aligned} L_q = \mu'_{1:1} &= \frac{\rho^2 [1 - N\rho^{N-1} + (N-1)\rho^N]}{(1 - \rho)(1 - \rho^{N+1})}, \quad \rho \neq 1 \\ &= \frac{N(N-1)}{2(N+1)}, \quad \rho = 1. \end{aligned} \quad (18)$$

Proof. Set $k=1$ in (17), we get the relation (18). ■

Theorem 6. The expected value of the minimum number of customers in the queue are given by

$$\mu'_{1:k} = \frac{\rho^{k(N+1)}}{(1 - \rho^{N+1})^k} \sum_{i=0}^k (-1)^{k-i} \binom{k}{i} \frac{1 - \rho^{i(N-1)}}{\rho^{i(N-1)}(1 - \rho^i)}, \quad \rho \neq 1,$$

$$\mu'_{1:k} = \frac{S(N-1, k)}{(N+1)^k}, \quad \rho = 1, \quad (19)$$

where $k=1, 2, \dots$ represent the busy period and $S(N, k)$ defined in (14).

The second moment is then given by

$$\begin{aligned} \mu_{1:k}^{(2)} &= \frac{\rho^{k(N+1)}}{(1 - \rho^{N+1})^k} \sum_{i=0}^k (-1)^{k-i} \binom{k}{i} \frac{3 - (2N+1)\rho^{i(N-1)} + (2N-1)\rho^{iN}}{\rho^{i(N-1)}(1 - \rho^i)^2}, \\ &\quad \rho \neq 1 \\ \mu_{1:k}^{(2)} &= \frac{(2N+1)S(N-1, k) - 2S(N-1, k+1)}{(N+1)^k}, \quad \rho = 1 \end{aligned}$$

Proof. Applying the definition, at $\rho \neq 1$,

$$\begin{aligned} \mu'_{1:k} &= \sum_{x=1}^N [1 - F(x)]^k = \sum_{x=0}^{N-1} \left(1 - \frac{1 - \rho^{x+1}}{1 - \rho^{N+1}} \right)^k \\ &= \frac{1}{(1 - \rho^{N+1})^k} \sum_{x=0}^{N-1} (\rho^{x+1} - \rho^{N+1})^k \end{aligned}$$

$$\mu'_{1:k} = \frac{\rho^{k(N+1)}}{(1 - \rho^{N+1})^k} \sum_{i=0}^k (-1)^{k-i} \binom{k}{i} \frac{1 - \rho^{i(N-1)}}{\rho^{i(N-1)}(1 - \rho^i)}.$$

At $\rho = 1$, we have

$$\begin{aligned} \mu'_{1:k} &= \sum_{x=1}^{N-1} \left(1 - \frac{x+1}{N+1} \right)^k \\ &= \frac{1}{(N+1)^k} \sum_{x=1}^{N-1} x^k = \frac{S(N-1, k)}{(N+1)^k}. \end{aligned}$$

The second moment is then given by, at $\rho \neq 1$,

$$\begin{aligned} \mu_{1:k}^{(2)} &= 2 \sum_{x=0}^N x [1 - F_{1:k}(x)] + \mu'_{1:k} \\ &= 2 \sum_{x=0}^N x [1 - F(x)]^k + \mu'_{1:k} \\ &= 2 \sum_{x=1}^{N-1} x \left[1 - \frac{1 - \rho^{x+1}}{1 - \rho^{N+1}} \right]^k + \mu'_{1:k} \\ &= \frac{2}{(1 - \rho^{N+1})^k} \sum_{x=1}^{N-1} x (\rho^{x+1} - \rho^{N+1})^k + \mu'_{1:k} \\ &= \frac{2\rho^{k(N+1)}}{(1 - \rho^{N+1})^k} \sum_{i=0}^k (-1)^{k-i} \binom{k}{i} \frac{1 - N\rho^{i(N-1)} + (N-1)\rho^{iN}}{\rho^{i(N-1)}(1 - \rho^i)^2} \\ &\quad + \mu'_{1:k} \\ &= \frac{\rho^{k(N+1)}}{(1 - \rho^{N+1})^k} \sum_{i=0}^k (-1)^{k-i} \binom{k}{i} \frac{3 - (2N+1)\rho^{i(N-1)} + (2N-1)\rho^{iN}}{\rho^{i(N-1)}(1 - \rho^i)^2}. \end{aligned}$$

At $\rho = 1$, we have

$$\begin{aligned} \mu'_{1:k}^{(2)} &= 2 \sum_{x=1}^{N-1} x \left(1 - \frac{x+1}{N+1}\right)^k + \mu'_{1:k} \\ &= \frac{2}{(N+1)^k} \sum_{x=1}^{N-1} x(N-x)^k + \mu'_{1:k} \\ &= \frac{(2N+1)S(N-1, k) - 2S(N-1, k+1)}{(N+1)^k}, \quad \rho = 1 \end{aligned}$$

hence the proof. ■

4. Waiting time Distribution for M/M/1/N model

Other useful performance measures are the r th moments of the minimum and maximum waiting time in the queue. The cumulative probability distribution of the waiting time in the queue for the $M/M/1/N$ queue is given by

$$W_q(t) = \begin{cases} 1 - \frac{1-\rho}{1-\rho^N} \sum_{n=1}^{N-1} \rho^n \sum_{j=0}^{n-1} \frac{e^{-\mu t} (\mu t)^j}{j!}, & \rho \neq 1 \\ 1 - \frac{1}{N} \sum_{n=1}^{N-1} \sum_{j=0}^{n-1} \frac{e^{-\mu t} (\mu t)^j}{j!}, & \rho = 1. \end{cases} \quad (20)$$

The expected waiting time is given by

$$W_q = \begin{cases} \frac{\rho[1 - N\rho^{N-1} + (N-1)\rho^N]}{\mu(1-\rho)(1-\rho^N)}, & \rho \neq 1 \\ \frac{N-1}{2\mu}, & \rho = 1. \end{cases}$$

In the following two theorems are established. The first one deals with the r th moments of the minimum waiting time while the second deals with r th moments of the maximum waiting time.

Theorem 7. The r th moments of the minimum waiting time in the queue is given by

$$v_{1:k}^{(r)} = r \left(\frac{\rho}{1-\rho^N}\right)^k \sum_{i=0}^k (-1)^i \binom{k}{i} \cdot \rho^{(N-1)i} \sum_{m=0}^{(k-i)(N-2)} \sum_{s=0}^{i(N-2)} a_{s,i} b_{m,k-i} \times \frac{\rho^m \Gamma(r+m+s)}{\mu^r k^{m+s+1}} \rho \neq 1, \quad (21)$$

$$v_{1:k}^{(r)} = \frac{r}{N} \sum_{i=0}^k (-1)^{k-i} \binom{k}{i} \cdot (N - 1)^i \sum_{m=0}^{(k-i)(N-3)} \sum_{s=0}^{i(N-2)} d_{s,i} c_{m,k-i} \frac{\Gamma(r+m+s+k-i)}{\mu^r k^{r+m+s+k-i}}, \quad \rho = 1, \quad (22)$$

where $b_{m,k-i}$ is a coefficient of $(\lambda t)^m$ in the expansion $\left[\sum_{\ell=0}^{N-2} \frac{(\lambda t)^\ell}{\ell!}\right]^{k-i}$,

i.e.,

$$\left[\sum_{\ell=0}^{N-2} \frac{(\lambda t)^\ell}{\ell!}\right]^{k-i} = \sum_{m=0}^{(k-i)(N-2)} b_{m,k-i} (\lambda t)^m,$$

$a_{s,i}$ is a coefficient of $(\mu t)^s$ in the expansion $\left[\sum_{j=0}^{N-2} \frac{(\mu t)^j}{j!}\right]^i$, $c_{m,k-i}$ is a coefficient of $(\mu t)^m$ in the expansion $\left[\sum_{\ell=0}^{N-3} \frac{(\lambda t)^\ell}{\ell!}\right]^{k-i}$, and $d_{s,i}$ is a coefficient of $(\mu t)^s$ in the expansion $\left[\sum_{j=0}^{N-2} \frac{(\mu t)^j}{j!}\right]^i$, see, Ahsanullah (1995) and Balakrishnan and Chan (1998).

Proof. Using equation (10) in Theorem 2 and the definition of $W_q(t)$ in equation (20) we have

$$\begin{aligned} v_{1:k}^{(r)} &= r \int_0^\infty t^{r-1} [1 - W_q(t)]^k dt \\ &= r \int_0^\infty t^{r-1} \left(\frac{1-\rho}{1-\rho^N}\right)^k \left[\sum_{n=1}^{N-1} \rho^n \sum_{j=0}^{n-1} e^{-\mu t} \frac{(\mu t)^j}{j!}\right]^k dt \\ &= r \left(\frac{1-\rho}{1-\rho^N}\right)^k \int_0^\infty t^{r-1} e^{-\mu kt} \left[\rho \sum_{n=1}^{N-1} \rho^{n-1} \sum_{j=0}^{n-1} \frac{(\mu t)^j}{j!}\right]^k dt \\ &= r \left(\frac{\rho(1-\rho)}{1-\rho^N}\right)^k \int_0^\infty t^{r-1} e^{-\mu kt} \left[\sum_{j=0}^{N-2} \sum_{i=j}^{N-2} \frac{\rho^i (\mu t)^j}{j!}\right]^k dt \\ &= r \left(\frac{\rho(1-\rho)}{1-\rho^N}\right)^k \int_0^\infty t^{r-1} e^{-\mu kt} \left[\sum_{j=0}^{N-2} \frac{\rho^j (\mu t)^j}{j!} \cdot \frac{1-\rho^{N-1-j}}{1-\rho}\right]^k dt \\ &= r \left(\frac{\rho}{1-\rho^N}\right)^k \int_0^\infty t^{r-1} e^{-\mu kt} \left[\sum_{j=0}^{N-2} \frac{(\lambda t)^j}{j!} - \rho^{N-1} \sum_{j=0}^{N-2} \frac{(\mu t)^j}{j!}\right]^k dt \\ &= r \left(\frac{\rho}{1-\rho^N}\right)^k \int_0^\infty t^{r-1} e^{-\mu kt} \sum_{i=0}^k (-1)^i \binom{k}{i} \left[\sum_{\ell=0}^{N-2} \frac{(\lambda t)^\ell}{\ell!}\right]^{k-i} \cdot \rho^{(N-1)i} \left[\sum_{j=0}^{N-2} \frac{(\mu t)^j}{j!}\right]^i dt \\ &= r \left(\frac{\rho}{1-\rho^N}\right)^k \sum_{i=0}^k (-1)^i \binom{k}{i} \cdot \rho^{(N-1)i} \sum_{m=0}^{(k-i)(N-2)} \sum_{s=0}^{i(N-2)} a_{s,i} b_{m,k-i} \lambda^m \mu^s \int_0^\infty t^{r+m+s-1} e^{-\mu kt} dt \\ &= r \left(\frac{\rho}{1-\rho^N}\right)^k \sum_{i=0}^k (-1)^i \binom{k}{i} \cdot \rho^{(N-1)i} \sum_{m=0}^{(k-i)(N-2)} \sum_{s=0}^{i(N-2)} a_{s,i} b_{m,k-i} \frac{\rho^m \Gamma(r+m+s)}{\mu^r k^{m+s+1}} \\ &= r \left(\frac{\rho}{1-\rho^N}\right)^k \sum_{i=0}^k (-1)^i \binom{k}{i} \cdot \rho^{(N-1)i} \sum_{m=0}^{(k-i)(N-2)} \sum_{s=0}^{i(N-2)} \frac{a_{s,i} b_{m,k-i} \rho^m \Gamma(r+m+s)}{\mu^r k^{m+s+1}}, \quad \rho \neq 1. \end{aligned}$$

At $\rho = 1$, from equation (10) and the second part of equation (20) we obtain

$$\begin{aligned} v_{1:k}^{(r)} &= \frac{r}{N} \int_0^\infty t^{r-1} \left[\sum_{n=1}^{N-1} \sum_{j=0}^{n-1} e^{-\mu t} \frac{(\mu t)^j}{j!}\right]^k dt \\ &= \frac{r}{N} \int_0^\infty t^{r-1} e^{-\mu kt} \left[\sum_{j=0}^{N-2} \sum_{i=j}^{N-2} \frac{(\mu t)^j}{j!}\right]^k dt \\ &= \frac{r}{N} \int_0^\infty t^{r-1} e^{-\mu kt} \left[(N-1) \sum_{j=0}^{N-2} \frac{(\mu t)^j}{j!} - \mu t \sum_{\ell=0}^{N-3} \frac{(\mu t)^\ell}{\ell!}\right]^k dt \end{aligned}$$

$$\begin{aligned}
 v_{1:k}^{(r)} &= \frac{r}{N} \int_0^\infty t^{r-1} e^{-\mu kt} \sum_{i=0}^k (-1)^{k-i} \binom{k}{i} \\
 &\quad \left[\mu t \sum_{\ell=0}^{N-3} \frac{(\mu t)^\ell}{\ell!} \right]^{k-i} (N-1)^i \left[\sum_{j=0}^{N-2} \frac{(\mu t)^j}{j!} \right]^i dt \\
 &= \frac{r}{N} \sum_{i=0}^k (-1)^{k-i} \binom{k}{i} \cdot (N-1)^i \times \\
 &\quad \sum_{m=0}^{(k-i)(N-3)} \sum_{s=0}^{i(N-2)} d_{s,i} c_{m,k-i} \mu^{m+s+k-i} \times \\
 &\quad \int_0^\infty t^{r+m+s+k-i-1} e^{-\mu kt} dt \\
 v_{1:k}^{(r)} &= \frac{r}{N} \sum_{i=0}^k (-1)^{k-i} \binom{k}{i} \cdot (N-1)^i \\
 &\quad \sum_{m=0}^{(k-i)(N-3)} \sum_{s=0}^{i(N-2)} d_{s,i} c_{m,k-i} \mu^{m+s+k-i} \frac{\Gamma(r+m+s+k-i)}{\mu^r k^{r+m+s+k-i}}.
 \end{aligned}$$

In particular, when $r=k=1$, from (21) we get

$$\begin{aligned}
 v_{1:1} &= W_q = \frac{\rho}{1-\rho^N} \sum_{i=0}^1 (-1)^i \binom{1}{i} \cdot \rho^{(N-1)i} \times \\
 &\quad \sum_{m=0}^{(1-i)(N-2)} \sum_{s=0}^{i(N-2)} \frac{a_{s,i} b_{m,1-i} \rho^m \Gamma(m+s+1)}{\mu^{km+s+1}} \\
 &= \frac{\rho}{\mu(1-\rho^N)} [\sum_{m=0}^{N-2} a_{s,0} b_{m,0} \rho^m \Gamma(m+1) - \\
 &\quad \rho^{N-1} \sum_{s=0}^{N-2} a_{s,1} b_{0,0} \Gamma(s+1)], \\
 b_{m,1} &= \frac{1}{m!}, \quad a_{s,1} = \frac{1}{s!}, \quad b_{0,0} = 1, \quad a_{0,0} = 1 \\
 v_{1:1} &= \frac{\rho}{\mu(1-\rho^N)} [\sum_{m=0}^{N-2} \rho^m - \rho^{N-1} \sum_{s=0}^{N-2} 1] \\
 &= \frac{\rho}{\mu(1-\rho^N)} \left[\frac{1-\rho^{N-1}}{1-\rho} - (N-1)\rho^{N-1} \right] \\
 &= \frac{\rho}{\mu(1-\rho)(1-\rho^N)} [1 - N\rho^{N-1} + (N-1)\rho^N], \quad \rho \neq 1.
 \end{aligned}$$

Also, from (22), we obtain

$$\begin{aligned}
 v_{1:1} &= W_q \\
 &= \frac{1}{N\mu} \left[- \sum_{m=0}^{N-3} d_{0,1} c_{m,1} \Gamma(m+1) \right. \\
 &\quad \left. + (N-1) \sum_{s=0}^{N-2} d_{s,1} c_{0,0} \Gamma(s+1) \right] \\
 &= \frac{N-1}{2\mu}, \quad \rho = 1,
 \end{aligned}$$

Such that $c_{m,1} = \frac{1}{m!}$, $d_{s,1} = \frac{1}{s!}$, $c_{0,0} = 1$, and $d_{0,1} = 1$. ■
Theorem 8. The r th moments of the maximum waiting time in the queue is given by

$$\begin{aligned}
 v_{k:k}^{(r)} &= r \sum_{i=1}^k (-1)^{i+1} \binom{k}{i} \cdot \frac{\rho^i}{(1-\rho^N)^i} \sum_{s=0}^i (-1)^s \binom{i}{s} \cdot \rho^{(N-1)s} \times \\
 &\quad \sum_{m=0}^{(i-s)(N-2)} \sum_{\eta=0}^{s(N-2)} a_{\eta,s} b_{m,i-s} \lambda^m \mu^\eta \frac{\Gamma(r+m+\eta)}{(\mu i)^{r+m+\eta}}, \\
 &\quad \rho \neq 1, \quad (23)
 \end{aligned}$$

$$\begin{aligned}
 v_{k:k}^{(r)} &= r \sum_{i=1}^k (-1)^{i+1} \binom{k}{i} \cdot \frac{1}{N^i} \sum_{s=0}^i (-1)^s \binom{i}{s} (N-1)^{i-s} \\
 &\quad \sum_{m=0}^{(i-s)(N-2)} \sum_{\eta=0}^{s(N-2)} g_{\eta,s} e_{m,i-s} \frac{\Gamma(r+m+\eta+s)}{\mu^r i^{r+m+\eta+s}}, \\
 &\quad \rho = 1, \quad (24)
 \end{aligned}$$

where $b_{m,i-s}$ coefficient of $(\lambda t)^m$ in $(\sum_{\ell=0}^{N-2} \frac{(\lambda t)^\ell}{\ell!})^{i-s}$, $a_{\eta,s}$ coefficient of $(\mu t)^\eta$ in $(\sum_{j=0}^{N-2} \frac{(\mu t)^j}{j!})^s$, $e_{m,i-s}$ coefficient of $(\mu t)^m$ in $(\sum_{j=0}^{N-2} \frac{(\mu t)^j}{j!})^{i-s}$ and $g_{\eta,s}$ coefficient of $(\mu t)^\eta$ in $(\sum_{\ell=0}^{N-3} \frac{(\lambda t)^\ell}{\ell!})^s$.

Proof.

Using equation (9) in Theorem 2 and the definition of $W_q(t)$ in equation (20) we have

$$\begin{aligned}
 v_{k:k}^{(r)} &= r \int_0^\infty t^{r-1} [1 - (W_q(t))^k] dt \\
 &= r \int_0^\infty t^{r-1} \times \\
 &\quad \left[1 - \left(1 - \frac{1}{1-P_N} \sum_{n=1}^{N-1} P_n \sum_{j=0}^{n-1} \frac{e^{-\mu t} (\mu t)^j}{j!} \right)^k \right] dt \\
 &= r \int_0^\infty t^{r-1} \left[1 - \left(1 - \frac{1-\rho}{1-\rho^N} \sum_{n=1}^{N-1} \rho^n \sum_{j=0}^{n-1} e^{-\mu t} \frac{(\mu t)^j}{j!} \right)^k \right] dt \\
 &= r \int_0^\infty t^{r-1} \left[1 - \left(1 - \frac{\rho(1-\rho)}{1-\rho^N} \sum_{j=0}^{N-2} \sum_{i=j}^{N-2} \frac{\rho^i (\mu t)^j}{j!} \right)^k \right] dt \\
 &= r \int_0^\infty t^{r-1} \left[1 - \left(1 - \frac{\rho(1-\rho)}{1-\rho^N} \sum_{j=0}^{N-2} \frac{(\mu t)^j}{j!} \cdot \frac{\rho^j (1-\rho^{N-1-j})}{1-\rho} \right)^k \right] dt
 \end{aligned}$$

$$v_{k:k}^{(r)} = r \int_0^\infty t^{r-1} \left[1 - \left(1 - \frac{\rho e^{-\mu t}}{1 - \rho^N} \sum_{j=0}^{N-2} \frac{(\mu t)^j}{j!} \cdot \rho^j (1 - \rho^{N-1-j}) \right)^k \right] dt$$

$$= r \int_0^\infty t^{r-1} \left[1 - \sum_{i=0}^k (-1)^i \binom{k}{i} \left(\frac{\rho e^{-\mu t}}{1 - \rho^N} \sum_{j=0}^{N-2} \frac{(\lambda t)^j}{j!} \cdot (1 - \rho^{N-1-j}) \right)^i \right] dt$$

$$= r \int_0^\infty t^{r-1} \left[1 - \sum_{i=0}^k (-1)^i \binom{k}{i} \cdot \frac{\rho^i e^{-\mu i t}}{(1 - \rho^N)^i} \left(\sum_{j=0}^{N-2} \frac{(\lambda t)^j}{j!} \cdot (1 - \rho^{N-1-j}) \right)^i \right] dt$$

$$= r \int_0^\infty t^{r-1} \left[1 - \sum_{i=0}^k (-1)^i \binom{k}{i} \cdot \frac{\rho^i e^{-\mu i t}}{(1 - \rho^N)^i} \left(\sum_{\ell=0}^{N-2} \frac{(\lambda t)^\ell}{\ell!} - \rho^{N-1} \sum_{j=0}^{N-2} \frac{(\mu t)^j}{j!} \right)^i \right] dt$$

$$= r \int_0^\infty t^{r-1} \left[1 - \sum_{i=0}^k (-1)^i \binom{k}{i} \cdot \frac{\rho^i e^{-\mu i t}}{(1 - \rho^N)^i} \sum_{s=0}^i (-1)^s \binom{i}{s} \cdot \left(\sum_{\ell=0}^{N-2} \frac{(\lambda t)^\ell}{\ell!} \right)^{i-s} \rho^{(N-1)s} \left(\sum_{j=0}^{N-2} \frac{(\mu t)^j}{j!} \right)^s \right] dt$$

$$= r \int_0^\infty t^{r-1} \left[1 - \sum_{i=0}^k (-1)^i \binom{k}{i} \cdot \frac{\rho^i e^{-\mu i t}}{(1 - \rho^N)^i} \sum_{s=0}^i (-1)^s \binom{i}{s} \times \sum_{m=0}^{(i-s)(N-2)} b_{m,i-s} (\lambda t)^m \rho^{(N-1)s} \sum_{\eta=0}^{s(N-2)} a_{\eta,s} (\mu t)^\eta \right] dt$$

where $b_{m,i-s}$ coefficient of $(\lambda t)^m$ in $\left(\sum_{\ell=0}^{N-2} \frac{(\lambda t)^\ell}{\ell!} \right)^{i-s}$ and $a_{\eta,s}$ coefficient of $(\mu t)^\eta$ in $\left(\sum_{j=0}^{N-2} \frac{(\mu t)^j}{j!} \right)^s$. Thus

$$v_{k:k}^{(r)} = r \int_0^\infty t^{r-1} \sum_{i=1}^k (-1)^{i+1} \binom{k}{i} \cdot \frac{\rho^i e^{-\mu i t}}{(1 - \rho^N)^i} \sum_{s=0}^i (-1)^s \binom{i}{s} \cdot \rho^{(N-1)s} \times \sum_{m=0}^{(i-s)(N-2)} \sum_{\eta=0}^{s(N-2)} a_{\eta,s} b_{m,i-s} \lambda^m \mu^\eta t^{m+\eta} dt$$

$$= r \sum_{i=1}^k (-1)^{i+1} \binom{k}{i} \cdot \frac{\rho^i}{(1 - \rho^N)^i} \sum_{s=0}^i (-1)^s \binom{i}{s} \cdot \rho^{(N-1)s} \times \sum_{m=0}^{(i-s)(N-2)} \sum_{\eta=0}^{s(N-2)} a_{\eta,s} b_{m,i-s} \lambda^m \mu^\eta \frac{\Gamma(r+m+\eta)}{(\mu i)^{r+m+\eta}}, \rho \neq 1.$$

At $\rho = 1$, from equation (9) and the second equation of (20) we obtain

$$v_{1:k}^{(r)} = r \int_0^\infty t^{r-1} \left[1 - \left(1 - \frac{1}{N} \sum_{n=1}^{N-1} \sum_{j=0}^{n-1} e^{-\mu t} \frac{(\mu t)^j}{j!} \right)^k \right] dt$$

$$= r \int_0^\infty t^{r-1} \left[1 - \left(1 - \frac{e^{-\mu t}}{N} \sum_{j=0}^{N-2} \sum_{i=j}^{N-2} \frac{(\mu t)^j}{j!} \right)^k \right] dt$$

$$= r \int_0^\infty t^{r-1} \sum_{i=1}^k (-1)^{i+1} \binom{k}{i} \frac{e^{-i\mu t}}{N^i} \times \left((N-1) \sum_{j=0}^{N-2} \frac{(\mu t)^j}{j!} - \mu t \sum_{j=1}^{N-2} \frac{(\mu t)^{j-1}}{(j-1)!} \right)^i dt$$

$$= r \int_0^\infty t^{r-1} \sum_{i=1}^k (-1)^{i+1} \binom{k}{i} \frac{e^{-i\mu t}}{N^i} \sum_{s=0}^i (-1)^s \binom{i}{s} (\mu t)^s \times (N-1)^{i-s} \sum_{m=0}^{(i-s)(N-2)} e_{m,i-s} (\mu t)^m \sum_{\eta=0}^{s(N-2)} g_{\eta,s} (\mu t)^\eta dt$$

$$= r \sum_{i=1}^k (-1)^{i+1} \binom{k}{i} \cdot \frac{1}{N^i} \sum_{s=0}^i (-1)^s \binom{i}{s} \times (N-1)^{i-s} \sum_{m=0}^{(i-s)(N-2)} \sum_{\eta=0}^{s(N-2)} e_{m,i-s} g_{\eta,s} \frac{\Gamma(r+m+\eta+s)}{\mu^r i^{r+m+\eta+s}}, \rho = 1.$$

In particular, when $r=k=1$, from (23) we get

$$v_{1:1} = \frac{\rho}{\mu(1-\rho^N)} \left[\frac{1-\rho^{N-1}}{1-\rho} - (N-1)\rho^{N-1} \right]$$

$$= \frac{\rho}{\mu(1-\rho)(1-\rho^N)} [1 - N\rho^{N-1} + (N-1)\rho^N] = W_q, \rho \neq 1.$$

Also, from (24), we obtain

$$v_{1:1} = \frac{1}{\mu N} \left\{ (N-1) \sum_{m=0}^{N-2} e_{m,1} g_{0,0} \Gamma(m+1) - \sum_{\eta=0}^{N-3} e_{0,0} g_{\eta,0} \Gamma(\eta+2) \right\} = \frac{N-1}{2\mu}$$

$$= W_q, \rho = 1,$$

such that $e_{0,0} = g_{0,0} = 1, e_{m,1} = \frac{1}{m!}$ and $g_{\eta,0} = \frac{1}{\eta!}$.

Since $\mu_{k:k}$ and $\mu'_{k:k}$ give the sum of the expected value of the maximum number of customers in the system and queue, we compute the expected value of the maximum number of customers in the system and queue during the busy period k , respectively, by

$$\Delta \mu_k = \mu_{k:k} - \mu_{k-1:k-1},$$

$$\Delta \mu'_k = \mu'_{k:k} - \mu'_{k-1:k-1},$$

with conventional $\mu_{0:k} = \mu'_{0:k} = 0$ and $\rho = \Delta \mu_k - \Delta \mu'_k$.

Similarly, the expected value of the maximum waiting time in the queue during the busy period k is computed by

$$\Delta v_k = v_{k:k} - v_{k-1:k-1}.$$

5. Conclusion

In this paper, we have considered the Markovian queueing model with a single-server and finite system capacity. The paper presents some complementary measures of performance which are depending on the methods of order statistics. The expected value and the variance of the minimum (maximum) number of customers in the system (queue) as well as the r th moments of the minimum (maximum) waiting time in

the queue are derived. When $N \rightarrow \infty$ the results of Abdelkader and Al-Wohaibi (2011) can be obtained. Clearly, the expected value and the variance of the number of customers in the system (queue) as well as the r th moment waiting time can be obtained as special cases from our proposed measures when $k=1$. Although this work is currently restricted to the M/M/1/N model, it can be applied to other queueing models.

Corresponding author

Yousry H. Abdelkader

Math. Dept., Faculty of Science, Alexandria University, Egypt

yabdelkader@yahoo.com

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Effect of Counseling on Self-Care Management among Adult Patients with Pulmonary Tuberculosis

Howyida, S. Abd ElHameed¹, Heba, A.Aly², Abeer, Y.Mahdy²

¹Community Health Nursing Department, ²Medical Surgical Nursing Department, Faculty of Nursing, Benha University
elmokhtar.mohamed@yahoo.com

Abstract: Today, tuberculosis is still one of the major public health problems in many places in the world especially in Africa and Asia. The aim of this study was to evaluate the effect of counseling on self-care management among adult patients with pulmonary tuberculosis (T.B). An intervention design was conducted at homes of pulmonary patients with tuberculosis who attended at Benha Chest Hospital during the years 2010. The sample was composed of 100 adult patients with tuberculosis. Two tools were utilized in this study: 1) A structured interview questionnaire to collect data about general characteristics of patients and their knowledge about the disease. 2) An observational checklist for assessing T.B patient's self-care and home condition. Results revealed that significant improvement in patient's knowledge and practice post implementation of the health educational counseling. This study concluded that after the counseling implementation there was a significant improvement on participants' physical, social and psychological conditions. The study recommended that health education intervention should be carried out for all new T.B cases at the chest clinics about T.B self-care, and continuous health education to all old cases attending at chest clinics for follow up to upgrade their knowledge and practice related to T.B self-care management at home [Howyida, S. Abd ElHameed, Heba, A.Aly and Abeer, Y.Mahdy. **Effect of Counselling on Self-Care Management among Adult Patients with Pulmonary Tuberculosis**. Life Science Journal 2012;9(1):956-964]. (ISSN: 1097-8135). <http://www.lifesciencesite.com>. 139

Key word: Counseling, Pulmonary Tuberculosis, Adult patients.

1. Introduction

The world health organization declared tuberculosis as a global emergency in recognition of its increasing infection and mortality rates.⁽¹⁾ Tuberculosis is a common and often deadly infectious disease caused by mycobacteria; tuberculosis usually attacks the lungs (as pulmonary tuberculosis) but can also affect other body systems.⁽²⁾

Globally, there were an estimated 9.27 million incident cases of tuberculosis in 2007. Most of the estimated number of cases was in Asia 55% and Africa 31%, with small proportions of cases in the Eastern Mediterranean Region 6%, the European Region 5% and the Region of the Americas 3%.⁽³⁾

In Egypt, there are 16 thousand patients annually, and the number of patients currently at around 23 thousand patients.⁽⁴⁾ Tuberculosis is spread through the air, when people suffering from active pulmonary tuberculosis cough, sneeze, speak, or spit, they expel infectious aerosol droplets 0.5 to 5 µm in diameter. A single sneeze can release up to 40,000 droplets. Each one of these droplets may transmit the disease, since the infectious dose of tuberculosis is very low and the inhalation of just a single bacterium can cause a new infection.⁽⁵⁾

Main symptoms of pulmonary tuberculosis include chest pain, coughing up blood, and prolonged cough for more than three weeks. Systemic symptoms include fever, chills, night sweats, appetite loss,

weight loss, pallor, and often a tendency to fatigue very easily.⁽⁶⁾

The previous researches identified that the most common complication of pulmonary T.B are lung abscess, chronic obstructive pulmonary disease, spread of infection to other organs, respiratory failure and bronchitis in addition to malnutrition, military T.B, pleural effusion, pneumonia, empyema, fibrosis and Haemoptysis.⁽⁷⁾

Treatment for tuberculosis uses antibiotics to kill the bacteria. The two antibiotics most commonly used are rifampicin and isoniazid.⁽⁸⁾

Tuberculosis prevention and control takes two parallel approaches. In the first, people with tuberculosis and their contacts are identified and then treated. Identification of infections often involves testing high-risk groups for tuberculosis. In the second approach, children are vaccinated to protect them from tuberculosis. Unfortunately, no vaccine is available that provides reliable protection for adults.⁽⁹⁾

Home health care is provided to individuals and families in their place of residence for the purpose of promoting, maintaining or restoring health for maximizing the level of independence while minimizing the effects of disability and illness including terminal illness.⁽¹⁰⁾

Counseling refers to face to face communication between counselor (care provider) and clients to make free and informed choice about their life and act on their choice.⁽¹¹⁾ It is also a process through which one

person helps another by purposeful conversation in understanding atmospheres.⁽³⁾

According to *Orem et al.*⁽¹²⁾, self-care is the practice of activities that individuals initiate and perform on their own behalf in maintaining life, health and well being. It is also defined as any action or psychological process undertaken to promote, assess, maintain or restore through as self-help.⁽¹³⁾

Nurses who work in the community can control communicable diseases in several ways as education, immunization, early detection through case finding and contact notification, initiation of appropriate treatment, support, encouragement and referral, Nurses can educate members of the community about ways to prevent such a communicable disease, how to detect signs and symptoms of communicable disease, actions that people can take to reduce the transmission of the disease, and when to seek help.⁽¹⁰⁾

Significance of the study

Tuberculosis is an important public health problem in the world, more people will die from tuberculosis than in any other disease and almost all deaths from tuberculosis are preventable. In relation to the incidence of tuberculosis, Egypt is ranked among the mid-level incidence countries; there are 16 thousand patients annually and the number of patients currently at around 23 thousand patients. Tuberculosis is considered the second most important public health problem after bilharziasis in Egypt. So, this study will be carried out trying to fulfill the health needs, upgrade knowledge and practice for this group and increase their health awareness about the importance of tuberculosis treatment and prevention.

Aim of the study:

The present study aimed to evaluate the effect of counseling on self-care management among adult patients with pulmonary tuberculosis.

Research Hypothesis:

Participants who will receive the health educational counseling about T.B self-care management will have better knowledge and practice post intervention.

2. Subjects & Methods:

Research design

An intervention design was used in carrying out this study.

Setting

The study was conducted at the Outpatient Clinic in Benha Chest Hospital which is the only specialized chest hospital in Kalyobia Governorate, the study subjects were followed by conducting visits

to them at their homes. The researchers provided them with counseling and health education about T.B self-care management.

Sample

Patients who attended to the Outpatient Chest Clinic during the years 2010 were 301 patients. The sample size was composed of 1/3 of the attendants (100 patients) who had fulfilled the following inclusion criteria: (diagnosed pulmonary T.B since 6 months, aged 20-45 years and of both genders).

Research instrument (tools): Two tools were used to collect study data:

1- An interviewing Questionnaire: It was developed by the researchers to collect data about: a) A study subjects' characteristics as age, gender, education level, marital status, occupation and income. b) Patient's knowledge about T.B disease signs and symptoms, types and mode of transmission, incubation period, investigations, vaccination, complications, treatment and follow up. 5 closed-ended questions were included dealing with knowledge related practice these were isolation technique, feeding & treatment system, preventive methods, follow up schedule and handling body secretion. The scoring for patient's knowledge and practical knowledge for pre/post/test is one point for the correct answer and zero for wrong or incorrect one. The total score was summed up and divided by the number of the items, giving a mean score for the variable. These scores were converted into percent and the patient was considered having good knowledge if > 65%, while average from 50-65% and poor < 50%. The questionnaire included also participant's knowledge toward their Common physical symptoms from their disease (T.B) as: loss of weight, chest pain, cough, general fatigue, fever, muscle cramps, diarrhea, constipation, skin irritation and dyspnoea. The responses were on a three levels of answers (always), (sometimes) and (rarely), these were respectively scored 3, 2 and 1. The total scores were summed up and divided by the number of items. Scores were converted into percent, mean and standard deviation. As regards common social and psychological study subjects, responses in relation to their disease pre/ post counseling intervention, they were adapted from the Social as Psychological Stress symptoms Scale of Burn.⁽¹¹⁾ Translated and modified by the researchers, it consisted of (11) items for social systems and (13) items for psychological reactions regarding disease. Responses were checked by (either) (always), (sometimes) or (rarely) and they were coded 3, 2 and 1.

2- An Observational Checklist, it was designed by to observe the study participants practices regarding their self-care management. It comprised preventive measures as; isolation, cleanliness, health habits, diet patterns, treatment and follow up; and self-care regarding common physical problems from T.B as; fever, loss of weight, productive bloody cough and sputum, dyspnea, chest pain, general weakness and night sweating. The observational checklist was scored as (3) if completely done, (2) if incompletely done and (1) if not done.

The observational checklist was used also for assessment of home environment of the study subjects, it was adapted from *Maurer and Smith* ⁽¹⁴⁾, it was composed of (12) closed ended questions, to observe participants by a checklist and assessing the home condition. Items included as residence, type of home, ventilations, room number, water source, sewage disposal and garbage refuse. Each item was assigned score (1) if present or zero if absent. Validity of contents was measured by four experts in the field of medical and community health nursing to test relevance and completeness.

Pilot study

A pilot study was carried out on ten adult patients with pulmonary tuberculosis (10%), to test content relevance, tools applicability, clarity of items needed to fill in the sheets using the interviewing questionnaire and observational checklist applied as pre/post-tests. Those participants excluded from the main study sample. Some modifications and rephrasing of certain tools questions were done accordingly. The content validity of the tools were revised by six consultant in community health nursing, medical surgical nursing and by chest specialists.

Ethical considerations and administrative issues:

An oral consent was obtained from each study subject, who agreed to participate in this study in order to gain their cooperation. They were assured about confidentiality of information given and that will be used only for the purpose of the study. They were also informed about their right to withdraw from the study at any time without giving any reason.

Procedures (field work)

Approval was taken from Benha Chest Hospital Director upon a letter issued from the Faculty of Nursing Dean to obtain permission for conduction of the study. Based on the results obtained from interviewing questionnaire and observational checklist as well as literature review, the counseling intervention was developed by the researchers. Contents of counseling were selected to meet study

subject's needs. All participant members received the same contents. Methods of the teaching were: lectures, discussion, demonstration and redemonstration; and presentation were done using suitable teaching aids real objects (posters / pictures). The counseling was implemented on 100 adult patients with pulmonary tuberculosis in the form of individual visits at their homes from January 2010 up to December 2010. The duration of home sessions lasted 1-2 hours in each visit, at least two sessions were covered. Home visits were paid 4 times/week. Each session started by a summary about the previous session and the objectives of the new one, taking into consideration the use of simple language that suite the adult patient T.B's level of understanding. The study subjects were interviewed immediately after counseling implementation. Evaluation was based on the score of the acquired knowledge, practice and self-care management in the pre-test and immediately post-test.

Statistical analysis

Statistical presentation and analysis of the present studied data were carried out, using the mean, standard deviation, student t-test, paired t-test, chi-square, linear correlation coefficient and analysis of variance (ANOVA) test by using the Statistical Package for Social Science (SPSS), version 17.

3-Result

Table (1) shows personal characteristics of the studied sample, 59.0% of patients were males, regarding to age, 66.0% of them were 40 years and more, with a mean age of 41.20 ± 5.646 years. As for marital status, 77.0% of them were married. Regarding to educational levels, 30.0% of them were secondary school, 35.0% of them were employees and 74.0% of them their income was inadequate.

Table (2) shows that, there were highly statistically significant improvements in all items related to knowledge of the T.B patients after implementation of the counseling. ($P < 0.001$).

Table (3) indicates that there were highly statistically significant improvements in all practice items toward T.B disease after implementations of the counseling. ($P < 0.001$).

Table (4) reveals that, there were highly statistically significant improvements in all items of the physical symptoms after counseling implementation ($P < 0.001$). Table (5) reveals that, there were highly statistically significant improvements in all items of the social condition after implementation of the counseling except for the items of change the working relationship, and insecurity, which showed statistically insignificant differences pre/post-implementation of the counseling. ($P > 0.05$).

Table (6) shows that the majority of the studied sample(87.0%), were always feeling anger at pre-test, while, post-implementation, 7.0% were rarely complaining from it. Concerning feeling of depression at pre-program, it represented 87.0%, however; after counseling implementation at post-test 26.0% rarely complained. The same table reveals that, there were highly statistically significant differences between pre/post counseling regarding to all psychological stress symptoms of T.B patients.

As evident from Table (7), there were highly statistically significant relationships between patients' total knowledge and their educational level(illiterate/read and write), but it shows insignificant relationships between patients' total knowledge and patient's age (20-<30 and 30<40) and inadequate income. There was highly statistically significant relationship between patients' total practice and their educational level ($P < 0.001$) and adequate income but not for their age. As regards total self-care management, the same table shows that significant relationships with both age and educational level but for patient's income it shows an insignificant relationship.

Table (8) shows that, there was a positive highly significant correlation between patient's total practices and total self-care management.

Table (9) shows positive highly statistically significant correlations between knowledge, practice, self-care management and the patient's home environmental condition.

Table (1): Frequency of personal characteristics studied sample (n= 100).

Items	%
Sex :	
Male	59.0
Female	41.0
Age	
20-	4.0
30-	30.0
40 +	66.0
Mean±SD	41.20±5.646
Marital status	
Single	15.00
Married	77.00
Widowed	8.00
Educational level	
Illiterate	29.00
Read and write	21.00
Basic education	13.00
Secondary/Diploma	30.00
University education	7.00
Occupation	
Employed	35.00
Housewife	30.00
Private worker	19.00
Unemployed	16.00
Income	
adequate and saving	14.00
Adequate	12.00
inadequate	74.00

Table (2): Frequency of the studied sample knowledge pre /post counseling implementation (n = 100).

Knowledge	Pre- program (%)			Post- program (%)			X ²	P-value
	Good	Average	Poor	Good	Average	Poor		
Definition of T.B	1.00	9.00	90.00	93.00	7.00	0.00	180.29	<0.000**
Causes	6.00	43.00	51.00	99.00	1.00	0.00	173.46	<0.000**
Types of T.B	12.00	21.00	67.00	100.00	0.00	0.00	157.14	<0.000**
Modes of transmission	5.00	22.00	73.00	96.00	4.00	0.00	167.45	<0.000**
BCG immunization	58.00	0.00	42.00	97.00	0.00	3.00	43.61	<0.000**
Symptoms	61.00	0.00	39.00	97.00	0.00	3.00	39.06	<0.000**
Treatment	11.00	35.00	54.00	99.00	1.00	0.00	156.51	<0.000**
Nutritional patterns	1.00	9.00	90.00	91.00	3.00	6.00	134.87	<0.000**
Complication	0.00	6.00	94.00	100.00	0.00	0.00	200.00	<0.000**
Clinical investigation	0.00	9.00	91.00	90.00	9.00	1.00	178.04	<0.000**
Contra indication drugs	0.00	2.00	98.00	88.00	12.00	0.00	193.14	<0.000**
Follow up	2.00	20.00	78.00	97.00	3.00	0.00	181.727	<0.000**
Health habits(smoking, shisha)	54.00	0.00	46.00	86.00	0.00	14.00	24.00	<0.000**
Common health symptom from disease as:								
*Productive bloody cough and sputum.	51.00	0.00	49.00	70.00	0.00	30.00	7.553	<0.006**
*Loss of weight and appetite.	6.00	15.00	79.00	90.00	10.00	0.00	153.500	<0.001**
Preventive methods	2.00	8.00	90.00	96.00	4.00	0.00	181.497	<0.001**
Total Knowledge	Mean ± SD	Range	Mean ± SD	Range	Paired t- test	p-value		
	35.59 ±7.342	0 - 48	59.33 ± 2.731	48 - 63	30.712	<0.001**		

** Highly statistically significant difference (P ≤ 0.001)

Table (3): Frequency of the studied sample practices pre/ post counseling implementation (n = 100)

Practice	Program (%)				X ²	P-value
	Pre		Post			
Isolation						
• Special and good ventilated room for T.B patient.	55.00		90.00		30.72	<0.001**
• Private personal utensils to control droplet infection, drinking and eating equipments (Spoons, plate, & bottles) clothes, towel bed linens, musk, thermometer, and tooth brush.	39.00		97.00		81.411	<0.001**
Prevention						
• Hand washing after coughing or sneezing.	8.00		63.00		68.946	<0.001**
• Put face mask or paper tissue during coughing or sneezing.	7.00		58.00		62.532	<0.001**
• Remove the sputum discharge in special plastic container and despoiled it in waste basket.	6.00		56.00		58.913	<0.001**
• Follow good health habits (continue personal hygiene, change and wear clean cloths.	10.00		53.00		52.105	<0.001**
• Refrain on cigarette smoking or shisha.	30.00		92.00		81.742	<0.001**
Nutrition						
Take balanced diet (protein, vitamins, carbohydrate, fat)	32.00		91.00		80.028	<0.001**
Increase protein intake	48.00		92.00		47.654	<0.001**
Increase number of meal/day	30.00		95.00		96.857	<0.001**
Treatment						
Taking regular ways and proportions.	51.00		90.00		36.567	<0.001**
Continuous follow up	46.00		95.00		57.723	<0.001**
Breathing exercise practice	21.00		89.00		93.414	<0.001**
Total Practice	Mean ± SD	Range	Mean ± SD	Range	Paired t-test	p-value
	15.59 ± 2.582	0 - 15	25.72±0.74	9 - 24	22.901	<0.001**

**Highly statistically significant difference ($P \leq 0.001$)

Table (4): Frequency of the studied sample self-care management toward common physical symptoms pre /post counseling implementation (n = 100).

Physical Symptoms	Pre-program (%)			Post-program (%)			X ²	P-value
	Always	Sometimes	Rarely	Always	Sometimes	Rarely		
Headache	69.00	26.00	5.00	0.00	5.00	95.00	164.226	<0.001**
Pain in the muscles	70.00	11.00	19.00	0.00	4.00	96.00	124.823	<0.001**
Pain in the joints	80.00	7.00	13.00	0.00	0.00	100.00	153.982	<0.001**
Weight loss	85.00	8.00	7.00	0.00	0.00	100.00	173.832	<0.001**
Productive and bloody cough	87.00	6.00	7.00	0.00	3.00	97.00	165.885	<0.001**
General weakness	10.00	12.00	78.00	0.00	1.00	99.00	21.799	<0.001**
Yellowish, bluish or bloody sputum	43.00	0.00	57.00	0.00	6.00	94.00	58.066	<0.001**
Loss of appetite	87.00	0.00	13.00	0.00	0.00	100.00	153.982	<0.001**
Fever	87.00	2.00	11.00	0.00	2.00	98.00	156.440	<0.001**
Nausea	43.00	0.00	57.00	0.00	0.00	100.00	54.777	<0.001**
Vomiting	75.00	12.00	13.00	0.00	7.00	93.00	136.693	<0.001**
Constipation	11.00	12.00	77.00	0.00	0.00	100.00	25.989	<0.001**
Diarrhea	87.00	0.00	13.00	0.00	5.00	95.00	154.259	<0.001**
Dyspnea	69.00	0.00	31.00	0.00	6.00	94.00	106.752	<0.001**
Night sweats	75.00	2.00	23.00	0.00	2.00	98.00	121.488	<0.001**
Itching	87.00	7.00	6.00	0.00	0.00	100.00	177.358	<0.001**
Chest pain	75.00	0.00	25.00	0.00	1.00	99.00	120.161	<0.001**
Skin irritation	70.00	11.00	19.00	0.00	0.00	100.00	136.134	<0.001**
Influenza	75.00	11.00	14.00	0.00	2.00	98.00	144.231	<0.001**
Slow heart beat	85.00	8.00	7.00	0.00	0.00	100.00	173.832	<0.001**
Drowsy	70.00	12.00	18.00	0.00	3.00	97.00	129.670	<0.001**
Total Physical Symptoms Level	Mean ± SD	Range	Mean ± SD	Range	Paired t-test	p-value		
	68.413±14.735	32 - 89	28.626 ± 3.250	69 - 82	24.637	<0.001**		

**Highly statistically significant difference ($P \leq 0.001$)

Table (5): Frequency of the studied sample according to self-care management regarding their social condition pre / post counseling implementation (n=100).

Social Symptoms	Pre-program (%)		Post-program (%)		X ²	P-value
	Yes	No	Yes	No		
Change the work's relationship	47.00	53.00	49.00	51.00	0.080	>0.05
Improvement in social relations	7.00	93.00	56.00	44.00	55.637	<0.001**
Loneliness	8.00	92.00	28.00	72.00	13.550	0.001**
Insecurity	0.00	100.00	2.00	98.00	2.020	>0.05
Difficulty in confrontation	94.00	6.00	28.00	72.00	17.151	<0.001**
Total Social Symptoms Level	Mean ± SD	Range	Mean± SD	Range	Paired t- test	p-value
	10.01± 1.352	6 - 12	7.560±1.713	4 - 12	8.769	<0.001**

**Highly statistically significant difference ($P \leq 0.001$)

Table (6): Frequency of the studied sample regarding self-care management toward their psychological stress pre / post counselling implementation (n = 100).

Psychological stress symptoms	Pre-program (%)			Post program (%)			X ²	P-value
	Always	Sometime	Rarely	Always	Sometime	Rarely		
Feeling confused	0.00	8.00	55.00	0.00	28.00	25.00	21.660	<0.001*
Feeling worried	85.00	5.00	2.00	0.00	24.00	2.00	88.092	<0.001*
Feeling anger	87.00	8.00	0.00	0.00	44.00	7.00	116.218	<0.001*
Feeling lazy and tired	5.00	2.00	44.00	0.00	12.00	32.00	13.596	<0.001*
Constantly frustrated	75.00	0.00	20.00	0.00	30.00	31.00	104.947	<0.001*
Feeling depressed	87.00	2.00	6.00	0.00	12.00	26.00	100.713	<0.001*
Feeling stress	82.00	11.00	0.00	0.00	56.00	0.00	109.807	<0.001*
Total Psychological Stress Symptoms Level	Mean ± SD	Range	Mean ± SD	Range	Paired t- test	p-value		
	67.760±13.238	26 - 77	33.900±7.096	23 - 43	23.749	<0.001*		

**Highly statistically significant difference ($P \leq 0.001$)

Table (7): Relationship between total mean score of studied sample knowledge, practice & self-care management, and their characteristics (n=100).

Item	Knowledge Mean ±SD	Practice Mean ±SD	Self-Care Management Mean ±SD	ANOVA	
				F	P-value
Personal Characteristics					
Age :					
20-	37.00±6.06	11.75±2.22	54.17±7.55	1.075	0.345
30-	37.10±6.82	13.07±2.61	53.89±7.52	2.294	0.106
40 +	34.82 ±7.61	13.49±2.54	48.39±7.91	4.29	0.021*
Educational level :					
Illiterate	32.83 ± 6.95	10.31±0.81	46.36±3.20	6.644	<0.001**
Read and write	30.10±10.12	10.00±1.41	51.50±8.12	10.009	<0.001**
Basic education	35.08±5.66	9.77±1.69	52.50±4.44	8.66	0.04*
Secondary/Diploma	38.10±6.37	9.93±1.46	52.37±8.79	9.45	0.03
University education	39.43±6.21	9.57±1.90	55.88±5.33	10.63	<0.001**
Income :					
Enough and saving	37.86±5.40	6.86±2.85	48.64 ± 8.02	0.809	0.448
Enough	35.14±7.50	8.64±2.56	52.66 ± 7.97	5.395	0.007*
Insufficient	35.75±8.35	11.00±2.56	53.00±5.20	1.684	0.191

* Statistically significant difference ($p \leq 0.05$)

**Highly statistically significant difference ($P \leq 0.001$)

Table (8): Correlation between total practice of the studied sample and their total self-care management.

Items	Self-Care Management	
	r	P-value
Total Practices	0.731	<0.001**

**Highly statistically significant difference ($P \leq 0.001$)

Table (9): Correlation between total knowledge, practice and self-care management of the studied sample and their home environment condition.

Items	Environmental condition	
	r	P-value
Knowledge	0.804	<0.001**
Practice	0.775	<0.001**
Self-care management	0.77	<0.001**

**Highly statistically significant difference ($P \leq 0.001$)

4. Discussion:

The aim of the current study was to evaluate the counseling about self-care management of adult patients with pulmonary tuberculosis. This aim was achieved through; assessing the health needs (knowledge and practice) related to their self-care management, designing, implementing and evaluating the outcome of self-care management improvement after counseling implementation. The study sample was 100 (T.B) patients, the mean age was 41.20 ± 5.646 years; this finding can be referred to the high prevalence of smoking especially shisha among males. This finding was supported by *Bam study*¹⁵, which found that the mean age of the studied group was 41.06 ± 931 . On the same context, a recent study carried out by *Yadav and Bhatt et al.*,¹⁶ also showed that the majority of T.B patients were in the age group of 15-45 years. This finding suggests that T.B is common among economically active group and this lead to direct impact to the family economic status. And most of them married and the majority of them have got secondary diploma. As regards sex, the present study results showed that slightly less than three fifth of the T.B patients were males, this finding was consistent with, *Darwish et al., (2008)* who reported that the prevalence of T.B patients was higher among males than females and among low educated patients, also residents in rural and industry areas and also among low income patients.

According to studied sample knowledge related to tuberculosis (definition, causes, types, and mode of transmission, immunization, symptoms, treatment, complications, investigations, follow-up nutrition patterns, drug contraindications, preventive methods and common health symptoms from disease) improved after the counseling implementation. This agrees with the study conducted by *Hashim, et al.*,¹⁸ who reported that the majority of T.B patients, knew most knowledge about their disease as causes, mode of transmission, predisposing factors, common

symptoms, nutritional needs and methods of prevention.

On the other hand, regarding the study sample practices related to isolation technique, the findings of the current study revealed that most of the T.B patients had special and good ventilated room and personal utensils after the counseling intervention. Regarding to practice toward methods of prevention, the finding of the study demonstrated that, more than half of T.B patients were using paper tissue or face mask, to cover mouth and nose during coughing and sneezing, put the sputum in special plastic container and despoiled it in waste basket or in the trash, wearing washed and clean clothes continuously, while more than half of the T.B patients were washing hands after coughing or sneezing while most of the study sample were refraining on cigarette smoking or shisha after counseling implementation.

As regards T.B patients nutritional practice, the study results revealed that, less than half of patients were eating three meals/day and at regular intervals while regarding the types of food during the period of disease less than half were eating integrated meals, this ratio was improved considerably after counseling implementation to be followed by most of them.

Regarding to practice toward treatment systems before counseling, almost half of T.B patients were taking treatment regularly, more than two fifth continue for follow up, and also one fifth of patients were practicing breathing exercises to reduce shortness of breath. All these ratios increased after counseling implementation to reach most of them.

According to T.B patients self-care management toward common physical symptoms as general weakness, underweight, loss of appetite; pain (chest, abdomen, muscles, joints), digestive disorders (vomits – nausea- vomiting and constipation) sweat, dyspnea and coughing. diet, preventive methods as (hand wash- isolate sputum), there were highly statistically significant improvements, in all the previous items after implementation of counseling. This findings are

in agreement with *Hashim, et al.*,¹⁸ and *Mangesho, et al.*,¹⁹ who reported that more than three quarters of T.B patients presented with cough, fever, chest pain and weight loss as main complains of T.B, this condition improved post health education intervention.

Patients with T.B had social effect on their relation pre intervention but post counseling, results revealed that, there were highly statistically significant improvement in all items of the social complaints after counseling except for the items of change the work's relationship, and insecurity, were statistically insignificant differences pre/ post counseling were detected. This insignificant difference in improvement can be explained by the competitive nature of the work environment, official and rigid rules that regulate work but cannot be easily changed. The same can be said about patients insecurity as, patients security is a complex issue that has multifactor patterns not only the home security can affect the patients but also the community security can do. This result supported by *Zacks et al.*,²⁰ who concluded in cross-sectional study that, the majority of T.B subjects reported financial insecurity, internalized shame, and social rejection, regardless of method of T.B acquisition or socioeconomic status?

Considering psychological stress, *Levensone et al.*,²¹ and *Wright*²² highlighted that chronic health problems are associated with psychological stress and depression. The present study finding was inconsistent with them. The majority of patients before implementation of self-care management counseling were complaining from many psychological symptoms like feeling worried, anger, difficult to relax, difficult to concentrate during work, constantly frustrated, feeling stressed and feeling depressed. However, after implementation of the counseling there were significant improvements in all items of the psychological aspects. This was due to an increase in patients' moral spirit and there was decrease in patient's stress symptoms. In relation to personal characteristics of the studied sample and their knowledge, practices on self-care management, results revealed that highly statistically significant relationships were detected between patients' total knowledge and their educational level; however insignificant relationship were found between patients' total knowledge and their age and income.

There was highly statistically significant relationship between patients' total practice and their educational level and income but not for their age. As regards total self-care management, the same table shows that significant relationships with both age and educational level but for patient's income it shows an insignificant relationship. On the other hand, the current study result showed highly statistically

significant positive correlation between self-care management and the total practices. This result can clearly answer the stated research hypothesis in that when the counseling was conducted, it led to increase patient's awareness about their practices.

As regards correlations between patients total knowledge related practice and self-care management and their home environmental condition, results showed positive statistically significant correlations between knowledge, practice, self-care management and the patients home environmental condition after counseling intervention. This might be due to that the majority of studied sample were having a private and hygienic room, good ventilations and cleaning houses, good kitchens and proper collection of garbage refuse in special boxes.

Conclusion

The implemented counseling revealed significant improvements of patient's knowledge about T.B as (definition, causes, modes of transmission and preventive methods); practice as (isolation technique and prevention, nutrition pattern and treatment); and self-care management about T.B. There were highly statistically significant relationships between patients' total knowledge, practice, self-care management and their educational level. Significant improvements were detected for patient's physical, social and psychological symptoms after counseling implementation. as well, there were positive correlation between patient's knowledge, practice and self-care management.

Recommendations

- Based on the study finding, the following recommendation are suggest conducting health intervention counseling to all newly diagnosed T.B cases who attend the chest hospital.
- Continuous educational program to all patients and their families about T.B during the follow up visits to upgrade their knowledge and practices.
- Further researches are needed in other areas especially rural areas to implement intervention program about management and preventive measures of T.B.

Corresponding author

Howyida, S. Abd ElHameed,
Community Health Nursing Department
Faculty of Nursing, Benha University
elmokhtar.mohamed@yahoo.com

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Circulating Hematopoietic Stem Cell and Some Physiological Parameters in Different Training ProgramsMohammed Nader Shalaby¹, Jin Yu Liu², Mona Mahmoud Kassem³, Mohammed Saad⁴⁽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⁾Department of Health Sciences Faculty of Physical Education Port said University , Egypt⁽⁴⁾Faculty of Physical Education, Suez Canal University
dr.m.nader@hotmail.com

Abstract: Exercise is one of the most powerful non pharmacological strategies, which is able to affect nearly all cells and organs in the body. Changes in the behavior of adult stem cells have been shown to occur in response to exercise training. The aim of this study is to reveal the role of aerobic and anaerobic training programs on CD³⁴⁺ stem cells and some physiological parameters. 20 healthy male athletes aged (18-24 yrs) were recruited for this study. Healthy low active males and BMI matched participants (n=10) aged (20-22 yrs) were recruited as controls. Aerobic and anaerobic training programs for 12 weeks were used. VO_{2max}, pulse rate estimation using strand Rhythmic protocol. RBCs, WBCs, Hb and hematocrit were estimated using coulter counter, Lactate by accusport, CD³⁴⁺ stem cells by flow cytometer. Results revealed: VO_{2 max} was increased in case of aerobic training program compared to anaerobic one. Lactate concentration was decreased in case of aerobic training programs compared to anaerobic one. RBCs, WBCs, Hb and hematocrit were increased in anaerobic than aerobic training programs. CD³⁴⁺ stem cells were increased in case of anaerobic training program than aerobic one and control. It is concluded that training programs provoke better adaptation.

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Key words: Aerobic and anaerobic training programs, CD34⁺ stem cells, physiological parameters.

1. Introduction

Exercise is one of the most powerful non-pharmacological strategies, which is able to affect nearly all cells and organs in the body. In this context, a new research avenue focusing on the action of exercise on adult stem cells has emerged during the last decade. Changes in the behavior of adult stem cells from different regions including skeletal muscle and the cardiovascular system have been shown to occur in response to exercise training.

Through its action on adult stem cells, exercise may act on the regenerative potential of tissues by altering the ability to generate new stem cells and differentiated cells that are able to carry out tissue specific functions (Kado and Thonell, 2000). Strength and power are important aspects of fitness, sport and everyday activity. However, much debate remains as to how these qualities, should be assessed. Much of the debate originates from the definition of strength and power and the different terminology used across laboratories. Sale (1991) defined strength as the force exerted under a given set of conditions during a maximal voluntary contraction (MVC). Sale continued to define power as the rate at which mechanical work is performed under a specified set of conditions, or the product of force and velocity. Both definitions imply that strength and power are defined by conditions such

as velocity, contraction type, and posture and movement pattern specificity. That is, strength for one task may not imply strength for another. An associated problem with this is that strength and power are quite often measured in contexts dissimilar to the environment in which functional strength and power are needed (Fatourous *et al.*, 2000).

There are several training methods which are used to enhancement of strength and power of these methods is the complex circuit exercises together with the well known aerobic and anaerobic methods which are used in this thesis.

Guyton and Hall (2006), reported the effect of athletic training on muscles, they stated that muscles that function under no load, even if they are exercised for hours on end, increase little in strength. At the other extreme, muscles that contract at more than 50% maximal force of contraction will develop strength rapidly even if the contraction are performed only a few times each day. They also added that during muscle contraction blood flow increase about 13 fold but also the flow decrease during each muscle contraction, this decrease in flow is due to the compression of intramuscular blood vessel, but the blood flow to muscle increases during contraction.

Thomas Hawke (2005) stated that although endurance training is associated with high repetition

low resistance exercise, significant muscle damage can occur if the duration or mode of exercise is extreme, for example, both marathon running and downhill running can lead to significant muscle fiber damage. In contrast to endurance training, resistance exercise training is associated with high intensity. Low repetition work loading to increases in muscular strength, power and oxidative capacity, with little change in aerobic capacity. The workloads placed on skeletal muscle during resistance training are at or near maximal capacity, and as such produce significant perturbations to other skeletal muscle fibers and the associated extracellular matrix.

In a recent study, Burd *et al.* (2010), investigate the impact of two distinctly different exercise volumes on anabolic signaling myogenic gene expression, and rates of muscle protein synthesis (Mix, Myo, Sarg), specifically, they utilized a unilateral model in which subjects performed exercise at 90% IRM until failure (90 FAIL), 30% IRM in which the amount of external work was matched to 90 FAIL (30 WM), or 30% IRM to failure (30 FAIL). They reached the conclusion that low-load high volume resistance exercise is more effective in inducing acute muscle anabolism than high load low volume or work matched resistance exercises modes.

As for training induced adaptations, exercise induced neutrophilia was shown to become progressively blunted with training (Suzuki *et al.*, 1999), but no study ever tested whether circulating HPC counts may differ between trained and sedentary subjects. Circulating immature cells are likely involved in angiogenesis (Reyes *et al.*, 2002) and repair processes (Springer *et al.*, 2001) both mechanisms being possibly associated with strenuous exercise and progressive training. Given the large use of exercise based rehabilitation programs in several diseases, knowledge of the physiological effects of training on HPCs might be of potential clinical use.

Identification of EPCs on the cell surface expressions of various protein markers. There is no straight forward definition of an EPC marker because these cells seem to be a heterogeneous group associated with different cell surface antigen expression profiles. The most commonly described molecules that serve as biomarkers for recognition of an EPC population include CD³⁴⁺, CD133, and VEGFR2. The pioneer study of Asahara *et al.* (1999) recognized EPCs as CD³⁴⁺ mononuclear cells (MNCs). Hematopoietic stem cells that serve as a source of EPCs express CD³⁴⁺, however this marker is also present on the surface of mature endothelial cells (Fina *et al.*, 1990).

Human CD133 antigen is a membrane glycoprotein whose expression is related to hematopoietic stem cell differentiation into EPCs (Urbich and Dimmeler, 2004). The third marker proposed for EPC identification is VEGFR2, a protein

predominantly expressed on the endothelial cell surface. Urbich and Dimmeler, (2004) and Birn *et al.* (2005) claim that EPCs are positive for CD³⁴⁺, CD133 and VEGFR2 markers.

CD³⁴⁺ cells are multipotent progenitors that can engraft in several tissues (Krause *et al.*, 2001), circulating CD³⁴⁺ cells can be used to indirectly estimate hematopoiesis based on CD38, human leukocyte antigen (HLA) Dr, and CD33 markers..

Patrick and Stephane (2003) found that CD³⁴⁺ stem cell from elite triathletes to be significantly lower than in healthy sedentary subjects. They stated that the low CD³⁴⁺ counts and neutopenia and low lymphocyte counts could contribute to the increased upper respiratory tract infections observed in these sportsmen. They hypothesized three explanations (1) Aerobic training could induce deleterious effect on BM by inhibition of central CD³⁴⁺ SC growth (2) intense training could depress the mobilization of CD³⁴⁺ SC. (3) due to aetiology of the damage/ repair process. They conclude that CD³⁴⁺ SC quantification in elite sports men should be helpful for both basic science researches and sport clinicians.

The aim of this study is to reveal the role of aerobic and anaerobic training programs on CD³⁴⁺ stem cells and some physiological parameters.

2. Material and Methods

Participants:

Twenty healthy male athletes aged (18-24 yrs) with a training history of (4-9yrs) were recruited 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 non smokers, 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 II receptor antagonists, ACE inhibitors; peroxi some proliferators activated receptor (PPAR α) 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 weights 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.

Athletes were divided into two groups, one group was subjected to aerobic training program and the other group was subjected to anaerobic training program. The training program lasted for 12 weeks for each group each protocol was composed of warming up for several minutes, then the training cases which was ended by cooling down procedure for another minutes. $VO_2 \text{ max}$ value is obtained using Astrand Rhythmic nomogram 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 eosinophil (Guyton and Hall, 2006). All blood cells were counted using coulter counter which is easy to read numerical presentation. Lactate analysis was performed by using accusport after the training programs at rest compared to control at rest.

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 CD^{34+} 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.

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 u test (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 was 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.

Aerobic Training Program After Dr. Phil Esten (2010)

Physiologically speaking, the 3000, and 5000 meter events pull up to 80 percent of their performance energy from the oxidative energy system. Most of us know this system as the aerobic energy system. Therefore, 70 to 80 percent of training should be actual distance or aerobic running, which activates the oxidative energy system.

Anaerobic Training Program After Tom Green (2003)

Sprinting is a difficult combination of aggression, relaxation, technique and efficiency. The 100 meters is sometimes labeled as the easiest most complicated event in sport! And contrasting bodybuilding, gaining too much size can become a negative. Generally speaking world-class sprinters are not that large, anywhere from 155-180lbs. In fact, what's interesting is that some sprinters do not lift weights at all! But for those of us who aren't as genetically gifted, the ultimate goal is having incredible strength-to-weight ratios, lean body mass and a well-developed CNS (central nervous system) for fast reaction and the ability to explode on command.

3. Results

Subjects characteristics:

Twenty athletes 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 $VO_2 \text{ max}$ 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.

Twenty Athletes agreed to participate in 12 weeks of training sessions of aerobic and anaerobic exercises.

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): Haematopoietic stem cells for control, aerobic exercise training and anaerobic training courses of exercise for 12 weeks in the resting stages and Lactate

Variable	Control			Aerobic training			Anaerobic training		
CD ³⁴⁺ S cells	170.0	±	21.10	130	±	14.61	251.6	±	21,64
Lactate (mmol/L)	1.2	±	0.3	0.8	±	0.1	0.9	±	0.2

Table (2) reveals a significant changes after anaerobic training compared to aerobic and control in case of CD³⁴⁺ SC(values are means ±SE $P < 0.05$).

Table (3): Haematological values of RBCs, WBCs,HB and hematocrit (PCV) after aerobic and anaerobic training program (at rest) and control.

Variable	Control			Aerobic training			Anaerobic training			Sig
RBCs (million/mm ³)	4.7	±	0.9	4.9	±	0.2	5.3	±	0.3	S
WBCs (thousands/ mm ³)	4.8	±	0.7	6.1	±	0.4	6.6	±	0.5	S
Hb (g/dL)	12.8	±	0.8	14.2	±	0.5	15.4	±	0.4	S
Hematocrit (%)	42	±	3.2	44	±	1.1	46	±	1.2	S

Table (3) reveals a significant change between participant in aerobic program and anaerobic one in hematological value and control ($P < 0.05$).

Table (4): The variation in VO_{2 max} or participants healthy sedentary and after aerobic and anaerobic training programs.

Participants	VO _{2max} (mL/kg/min)		
Healthy sedentary (mL/kg/m)	36	±	1.7
Aerobic training program (mL/kg/m)	62	±	2.2
Anaerobic training program	54	±	2.1

The results are expressed as mean ± SE ($P < 0.05$).

Table (4) VO_{2 max} (mL/kg/min) results indicated an increased value between the healthy sedentary participants and after aerobic and anaerobic training programs.

4. Discussion

Sport's training is done for improving sports performance. The sports performance as any other type of human performance is not the product of one single system or aspect of human personality. On the contrary, it is the product of the total personality of the sports person. The personality of a person has several dimensions of physical, physiological, social and psychic. Sports training, therefore, directly and indirectly aim at improving the personality and fitness of the sport man. The specify of training principle states that the nature of tissue adaptation after training is dependent on the specific type of training practiced (Nieman, 2003).

Tables (1,2) revealed lower values of lactate after aerobic and anaerobic training programs which means a better fitness. Lactate is the end product of the anaerobic carbohydrate breakdown. It is the metabolite displaying the most spectacular concentration changes in muscle and the blood with exercise. As a result, its measurement offers a wealth of information regarding the effect of exercise on metabolism. Lactate determine in whole blood rather than plasma or serum. (Mougios, 2006) He also added that when measuring lactate in the blood after short hard or maximal exercise it is necessary to remember that it takes some minutes to peak. Thus, a blood sample taken right after the end of exercise will not produce the peak value. To trace it, it is needed to perform several minutes before taking the samples.

Programming training based on intensities dictated by blood lactate concentration is superior to programming training based on heart frequencies

because lactate relates directly to muscle metabolism and muscle adaptations. Thus, one could use lactate to determine training intensities at the beginning of a training program, monitor training through heart frequencies on a daily basis, and resort to lactate periodically every few weeks to fine tune intensities. Most investigators agree that intensities that hold below the blood lactate concentration below 4mmol/L are the most effective in improving aerobic endurance, cardiac function and the lipidemic profile (Greenhaff and Timmons, 1998; Mougios, 2006),

Barrett *et al.* (2010) in Ganong review of Medical physiology stated that blood consists of a protein rich fluid known as plasma, in which are suspended cellular elements: white blood cells, red blood cells and platelets. The normal total circulating blood volume is about 8% of the body weight (5600 ml in a 70 kg man). About 55% of this volume is plasma. They added that red cells, white cells and platelets are formed in the bone marrow, which is actually one of the largest organs in the body, approaching the size and weight of the liver. Hematopoietic stem cells (HSCs) are bone marrow cells that are capable of producing all types of blood cells. They differentiate into committed stem cells (Progenitors cells). The HSCs are derived from uncommitted, totipotent stem cells that can be stimulated to form any cell in the body, adults have a few of these, but they are more readily obtained from the blastocysts of Embryo.

Rothers and Roberts (1997) stated that the main functions of the cellular components of blood are the transport of oxygen and carbon dioxide, blood clotting, acid base buffering immune functions and tissue repair and destruction, and the function of plasma (liquid components) are blood clotting, circulating or cellular components and their contents, heat transfer and thermoregulation, water exchange and transport, circulation of hormones, acid base buffering, circulation of metabolites, nutrients and waste products.

Gillen *et al.* (1991), Burge *et al.* (1993) reported that acute effect of exercise on blood is to cause release of fluid from the vascular component, which decreases the volume of plasma and blood. This fluid loss from the plasma decreases plasma volume and cause hematocrit and plasma metabolite concentration to increase, which is termed hemoconcentration. In fact, a significant hemoconcentration occurs when a person moves from a supine to a vertical position. The added hemoconcentration of exercise is predominantly confined to the transition from rest to exercise. This response is followed by a more gradual hemoconcentration that occurs with increases in exercise intensity. And these changes are larger during the larger blood pressure associated with resistance exercise than during more prolonged dynamic exercise.

Spriet *et al.* (1986) added that prolonged exercise involving sweating increased fluid loss from the body,

and the degree of hemoconcentration can be measured by either directly measuring plasma volume or estimating relative changes in plasma volume from hemoglobin and hematocrit measurements. Also blood viscosity increases above what would be expected for hemoconcentration effects. In addition there is destruction of erythrocyte, termed hemolysis, which increases plasma hemoglobin concentration (Zierler *et al.*, 1992). This was in accordance with the increased cellular changes after training programs due to hemoconcentration (Table 3).

Endurance training increases the volume of blood the ventricle can hold and contributes to its maximum stroke, ventricular thickness is usually slightly increase. The blood cells, Rbs, Wbcs, platelets and Hematocrit and hemoglobin are slightly increase together with stem cells SC, CD³⁴⁺ (Tables 2,3).

As for the adaptive response to anaerobic exercise, blood cellular components of RBCs, WBCs, HCT and haemoglobin numbers and contents increased together with increase CD³⁴⁺SC compared to aerobic one and control CD³⁴⁺ (25,6±21,64) (130±14,61) and 170±21.10 (Tables 2.3), this was in accordance with the results of Bonsignore *et al.* (2002) Mobius – Winkler *et al.*, 2009 Bonsignore *et al.*, (2010), ,.

As for table(4), it indicated an increased Vo_{2max} value between sedentary, aerobic and anaerobic training programs participants. Also a significant change in aerobic compared to anaerobic training program which means a better cardiovascular adaptation for the aerobic group.

Amany and Mohamed (2011) reported the effect of concurrent training (endurance and resistance on CD³⁴⁺/CD⁴⁵⁺ stem cells, VO_{2 max}, certain physical variables and record level of 1500 m. running, they came to the results that there was a significant increase between pre and post measures in accounting of CD³⁴⁺/CD⁴⁵⁺ stem cells, power and strength, VO_{2 max} and record level of 1500m running for the sake of concurrent training group they concluded that the concurrent training for two months can improve physical, and the record level together with increased stem cells among young runners.

Resistance exercise stimulates the synthesis of skeletal muscle proteins (West *et al.*, 2009), which is expressed as muscle hypertrophy. It has recently been established that, myofibrillar (My) protein synthesis is already maximally stimulated at 60% IRM, in the post absorptive state, with no further increase at higher load intensities (ie 75 – 90 % IRM) (Kumar *et al.*, 2008).

Additionally, performance of low load contraction (~ 20 IMR) with vascular occlusion is sufficient to induce an increase in mixed muscle (Mix) protein synthesis (Fujita *et al.*, 2007).

In 2010, Burd *et al.*, reported that low-load high volume resistance exercise is more effective in inducing acute muscle anabolism than high-load low

volume or work matched resistance exercise modes. Fifteen young men (21 ± 1 years), performed 4 sets of unilateral leg extension exercise at different exercise loads and/or volumes. 90% of repetition maximum (IRM) until volitional failure (90 Fail) 30% IRM work matched to 90% fail (30 wM), or 30% IRM performed until volitional failure (30 FAIL).

Regular physical activity is associated with enhanced endothelial function which has been related to lower incidence of cardiovascular disease (Delp *et al.*, 1993; Delp 1995; Hambrecht *et al.*, 2003 and Haram *et al.*, 2006).

Bonsignore *et al.* (2002) suggested that increased HPCS reflect on adaptation response to recurrent, exercise-associated release of neutrophils and stress and inflammatory mediators, indicating modulation of bone marrow activity to habitual running. In 2004, Laufs *et al.*, measured EPCS in mice and patients with stable CAD. Mice engaged in 3 weeks of voluntary wheel running and humans underwent a 4 week training program of bicycle ergometer endurance exercise (60- 80% peak $\text{VO}_{2\text{max}}$), strength exercise, and walking. EPC number was significantly increased in the blood, bone marrow of mice after 7 days of exercise which persisted for the 28 days of the training program. In human the number increased $78 \pm 34\%$ compared to before the 4 week training program. EPC apoptosis was found to decrease $41 \pm 11\%$ after training. As for Steiner *et al.* (2005) who utilized a 12 week exercise program in patients with asymptomatic coronary artery disease (CAD). Results showed a 2.9 ± 0.4 fold increase in circulating EPCs in the exercise group. This increase was correlated with an increase in flow mediated dilation and no synthesis.

In another training study, Sandri *et al.* (2005) analyzed the responses of circulating ($\text{CD}^{34+}/\text{KDR}^{\pm}$) number and function in three patient groups, those with ischemic and training occurred for 4 weeks. Increases in CPC was increased in all three groups accompanied by an increase in the CXCR4, also VEGF levels were increased in the groups. They concluded that the ischemic exercise groups appeared to increase VEGF, which may have stimulated the increase in CPC numbers. Thijssen *et al.* (2006) reported no change in $\text{CD}^{34+}/\text{KD}^{\pm}$ cells in healthy young and older participants following 8 weeks of cycle exercise training for 20 minutes 3 time per weeks at 65% of heart rate reserve. Therefore, exercise training may not increase EPC number in healthy individuals. Vasankari *et al.* (1998), Hoetzer *et al.* (2007) reported that exercise may improve the number and function of EPCS while improving oxidative stress status.

Conclusion

It may be concluded that:

- $\text{VO}_{2\text{max}}$ was increased in case of aerobic training program compared to anaerobic one and control indicating a better cardiovascular adaptivity.

- Lactate concentration was decreased in case of aerobic training program and anaerobic one compared to control meaning a better fitness..
- Hb, RBCs, WBCs and hematocrit value were increased after anaerobic training program compared to aerobic one due to stress.
- CD^{34+} SC counts were increased in peripheral blood of anaerobic training program then aerobic one and control due to stress and indicating better adaptation.

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Corresponding author:

Mohammed Nader Mohammed Shalaby

Norman Bethune College of Medicine, Jilin University China And Faculty of Physical Education, Port said University Egypt

Phone number: 00201000400900

E-mail: dr.m.nader@hotmail.com

Prof. 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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Study of some Fibrosis Indices in Genotype 4 HCV Infected Egyptian Patients

Niveen Saady^{1*}, Doaa M. Elghannam¹, Raghada Farag², Mohamed Abd El-Maksoud², Mona A. El-Hussiny¹, Mohamed El-Malky³, Nader El-Malky⁴

Departments of ¹Clinical Pathology, ²Tropical Medicine, ³Medical Parasitology and ⁴Internal Medicine, Faculty of Medicine, Mansoura University, Egypt.

mona_clinic@hotmail.com

Abstract: Background and Aims: In HCV infected patients, liver biopsy is considered essential to stage liver fibrosis. Procedure of liver biopsy is invasive, expensive and not suitable for all patients. The present study aimed to evaluate the diagnostic accuracy of the readily available non-invasive fibrosis indexes for the fibrosis progression discrimination in chronic HCV mono infected and co-infected *Schistosoma mansoni* patients and to find a better combination of existing non invasive markers. **Methods:** The study included 100 genotype 4 HCV mono-infected and *S. mansoni* co-infected patients who underwent liver biopsy. The degree of fibrosis was scored according to the METAVIR staging system. The readily available AAR, APRI, FI, FCI, FT and FIB-4 serum indices. were tested in the patients. **Results:** There was a significant relationship between fibrosis stages and serum indexes except AAR and FCI ($P > 0.05$). AUROC of FT was higher than other indexes ($P < 0.05$) for differentiating minimal fibrosis (F1) from significant fibrosis (F2-F4). Also, FT showed high AUROC to predict cirrhosis. In HCV mono infected patients, minimal fibrosis can be identified using FCI and FT with sensitivity 57% for both, and specificity 58% and 57% respectively while cirrhosis can be identified using FI, FIB-4, APRI, FT, and AAR with sensitivity 100%, 75%, 100%, 50% and 100% and specificity 53%, 77%, 60%, 100%, and 59% respectively. In HCV/*S. mansoni* coinfection patients, minimal fibrosis can be identified using FT, FIB-4, APRI, FI, FCI and AAR with sensitivity 70%, 70%, 71%, 70% 60% and 60% and specificity 61%, 75%, 55%, 62%, 55% and 50% respectively while cirrhosis can be identified using FT, FI, FIB-4, and FCI with sensitivity 88%, 88%, 50% and 50% and specificity 52%, 70%, 60%, and 58% respectively. Moreover, *S. mansoni* anti-SEA was poorly significant with fibrosis stages. **Conclusion:** All methods used for predicting liver fibrosis were directly, and significantly, correlated with histological findings, but FT, FI, and APRI score had the strongest correlation with fibrosis severity while, AAR, and FCI showed significantly low 'r' index. These results suggest that the using FT as a first-line test in the social health centers seems feasible and effective.

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Key words: HCV, *Schistosoma mansoni*, fibrosis, Egypt,

1. Introduction

Viral hepatitis C is a serious liver disease affecting 180 million people worldwide (1). The severity of the disease associated with Hepatitis C Virus (HCV) infection varies from asymptomatic chronic infection to cirrhosis and hepatocellular carcinoma (2). Egypt has the highest HCV prevalence in the world, with an overall prevalence of 12% among the general population, 40% in persons above 40 years of age, and even higher among persons in rural areas (3). Genotype 4 is the predominant genotype of HCV in Egyptian patients (4).

Schistosomiasis and HCV co-infection is common in Egypt. Some authors postulated an evidence of the association between the schistosomiasis treatment campaigns and the high HCV sero-prevalence rates observed in Egypt (5). Patients with HCV/*Schistosoma mansoni* co-infection have a more rapid progression of HCV liver fibrosis than do those with HCV infection alone and exhibited higher titers of HCV RNA (6). Schistosomiasis per se may cause the persistence of viremia due to reduced

immunity (7). Prevalence of periportal thickening and fibrosis (PPT/F) increased significantly with increasing intensity of *S. mansoni* infection (8).

Staging liver fibrosis is considered to be an essential part in the management of patients with chronic hepatitis C (CHC), because it provides prognostic information and, in many cases, assists in therapeutic decisions (9). At present, liver biopsy is still most commonly used as reference standard for the assessment of liver fibrosis. However, its expense, risk of side-effects, and potential inaccuracy from sampling and observation errors reduce its utility for frequent liver fibrosis screening (10).

Currently, there are several non-invasive diagnostic methods for determining liver fibrosis that are being validated, such as blood markers and imaging methods (11). Several scoring systems like AST to ALT ratio (AAR), AST-Platelet ratio (APRI), Fibrotest (FT), Fibrosis Index (FI) and FIB-4 with different thresholds to predict presence or absence of fibrosis or cirrhosis in patients infected with HCV had been proposed (12-17).

Recently, a new marker FCI (fibrosis cirrhosis index) had been postulated to predict fibrosis in HCV infected patients (18).

The purpose of our study was to evaluate and compare the diagnostic performance of the readily available non-invasive serum indexes including FT, AAR, APRI, FI, FIB-4 and FCI to find accurate and reliable non-invasive markers for evaluating fibrosis progression in HCV with or without Co schistosomiasis infection.

2. Methods

Patients

We carried out a retrospective cross-sectional study of all patients with documented HCV who underwent a liver biopsy between January 2010 and June 2011 at the outpatient clinic of the Tropical Medicine Department of Mansoura University Hospital. Elevated aminotransferase (ALT greater than 45 IU/L for >6 months (measured on at least two separate occasions), detectable levels of HCV-RNA and compatible hepatic histology were mandatory for the diagnosis of chronic liver disease secondary to HCV infection. Liver biopsies were performed on patients who were potential candidates for interferon plus ribavirin therapy. Consents were obtained from subjects included in the study. The study was approved by ethical committee of Mansoura Faculty of Medicine, Egypt.

Exclusion criteria

Patients who received a previous course of INF or immunosuppressive therapy or who had clinical evidence of Hepatitis B infection, HIV infection, end-stage renal disease, autoimmune disorders, liver cancer or complication of portal hypertension (variceal bleeding, encephalopathy, ascites, Child-Pugh B or C), were excluded from the study. Also, patients who had hemoglobin lower than 12 g/dl, pregnancy, neoplastic disease, uncontrolled psychiatric disease, severe cardiac disease; alcohol or drug abuse and a contraindication for liver biopsy (low platelet count < 70,000 plt/mm³, prolonged prothrombin time or decompensated liver cirrhosis) were excluded. This study included 100 patients (M/F 84/16; mean age 43.9 ± 7.8 (range 27-62 years).

HCV RNA detection and quantitative PCR

HCV infection was first documented in all patients by third-generation enzyme-linked immunosorbent assay (Abbott anti-HCV ELISA, Abbott Lab, IL, USA). RNA was extracted from 140 µl serum samples using QIAamp viral RNA extraction kit (Qiagen USA cat # 52906) according to the manufacturer's protocol. The HCV viral load was measured by Real time PCR, Stratagene Mx3000P Real-Time PCR System with a sensitivity of approximately 15 IU/ml.

HCV genotyping

Samples positive for HCV-RNA by real time PCR were subjected to genotyping of HCV, by RT-PCR for the core domain using the primers modified by Ohno *et al.* (19).

Histological evaluation of biopsy samples

The histological evaluation of paraffin-embedded liver specimens was carried out at the Pathology Department, Mansoura faculty of Medicine, following the recommendations of the Patient Care Committee of the American Gastroenterological Association (20). Ultrasound was routinely used to determine the percutaneous biopsy site. Liver fibrosis was estimated according to METAVIR scoring system (21). Histological staging based on the degree of fibrosis have five degrees of fibrosis: as F0 (no fibrosis), F1 (mild portal fibrosis without septa), F2 (moderate periportal fibrosis with few septa), F3 (severe fibrosis, fibrous septa with architectural distortion but with no obvious cirrhosis (bridging fibrosis) and F4 (cirrhosis).

We further grouped fibrosis stages as F0-F1 (minimal fibrosis), F2-F3 (advanced fibrosis), and F4 (cirrhosis). F2, F3, F4 (significant fibrosis).

Clinical and Laboratory data of Biomarkers of fibrosis

A complete clinical evaluation was performed on each patient. Immediately prior to the liver biopsy, 3 venous blood samples were obtained from all subjects and were processed in our hospital's laboratories'. EDTA blood samples were subjected to complete blood counts as (HB, Platelets, WBCs), by automated Sysmex 800. Sera were separated and tested for Albumin, aspartate aminotransferase (AST), ALT, total bilirubin, gamma-glutamyl transpeptidase (GGT), alkaline phosphatases (ALP) and total cholesterol; using a Hitachi 902 Analyzer (Roche Diagnostics, Branchburg, NJ). Also, Prothrombin index, INR by Sysmex 540 coagulation analyzer (Dad Behring) was measured. Frozen serum stored at -80°C were analyzed for further assays to determine the special biomarkers designed to estimate the stage of fibrosis: Haptoglobin and Apolipoprotein A1 (Apo A1) concentration by Radial immunodiffusion (DIFFU-PLATE; Biocientifica®, SA, Buenos Aires), Alpha-2-macroglobulin: Quantitative determination using Turbidimetry technique (SPINREACT, S.A. Ctra, Santa Coloma)

Bilharisiasis ELISA

Serological detection of anti-*S. mansoni* IgG antibodies was done using the indirect ELISA technique where microtitration plates were sensitized using *Schistosoma* soluble egg antigen.

Anti-*Sm* IgG was tested by ELISA as follow;

Schistosoma mansoni soluble egg antigen (SEA) was prepared according to the method described (22). Total IgG responses to *S. mansoni* SEA was measured by indirect enzyme-linked immuno-sorbent assay

technique (ELISA) according to the general principles described by **Engvall and Perlmann (23)**. Briefly, Maxisorb polystyrene flat-bottomed micro-titration (Nunc, Roskilde, Denmark) plates were coated by overnight incubation (ON) at 4°C with 5 µg/ml antigen. The plates were washed six times in between each incubation step. Following the blocking step [0.1% (w/v) bovine serum albumin (BSA) (Fraction V, Sigma, MO, USA) in 0.035 M phosphate-buffered saline (PBS), pH 7.8, 1 h incubation at 37°C], the serum samples were loaded into the wells and incubated for 1 h at room temperature (RT). Sera were diluted 1/200 for IgG. All samples were tested in duplicate. As detecting antibodies, rabbit-anti-human IgG labeled with peroxides diluted 1/2000 (from Sigma, MO, USA) was used. Incubation times were 1 h at RT. Finally, the assays were developed using 3, 3', 5, 5' tetramethyl-benzidine (TMB) (Sigma, MO, USA), incubated for 15 min in the dark, and stopped by adding 20 % H₂SO₄. Absorbance at wavelength of 450 nm for substrate colour and 620 nm as reference was measured using ELISA Reader (Robbonic - India). Absorbance of the samples and the control plates without coating antigens were subtracted from the absorbance of the same samples. Antibodies concentration was calculated from optical densities.

The following scores were evaluated for predicting liver fibrosis: AAR, APRI (AST to platelet ratio index), Fibrosis Index (FI), Fibrotest (FT), Fibrosis / cirrhosis index (FCI) and FIB-4 indices.

- **AAR (12)** = AST (IU/l) / ALT (IU/l)
- **APRI (13)** = $\left\{ \frac{\text{AST (IU/l)}}{\text{ALT}_{\text{ULN}} \text{ (IU/l)}} \times 100 \right\} / \text{platelet count (10}^9\text{/l)}$
- **FI (15)** = $8.0 - 0.01 \times \text{PLT (10}^9\text{/l)} - \text{serum albumin (g/dl)}$
- **FIB-4 (16)** = $[\text{Age (Years)} \times \text{AST (IU/l)}] / [\text{Platelet count (} \times 10^9\text{/l)} \times \text{ALT (IU/l)}^{1/2}]$
- **FT (24)** = includes α₂-macroglobulin, apolipoprotein A1, haptoglobin, total bilirubin, and GGT, adjusted for age and gender. Fibrotest was calculated using the following formula that is available on the USPTO website (<http://www.uspto.gov>; Patent no. 6,631,330): $f = 4.467 \log [\alpha_2\text{-macroglobulin (g L}^{-1}\text{)}] + 1.357 \log [\text{haptoglobin (g L}^{-1}\text{)}] + 1.017 \cdot \log [\text{c-glutamyl transpeptidase (IU - L}^{-1}\text{)}] + 0.0281 [\text{age (years)}] + 1.737 \log [\text{bilirubin (l mol L}^{-1}\text{)}] + 1.184 [\text{apolipoprotein A1 (g L}^{-1}\text{)}] + 0.301 \cdot \text{sex (female} = 0; \text{male} = 1)$ 5.540 .
- **FCI (18)** = $(\text{ALP} \times \text{Bilirubin}) / (\text{Albumin} \times \text{Platelet count})$

Statistical analysis

The data was analyzed using statistical package SPSS version 16 for windows. A *P* value of 0.05 was considered statistically significant. All data was presented as mean values. Spearman's rank correlation

was used to assess the significant association between continuous variables and liver fibrosis stages. The student t-test was used to compare arithmetic means and parameters while Chi-square (X²) test was used to compare categorical data, correlation with Fisher's exact test was used when appropriate. Patients were divided into three main groups as, patients with no or minimal fibrosis (F1), patients with advanced fibrosis (F2-F3) and patients with cirrhosis (F4). The independently distinguished values of biochemical markers and AAR, APRI, FIB-4, FCI, FT and FI indices for the prediction of significant fibrosis and cirrhosis were evaluated using univariate regression analysis. Area under the receiver operating characteristic (ROC) curves (AUROCs) was used to compare and deduce the diagnostic accuracies of the selected biomarkers.

3. Results

The demographic and clinical outcomes of the 100 HCV genotype 4 infected patients explained in **Table 1**. The evaluation of chronic HCV activity (inflammatory grade) showed mild chronic hepatitis in 50 patients, moderate chronic hepatitis in 30 patients and severe chronic hepatitis in 20 patients. According to the Metavir scoring system, the severity of liver fibrosis in the study group of 100 patients with chronic hepatitis C was graded as follows: 50 patients had stage 1 fibrosis (F1); 29 patients had stage 2 fibrosis (F2); 9 patients had stage 3 fibrosis (F3); and 12 patients had cirrhosis (F4).

The different variables in fibrosis stages (**Table 2**). Most of studied patients were of mild and moderate fibrosis (F1-F2) (79/ 100). The distribution of liver fibrosis stages with regard to age and gender of patients showed no significant differences. Viral load was significant among fibrosis stages. It gradually increased in advanced fibrosis and cirrhosis. The discriminative values of the biochemical markers for the prediction of different fibrosis stages were determined by logistic regression analysis. By univariate analysis (*P* < 0.05), viral load, Hb level, bilirubin, ALT, AST, platelet count and haptoglobin levels were significantly associated with various fibrosis stages.

Schistosoma mansoni (*S. mansoni*) Soluble Egg antigen antibodies (anti-SEA) levels were significantly different between fibrotic stages with higher levels in cirrhotic patients

The relationship between the fibrosis stages and six serum indices: AAR, APRI, FI, FT, FCI and FIB-4 is illustrated in **Figure 1&Table 2**. There was a significant relationship between fibrosis stages and serum indexes (*P* < 0.05) except AAR and FCI. A gradual increase in the level of APRI, FI, FT and FIB-4 indexes was observed in fibrosis stages. The cutoff

values and AUROCs of the serum non-invasive indices scores are shown in **Table 3**.

In HCV patients (both *S. mansoni* positive and negative), we analyzed the sensitivity and specificity of each index for minimal (F1), advanced (F2-F3), and cirrhosis (F4). In HCV/*S. mansoni* coinfection patients, minimal fibrosis can be identified using FT, FIB-4, APRI, FI, FCI and AAR with sensitivity 70%, 70%, 71%, 70% 60% and 60% and specificity 61%, 75%, 55%, 62%, 55% and 50% respectively while cirrhosis can be identified using FT, FI, FIB-4, and FCI with sensitivity 88%, 88%, 50% and 50% and specificity 52%, 70%, 60%, and 58% respectively

In HCV mono infected patients, minimal fibrosis can be identified using FCI and FT with sensitivity 57% for both, and specificity 58% and 57% respectively while cirrhosis can be identified using FI, FIB-4, APRI, FT, and AAR with sensitivity 100%, 75%, 100%, 50% and 100% and specificity 53%, 77%, 60%, 100%, and 59% respectively.

AUROC of FT was higher than other indexes ($P < 0.05$) for differentiating minimal fibrosis (F1) from significant fibrosis (F2-F4) (**Figure 2**). As shown, the order of performances of blood tests for minimal or significant fibrosis were differed from that of cirrhosis.

Spearman correlation between each serum index score and fibrosis stages was high for FT, FI, and APRI **Table 4**.

All methods used for predicting liver fibrosis were directly, and significantly, correlated with histological findings, but FT ($r = 0.62$), FI ($r = 0.57$), and APRI score ($r = 0.51$) had the strongest correlation with fibrosis severity while FFIB-4, AAR, and FCI showed significantly low 'r' index. Moreover, *S. mansoni* anti-SEA was poorly significant correlated with fibrosis stages.

The mean values of serum indices for minimal fibrotic HCV patients were illustrated in **Tables 5**. The most reliable indices were FT, FI, FIB-4 and

APRI for distinguishing between different fibrotic stages in HCV/*S. mansoni* coinfection patients. As regard HCV mono infected patients.

Table 1: Demographic, clinical, and liver histological features of 100 chronic HCV genotype 4 infected patients.

Features	Patients Mean (\pm SD)
Sex (Male/Female)	84/16
Age (years)	43.9 \pm 7.8
Viral load (IU/ml)	1.46 $\times 10^6 \pm 3 \times 10^6$
Hb level (12-16g/dl)	13.6 \pm 1.46
Platelet count (140-450 $\times 10^9/l$)	170 \pm 46
ALT (0-45IU/l)	64.2 \pm 39.2
ALP (0-92IU/l)	60.67 \pm 26.1
AST (0-40IU/l)	63.8 \pm 45.7
GGT (0-40IU/l)	47.1 \pm 19.7
Total Bilirubin (0.1-1mg/dl)	1.24 \pm 0.3.7
Albumin (3.5-5.1 g/dl)	4.1 \pm 0.47
Apolipoprotein A1 (0.7-1.69 g/L)	1.7 \pm 0.82
Haptoglobin, (0.8-3 g/L)	0.96 \pm 0.69
Alfa2-Macroglobulin (1.3-3 g/L)	2.94 \pm 0.96
B eliza Positive (OD \geq 0.4)	0.6327 \pm 0.41
AAR	1.05 \pm 0.46
FT	0.42 \pm 1.41
APRI	0.68 \pm 0.6
FI	1.98 \pm 0.78
FIB-4	0.59 \pm 0.57
FCI	0.38 \pm 0.26
Histological Fibrosis stage	
F1(minimal fibrosis)	50
F2	29
F3	9
F2+F3 (advanced fibrosis)	38
F4 (Cirrhosis)	12
F2+F3+F4 (Significant fibrosis)	50

Table 2: The different variables in fibrosis stages.

Features	F1 (n=50)	F2 (n=29)	F3 (n=9)	F4 (n=12)	P value
Sex (Male/Female)	43/7	23/6	9/0	9/3	0.425
Age (years)	42.5 \pm 5	45.2 \pm 5.4	45.7 \pm 9.3	50.2 \pm 11.6	0.394
Viral load ($\times 10^6$ IU/ml)	0.76 \pm 0.87	1.72 \pm 3.39	0.35 \pm 0.66	4.49 \pm 5.92	0.005
Hb level (12-16g/dl)	14.6 \pm 1.4	13.9 \pm 1.6	13.1 \pm 1.2	12.8 \pm 0.8	0.008
Platelet count (140-450 $\times 10^9/l$)	203 \pm 46.2	169 \pm 37.1	146 \pm 25.3	125 \pm 57.3	0.004
ALT (0-45IU/l)	61.7 \pm 44	61.3 \pm 38	60.1 \pm 21	84.1 \pm 24	0.018
ALP (0-92IU/l)	57.4 \pm 18.8	59.7 \pm 30.5	70.8 \pm 48.8	70 \pm 19.1	0.215
AST (0-40IU/l)	62.1 \pm 56	58.3 \pm 35	68.7 \pm 24	80.7 \pm 16	0.005
GGT (0-40IU/l)	45 \pm 18.9	47 \pm 20	50 \pm 21.2	50.4 \pm 23.5	0.68
Total Bilirubin (0.1-1mg/dl)	0.9 \pm 0.28	1.2 \pm 0.43	1.4 \pm 0.13	1.5 \pm 0.3	0.000
Albumin (3.5-5.1 g/dl)	4.2 \pm 0.48	4.3 \pm 0.49	4 \pm 44	3.8 \pm 0.24	0.082

Apolipoprotein A1(0.7-1.69 g/L),,	1.8±0.76	1.7±0.77	1.3±0.29	1.6±0.54	0.251
Haptoglobin (0.8-3 g/L)	1.23±0.76	0.81±0.51	0.72±0.38	0.84±0.37	0.000
a2-macroglobulin (1.3-3 g/L)	2.83±0.88*	2.91±1.2	2.95±0.89	3.41±0.63*	0.276 (0.039*)
B Eliza Positive (79/100) (OD≥0.4)	$\frac{43}{50}$ 0.41±0.9	$\frac{23}{29}$ 0.45±0.5	$\frac{5}{9}$ 0.66±0.1	$\frac{8}{12}$ 0.81±0.2	0.002
AAR	1.05±0.49	1.03±0.51	1.15±0.77	1.02±0.33	0.935
FT	0.37±1.2	0.4±1.57	0.53±1.0	0.51±0.86	0.001
APRI	0.58±0.4	0.64±0.33	1.44±1.07	0.65±0.25	0.011
FI	1.81±0.83	1.92±0.73	2.6±0.77	2.38±0.34	0.013
FIB-4	0.49±0.35	0.58±0.32	1.18±1.64	0.63±0.31	0.015
FCI	0.34±0.24	0.45±0.31	0.41±0.39	0.39±0.28	0.315

Table 3: Performance indices of serum AAR, APRI, FIB-4, FCI, FT and FI in 100 chronic HCV genotype 4 infected patients.

		Minimal fibrosis (F1)				Advanced fibrosis (F2-F3)				Cirrhosis (F4)			
		cutoff	Sen %	Spe%	AUC [95% CI]	cutoff	Sen%	Spe%	AUC [95% CI]	cutoff	Sen%	Spe%	AUC [95% CI]
AAR	Total	1	57	48	0.523	1.01	65	68	0.59	1.06	50	63	0.489
	Neg B	1	50	43	0.337	1.05	100	60	0.588	1.02	100	59	0.588
	Pos B	1	60	50	0.591	1.07	58	70	0.574	0.81	75	35	0.400
APRI	Total	0.56	62	68	0.633	0.598	70	68	0.675	0.588	50	61	0.580
	Neg B	0.58	50	43	0.398	0.574	100	61	0.721	0.58	100	60	0.721
	Pos B	0.46	71	55	0.714	0.64	63	71	0.663	0.44	100	40	0.473
FIB-4	Total	0.48	62	66	0.649	0.500	70	64	0.677	0.471	60	51	0.607
	Neg B	0.751	43	85	0.388	0.59	75	78	0.735	0.75	75	77	0.735
	Pos B	0.479	70	75	0.725	0.48	75	62	0.674	0.51	50	60	0.541
FI	Total	2.13	59	64	0.614	2.29	85	62	0.706	2.39	83	66	0.679
	Neg B	1.86	43	32	0.367	2.35	75	89	0.794	1.87	100	53	0.794
	Pos B	2.125	70	62	0.67	2.29	88	65	0.682	2.36	88	70	0.621
FT	Total	0.409	74	54	0.73	0.460	80	62	0.76	0.462	75	58	0.702
	Neg B	0.478	57	57	0.53	0.57	50	100	0.66	0.58	50	100	0.66
	Pos B	0.420	70	61	0.74	0.42	88	57	0.77	0.42	88	52	0.70
FCI	Total	0.29	57	52	0.603	0.29	55	50	0.549	0.32	50	55	0.508
	Neg B	0.31	57	58	0.536	0.33	50	65	0.471	0.37	50	65	0.471
	Pos B	0.28	60	55	0.604	0.28	63	50	0.576	0.35	50	58	0.510

Table 4: Spearmanrank Correlation between different tests and liver fibrosis (assessed according Metavir score).

Test	Spearman's rank correlation coefficient	P value
FT	0.621	0.001
FI	0.57	0.007
FCI	0.32	0.04
AAR	0.29	0.043
APRI	0.51	0.000
FIB-4	0.47	0.005
Bilh antibodies	0.342	0.001

Table 5: Mean of values of six indices in minimal fibrotic HCV genotype 4 patients.

	Negative (21)	Mean	<i>p</i>	Positive (79)	mean	<i>p</i>
Fibrotest						
F1 (50)	7	0.45	0.856	43	0.361	<u>0.000</u>
F2-F4(50)	14	0.48		36	0.47	
AAR						
F1 (50)	7	1.30	0.255	43	1.01	0.167
F2-F4(50)	14	1.03		36	1.06	
FCI						
F1 (50)	7	0.33	0.799	43	0.34	0.116
F2-F4(50)	14	0.4		36	0.42	
APRI						
F1 (50)	7	0.795	0.488	43	0.54	<u>0.001</u>
F2-F4(50)	14	0.650		36	0.82	
FIB-4						
F1 (50)	7	0.687	0.443	43	0.46	<u>0.001</u>
F2-F4(50)	14	0.632		36	0.72	
FI						
F1 (50)	7	2.28	0.360	43	1.73	<u>0.012</u>
F2-F4(50)	14	1.94		36	2.23	

Fibrotest

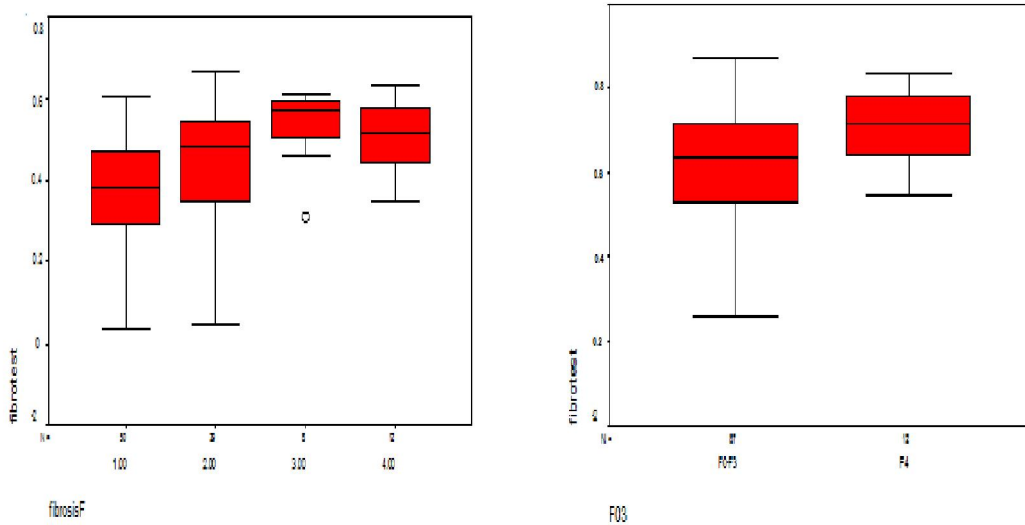
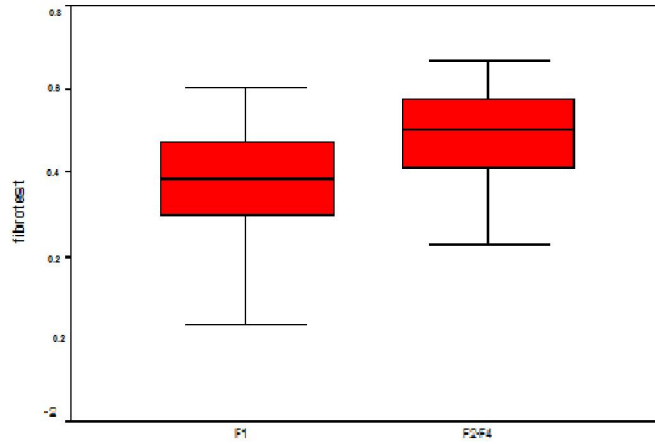
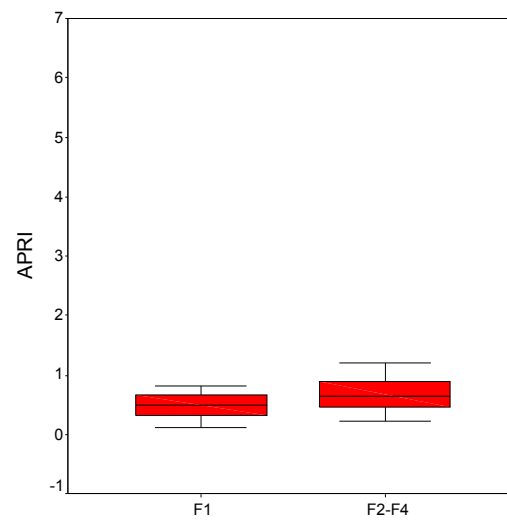
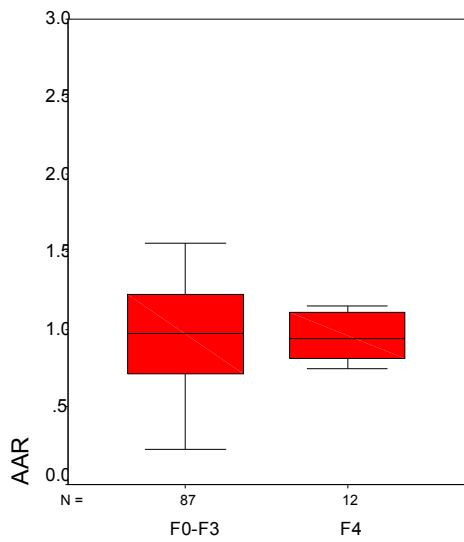


Figure 1 Box plots of the FT, FCI, AAR, APRI, FIB-4 and FI for different fibrosis stages. The horizontal line inside each box represents the median, while the top and bottom of boxes represent the 25th and 75th percentiles, respectively. Vertical lines from the ends of the box encompass the extreme data points.



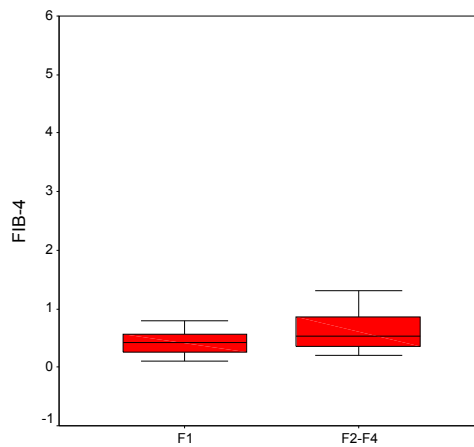
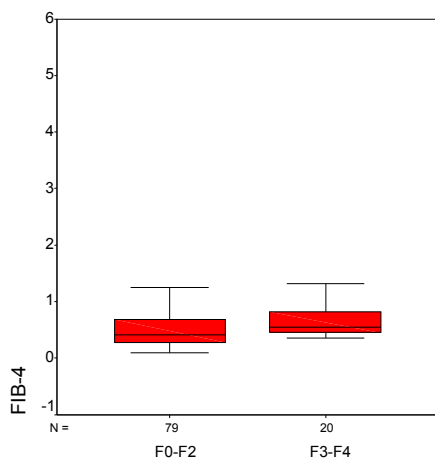
AAR

APRI

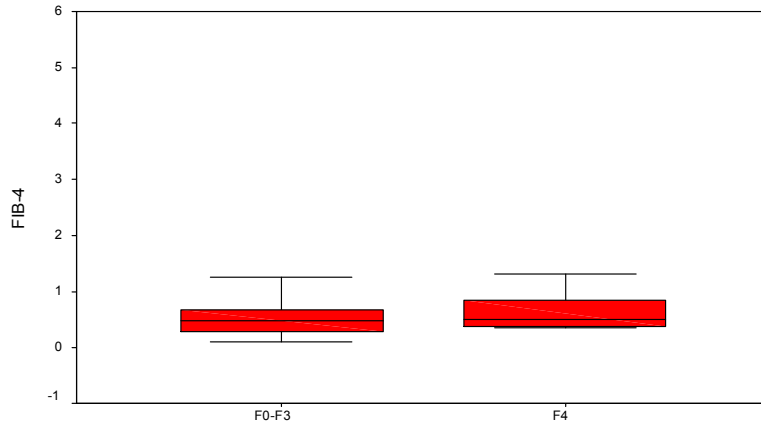


FIB-4

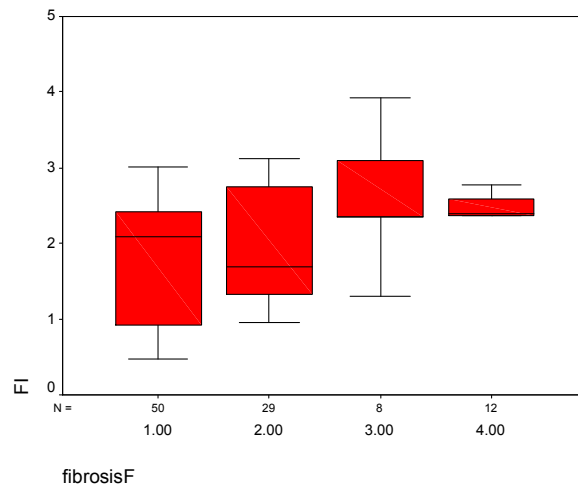
F03



F02



FI



FCI

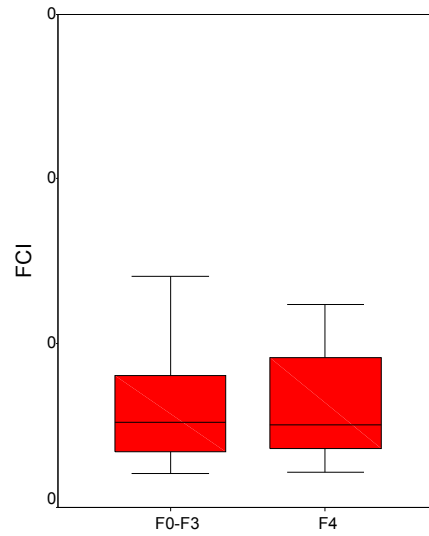
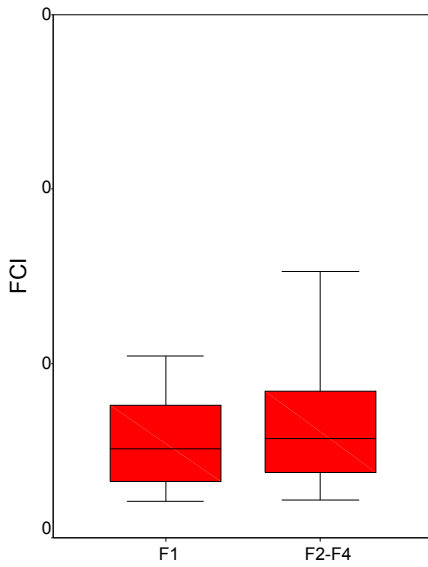
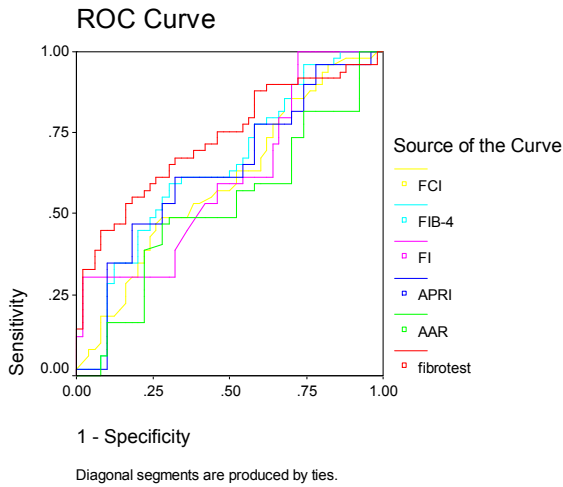


Figure 2: Receiver operating characteristic curves generated by six serum markers, AAR, APRI, FIB-4, FCI, FT and FI for differentiation between patients in fibrosis stage F1, F2-F3 and F4.

F1

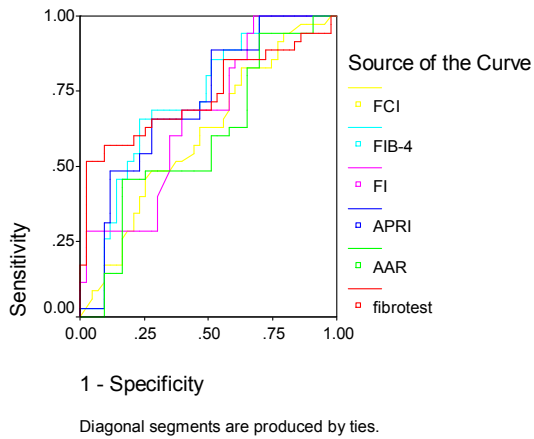


Area Under the Curve

Test Result Var	Area
fibrotest	.731
AAR	.523
APRI	.633
FI	.614
FIB-4	.649
FCI	.603

ROC Curve

ELISA2: 1.00 positive

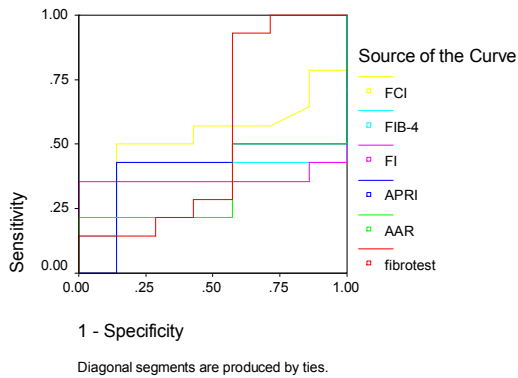


Area Under the Curve ^a

Test Result Variable(s)	Area
fibrotest	.736
AAR	.591
APRI	.714
FI	.665
FIB-4	.725
FCI	.604

ROC Curve

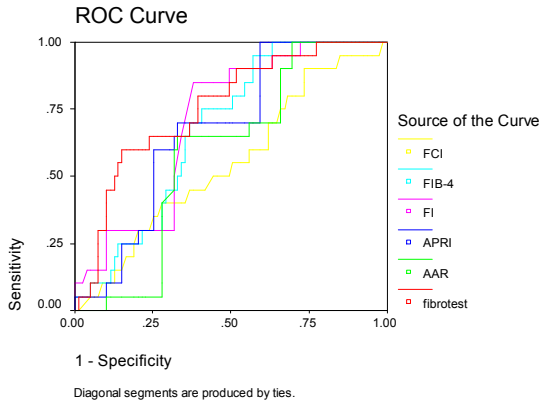
ELISA2: .00 negative



Area Under the Curve ^b

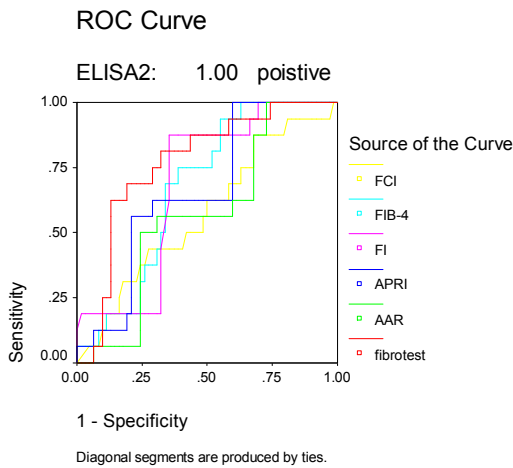
Test Result Variable(s)	Area
fibrotest	.531
AAR	.337
APRI	.398
FI	.367
FIB-4	.388
FCI	.536

F2-F3



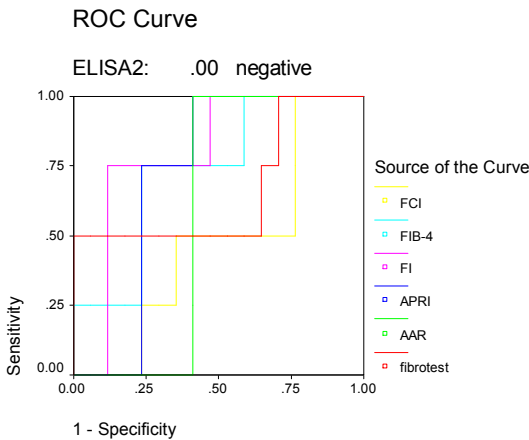
Area Under the Curve

Test Result Variable(s)	Area
fibrotest	.755
AAR	.590
APRI	.675
FI	.706
FIB-4	.677
FCI	.549



Area Under the Curve ^a

Test Result Variable(s)	Area
fibrotest	.769
AAR	.574
APRI	.663
FI	.682
FIB-4	.674
FCI	.576



Area Under the Curve ^e

Test Result Variable(s)	Area
fibrotest	.662
AAR	.588
APRI	.721
FI	.794
FIB-4	.735
FCI	.471

4. Discussion

Aiming to find accurate and reliable non-invasive markers for evaluating fibrosis progression in HCV with or without Co schistosomiasis infection is to avoid the use of invasive liver biopsy. The commonly used markers are: liver function tests (AST, ALT, bilirubin, alkaline phosphatase, albumin and PT). These tests only provide information about important aspects of liver function but they do not assess severity of liver fibrosis or cirrhosis (25). Other serum markers such as

α -2-Macroglobulin (26), apolipoprotein A1 (27), haptoglobin (28), are proposed as surrogate indices instead of liver biopsy (29). New researches indicated that these individuals' serum markers have limited accuracy in predicting hepatic fibrosis and proposed that the individual markers are useful for establishing the presence, but not absence, of fibrosis. Due to this limitations, algorithms or indices combining the results of panels of markers have been studied which improve

diagnostic accuracy and proposed as alternatives to liver biopsy (30).

All patients in our study were of genotype 4 to eliminate the HCV genotype effect on fibrosis progression. Co-infection with schistosomiasis in our studied HCV patients was high (79/100) among age group above 40 years which is in agreement with the hypothesis that increased prevalence and intensity of infection with *S. mansoni* in the populous Nile delta where the exposure to canal water was occurring in several million farmers treated with tarter emetic campaigns during 1980s and constituted the major silent reservoirs of HCV (31).

In this study, the impact of schistosomiasis on fibrosis staging was observed from the significant difference of the higher OD absorbance of anti SEA of *S. mansoni* in sever fibrotic and cirrhotic (F3-F4) HCV patients than others (F1-F2) (P ; 0.001), the linear correlation with fibrosis stages (r ; 0.342, P ; 0.001), and the significant difference of serum $\alpha 2$ macroglobulin levels between F1 and F4.

These findings are in agreement with Silveira *et al.*, (32) who reported increased levels of OD of IgG against SEA in patients with periportal fibrosis. Moreover, studies by Kamal *et al.*, (33) reported that Egyptian patients with co-infections had higher HCV-RNA titers, more advanced liver disease, more hepatic complications, and a greater mortality rate than those with HCV mono infection. Previously, the role of $\alpha 2$ macroglobulin has been discussed by Ahmed *et al.*, (34) as its high levels had an effect on granuloma formation around *S. mansoni* eggs in the rat and it is a reliable predictor of fibrosis in HCV patients. This is could be explained by its association with several growth factors as fibroblast, vascular endothelial, epidermal, transforming and platelet derived growth factors and fibrogenesis (35).

A debate has been raised regarding this role by Shiha and Zalata (36) who concluded that Schistosomal hepatic affection does not alter or interfere with assessment of fibrosis in mixed HCV-Schistosomal liver affection.

We evaluated the performance of AAR, APRI, FIB-4, FCI, FT and FI for staging liver fibrosis and to differentiate them from cirrhosis.

Similar to the poor performance of AAR reported by Lackner *et al.*, (37) our study revealed that it is less accurate in detection of mild fibrosis (F1) among HCV monoinfection and in HCV/*S. mansoni* co-infections. This is in contrary to that reported by Giannini *et al.*, (38), as a high diagnostic accuracy of AAR > 1.16 with 81.3% sensitivity and 55.3% specificity for the prediction of cirrhosis

We observed comparatively low values of APRI (0.58 ± 0.4) in mild fibrosis (F1) of total HCV patients with significant gradual increase in fibrosis stages (P ; 0.001). APRI was not accurate to detect mild fibrosis

(F1) among HCV mono-infection. Khan *et al.*, (39) reported that APRI < 0.42 predict mild fibrosis and > 1.2 , predict significant fibrosis in HCV patients with 90% NPV for absence of fibrosis and 91% PPV for fibrosis presence. Our results showed that APRI > 0.46 accurately diagnose fibrosis in HCV/*S. mansoni* co infection patients with 71% sensitivity, 55% specificity. Similarly, Ahmad *et al.*, (34) study revealed low cutoff values with significant direct correlation between APRI and fibrosis stage of the studied Egyptian HCV/*S. mansoni* co infection patients (sig, F2-F4; cutoff 0.60 sensitivity 82%, specificity 57% and F3-F4; cutoff 0.72 sensitivity 94%, specificity 67%).

Our results revealed a cutoff value of < 0.48 FIB-4 in diagnosis of mild fibrosis with sensitivity 62%, specificity 66%, while a cutoff value > 0.5 in the diagnosis of advanced fibrosis has sensitivity of 70%, sp 64%. Although, FIB-4 was not accurate to detect mild fibrosis (F1) among HCV mono-infection, it shows significant correlations of fibrosis and cirrhosis stages in HCV/ *S. mansoni* co infected patients. Similarly, Shaker and Khalifa (40) reported that FIB-4 was reliable in detecting significant fibrosis in Egyptian patients.

Fibrosis index (FI) showed high sensitivity, specificity, and AUROC for discriminating different fibrosis stages among all our studied groups. It was not sensitive to detect mild fibrosis (F1) among HCV mono-infected liver fibrosis stages. Ohta *et al.*, (15) developed this simple index and reported that at cutoff value < 2.1 for predicting F1 stage with 68% sensitivity and 63% specificity. At same cutoff, our data showed comparable results with AUROC 0.614 for the prediction of minimal fibrosis (F1). While for detection cirrhosis in HCV patients, he reported FI value > 3.30 , we observed a lower value (> 2.3) with 83% sensitivity and 66% specificity.

Recently, FCI was designed by Ahmad *et al.*, (18) and observed that it could better differentiate among fibrosis stages with high sensitivity, specificity, PPV and NPV. In our study, FCI was not able to detect minimal fibrosis (F1) among HCV mono-infected patients and HCV/ *S. mansoni* co infected patients. However, our lower cutoff values of FCI could be attributed to the inclusion criteria of studied patients who are candidate of interferon/rebaverin treatment as regard platelets $\geq 90,000$ cmm^3 and compensated cirrhosis with normal albumin levels ≥ 3.5 gm/dL .

An interesting finding from the present study was FT showed possibility of classifying all the stages of liver fibrosis with high sensitivity, specificity and AUROC for discriminating different stages among both HCV mono-infected and HCV/ *S. mansoni* co infected patients. El-Shabrawi *et al.*, (41) concluded that a highly significant linear correlation was found between FT-related fibrosis and fibrosis stage by

METAVIR scoring on histopathological examination. On the contrary of this, available data suggest that FT performs well in subjects with grade F1 or F4 of fibrosis, while it performs less well in the intermediate stage (F2). Other studies confirmed the causes of failure of the FT. The most frequent cause leading to false negative result was high haptoglobin in acute inflammation or sepsis. The most frequent cause of false positive results was extremely low haptoglobin associated with intravascular hemolysis and high bilirubin in hemolysis and Gilbert disease (42).

The readily available indexes are associated with some limitations like population discrepancy, not able to distinguish all fibrosis stages individually or some primarily developed for co-infected patients.

Although several non-invasive markers of liver fibrosis have been developed in the last few years, their use in the clinical practice is still limited. In fact, inter laboratory variability, lack of reproducibility and the risk of misdiagnosis (up to 20%), do not allow to recommend these methods in substitution of liver biopsy (43). One of the main limitations for the use of non-invasive markers is the difficult diagnosis of intermediate stages of liver fibrosis (44).

To the best of our knowledge, this is the first time noninvasive biomarkers have been used to assess the feasibility of using six non invasive biomarkers in HCV/S. *mansoni* co infected patients. In this study, we used a single cutoff which is an advantage over other biomarker studies using 2 different cutoffs. Among HCV/S. *mansoni* co-infected patients, FT, FIB-4, FI and FCI can detect all fibrotic and cirrhotic stages (F1, F2-F3,F4) while AAR, APRI can detect fibrotic stages only not the cirrhotic stage. Among HCV mono-infected patients, FT only can detect all fibrotic and cirrhotic stages, FIB-4, FI, AAR and APRI can detect significant fibrosis (F2-F4) and FCI can detect only mild fibrosis (F1). These results suggest that the using FT as a first-line test in the social health centers seems feasible and effective.

Corresponding author

Mona A. El-Hussiny

Department of Clinical Pathology, Faculty of Medicine, Mansoura University, Egypt

mona_clinic@hotmail.com

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Estimations and Prediction from the Inverse Rayleigh Model Based on Lower Record Statistics

A.I. Shawky¹M. M. Badr²

¹Department of Statistics, Faculty of Science, King Abdulaziz University, P.O. Box 80203, Jeddah 21589, Saudi Arabia.

²Department of Statistics, Faculty of Science for Girls, King Abdulaziz University, P.O. Box 70973, Jeddah 21577, Saudi Arabia.

aishawky@yahoo.com

Abstract: This article considers estimation of the unknown parameters for the inverse Rayleigh distribution (*IRD*) based on lower record values. We consider the maximum likelihood (*ML*) and Bayesian inference of the unknown parameters of the model, as well as the reliability and cumulative hazard rate functions. The Bayes estimators are obtained relative to both symmetric (squared error) and asymmetric (linear exponential (*LINEX*)) loss functions. It is noticed that the symmetric and asymmetric Bayes estimators are obtained in closed forms. Bayesian prediction interval of the future record values are obtained as well. Finally, practical examples using real record values are given to illustrate the application of the results.

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Key words: Bayesian inference; Squared error loss function; *LINEX* loss function; Maximum likelihood function; Reliability; Failure rate; Record values; Inverse Rayleigh distribution.

AMS Subject Classification: 62B10; 62E20; 62F15; 62G05; 62G30; 62M20.

1. Introduction

The Rayleigh distribution is a special case of the Weibull distribution, which provides a population model useful in several areas of statistics including life testing and reliability which age with time as its failure rate is a linear function of time. Various applications of this distribution are given in Siddiqui (1962), Polovko (1968), Gross and Clark (1975) and Lee *et al.* (1980). In the life distribution, if the random variable (*r.v.*) T has Rayleigh distribution, then the *r.v.* $X=1/T$ has *IRD*. The *IRD* was introduced in literature by Trayer (1964) (see, Mohsin and Shahbaz (2005)) and it has many applications in the area of reliability studies. Voda (1972) mentioned that the distribution of lifetimes of several types of exponential units can be approximated by the *IRD* and discussed some properties of maximum likelihood estimator (*MLE*) of the parameter θ . The probability density function (*p.d.f.*) of the *IRD* with scale parameter θ is

$$f(x; \theta) = \frac{2\theta}{x^3} e^{-\frac{\theta}{x^2}}, \quad x, \theta > 0, \quad (1.1)$$

and a cumulative distribution function (*c.d.f.*)

$$F(x; \theta) = e^{-\frac{\theta}{x^2}}, \quad x > 0, \quad \theta > 0. \quad (1.2)$$

The reliability, failure rate and the cumulative failure rate (hazard rate) functions of *IRD* are given, respectively, by

$$R(t; \theta) = 1 - F(t; \theta) = 1 - e^{-\frac{\theta}{t^2}}, \quad (1.3)$$

$$h(t; \theta) = \frac{f(t; \theta)}{R(t; \theta)}, \quad (1.4)$$

$$H(t; \theta) = -\ln R(t) = -\ln \left(1 - e^{-\frac{\theta}{t^2}} \right). \quad (1.5)$$

Record values are important in many real-life situations involving data relating to weather, sports, economics, and life-tests. The statistical study of record values have been pursued in different directions by several authors; see, Nagaraja (1988), Ahsanullah (1995) and Arnold *et al.* (1998). Some inferential methods based on record values for the Rayleigh and Weibull, generalized Pareto, Lomax, generalized exponential and power function distributions are studied by Balakrishnan and Chan (1993), Sultan and Moshref (2000), Sultan *et al.* (2001), Raqab (2002) and Sultan *et al.* (2002). Moreover, Abd-El-Hakim and Sultan (2001) have obtained the maximum likelihood estimators (*MLE*'s) of Weibull parameters based on record values. Also, Shawky and Bakoban (2010) have derived moments and moment generating functions from *EG* distribution and have made some statistical inferences based on record values.

In this paper, Bayesian and non-Bayesian estimators are derived for scale parameters, reliability and failure rate functions based on lower record values from *IR* distribution. Soliman *et al.* (2010) discussed the same problem with different prior distribution and another technique.

Now, let $\{X_n, n \geq 1\}$ be an infinite sequence of i.i.d. random variables from an absolutely continuous distribution function F , and probability density function f . Let $X_{i:j}$ denote the i^{th} order statistic of

the random sample X_1, X_2, \dots, X_j , and $F_{i,j}$ be its cumulative distribution function. Let $T_k = \min\{X_1, X_2, \dots, X_k\}$, $k \geq 1$. We say that X_j is a lower record value of this sequence if $T_j < T_{j-1}$, $j \geq 2$. By definition, X_1 is a record value. Let $L(n) = \min\{j: j > L(n-1), X_j < X_{L(n-1)}\}$, $n \geq 2$ with $L(1) = 1$. Then $X_{L(n)}$, $n \geq 1$, denotes the sequence of lower record values. From the above definition, the sequence of record statistics can be viewed as order statistics from a sample whose size is determined by the values and the order of occurrence of the observations.

In Bayesian estimation, we consider two types of loss functions. The first is the squared error loss function (quadratic loss) which is classified as a symmetric function and associates equal importance to the losses for overestimation and underestimation of equal magnitude. The second, introduced by Varian (1975), is the LINEX (linear-exponential) loss function which is known asymmetric. These loss functions were widely used by several authors; among of them Rojo (1987), Basu and Ebrahimi (1991), Pandey (1997), Soliman (2000), Nassar and Eissa (2004) and Shawky and Bakoban ((2008) & (2010)).

The quadratic loss for Bayes estimate of a parameter β , say, is the posterior mean assuming that exists, denoted by β_s . The LINEX loss function may be expressed as

$$l(\Delta) \propto e^{c\Delta} - c\Delta - 1, c \neq 0, \tag{1.6}$$

where $\Delta = \hat{\beta} - \beta$. The sign and magnitude of the shape parameter c reflects the direction and degree of asymmetry, respectively. If $c > 0$, the overestimation is more serious than underestimation, and vice-versa. For c closed to zero, the LINEX loss is approximately squared error loss and therefore almost symmetric.

The posterior expectation of the LINEX loss function Equation (1.6) is

$$E_{\beta}[l(\hat{\beta} - \beta)] \propto \exp(c\hat{\beta}) E_{\beta}[\exp(-c\beta)] - c(\hat{\beta} - E_{\beta}(\beta)) - 1, \tag{1.7}$$

where $E_{\beta}(\cdot)$ denotes the posterior expectation with respect to the posterior density of β . By a result of Zellner (1986), the (unique) Bayes estimator of β , denoted by $\hat{\beta}_L$ under the LINEX loss is the value $\hat{\beta}$ which minimizes (1.7), is given by

$$\hat{\beta}_L = -\frac{1}{c} \log\{E_{\beta}[\exp(-c\beta)]\}, \tag{1.8}$$

Provided that the expectation $E_{\beta}[\exp(-c\beta)]$ exists and is finite [Calabria and Pulcini (1996)]. We are interested with maximum likelihood estimation as a classical approach among non-Bayesian methods. The maximum likelihood is based on the information Provided by empirical data. The

invariant property was hold to obtain maximum likelihood estimators (MLE's) of reliability and failure rate functions.

In this paper, a discussion of the MLE's is considered in Section 2. In Section 3, Bayesian estimators is obtained. In Section 4, prediction of future records are derived. Numerical illustration and comparisons are presented in Section 5. Finally, conclusions are made in Section 6.

2. Maximum Likelihood Estimation

In this section, the maximum likelihood estimators (MLE's) of $IRD(\theta)$ are derived. We consider the case when θ is unknown. Let x_1, x_2, \dots be a sequence of i.i.d. random variables from $IRD(\theta)$, the joint density function of first n lower record values $\underline{x} = (x_{L(1)}, x_{L(2)}, \dots, x_{L(n)})$ is given by

$$f_{1,2,\dots,n}(x_{L(1)}, x_{L(2)}, \dots, x_{L(n)}) = \frac{\prod_{i=1}^n f(x_{L(i)})}{\prod_{i=1}^{n-1} F(x_{L(i)})}, \tag{2.1}$$

where $f(\cdot)$ and $F(\cdot)$ are given by (1.1) and (1.2), respectively. Abbreviation $x_{L(i)} = x_i$.

The likelihood function of (2.1), is given by

$$L(\theta|\underline{x}) = u \theta^n e^{-q\theta}, \tag{2.2}$$

where

$$q = x_n^{-2} \text{ and } u = \prod_{i=1}^n \frac{1}{x_i^3}. \tag{2.3}$$

Then the log-likelihood function, is given by

$$l = \ln L(\theta|\underline{x}) = \ln u + n \ln \theta - q\theta. \tag{2.4}$$

It follows, from (2.4), that the MLE of θ is

$$\hat{\theta} = n x_n^2. \tag{2.5}$$

For a given t , the MLE of $R(t)$ is obtained by replacing θ by $\hat{\theta}$ in Equation (1.3), then MLE of $H(t) = -\log R(t)$ can be obtained.

3. Bayesian Estimation

The natural family of conjugate prior for θ is a gamma distribution with p.d.f.

$$g(\theta) = \frac{b^a}{\Gamma(a)} \theta^{a-1} e^{-b\theta}, \theta > 0, a, b > 0. \tag{3.1}$$

Applying Bayes theorem, we obtain, from Equations (2.2) and (3.1), the posterior density of θ as

$$g(\theta|\underline{x}) = \frac{B^A}{\Gamma(A)} \theta^{A-1} e^{-B\theta}, \theta > 0, a, b > 0, \tag{3.2}$$

where $A = a + n$, $B = b + q$ and $q = x_n^{-2}$.

Estimation of θ :

The Bayes estimate $\hat{\theta}_{BS}$ of θ relative to squared error loss function is given by

$$\hat{\theta}_{BS} = \frac{A}{B}. \tag{3.3}$$

Under LINEX loss function, the Bayes estimate $\hat{\theta}_{BL}$ of θ using Equation (1.8) can be obtained as

$$\hat{\theta}_{BL} = \frac{A}{c} \ln \left(1 + \frac{c}{B} \right). \tag{3.4}$$

Estimation of $R(t)$:

The Bayes estimate $\hat{R}_{BS}(t)$ of $R(t)$ relative to squared error loss function is given by

$$\hat{R}_{BS}(t) = 1 - \left(1 + \frac{t^{-2}}{B} \right)^{-A}. \tag{3.5}$$

Under LINEX loss function, the Bayes estimate of $R(t)$ using Equation (1.8) is

$$\hat{R}_{BL}(t) = 1 - \frac{1}{c} \ln \left\{ \sum_{i=0}^{\infty} \frac{c^i}{i!} \left(1 + \frac{t^{-2}}{B} \right)^{-A} \right\}. \tag{3.6}$$

Estimation of $H(t)$:

The Bayes estimate of the cumulative failure rate function $H(t) = -\ln R(t)$ relative to quadratic loss function is

$$\hat{H}_{BS}(t) = \sum_{j=1}^{\infty} \frac{1}{j} \left(1 + \frac{j t^{-2}}{B} \right)^{-A}. \tag{3.7}$$

When the LINEX loss function is appropriate, the Bayes estimate of $H(t)$ is

$$\hat{H}_{BL}(t) = \frac{-1}{c} \ln \left\{ \sum_{j=0}^{\infty} (-1)^j \binom{c}{j} \left(1 + \frac{j t^{-2}}{B} \right)^{-A} \right\}. \tag{3.8}$$

4. Prediction of the Future Records

In the context of prediction of the future record observations, the prediction intervals provide bounds to contain the results of a future record, which is based on the previous record observed from the same sample.

Let the first n lower record observations $\underline{x} = (x_{L(1)}, x_{L(2)}, \dots, x_{L(n)})$, then the conditional density function of the s^{th} future lower record $Y = X_{L(s)}$, $1 \leq n < s$, for given $x_n = x_{L(n)}$ is given (see Arnold *et al.*, 1998) by

$$f(y|x_n; \theta) = \frac{[G(y) - G(x_n)]^{s-n-1}}{\Gamma(s-n)} \cdot \frac{f(y)}{F(x_n)}, \tag{4.1}$$

$0 < y < x_n < \infty$,

where $G(x) = -\ln F(x) = \theta x^{-2}$, thus, from (1.1) and (1.2), relation (4.1) can be written as

$$f(y|x_n; \theta) = \frac{2 \theta^{s-n}}{y^3 \Gamma(s-n)} \cdot [\xi(y)]^{s-n-1} e^{-\theta \xi(y)}, \tag{4.2}$$

where

$$\xi(y) = y^{-2} - x_n^{-2}. \tag{4.3}$$

The Bayes predictive density function of $Y = X_{L(s)}$ given the observed record x_n is given by

$$f^*(y|x_n) = 2 C1 \cdot \frac{[\xi(y)]^{s-n-1}}{y^3 [B + \xi(y)]^{s+a}}, \tag{4.4}$$

$0 < y < x_n$,

where $C1 = \frac{B^A}{B(s-n,A)}$ and $B(s-n,A)$ is a beta function.

Thus, the Bayesian prediction bounds for $Y = X_s$, given the previous data are obtained by evaluation

the following predictive survival function, for some positive λ ,

$$f(Y > \lambda | x_n) = \int_{\lambda}^{x_n} f^*(y|x_n) dy = \frac{\text{InBeta}(s-n,A,\delta)}{\text{Beta}(s-n,A)}, \tag{4.5}$$

where $\delta = \frac{x_n^2 - \lambda^2}{B \lambda^2 x_n^2} = \frac{x_n^2 - \lambda^2}{\lambda^2 (b x_n^2 + 1)}$ and $\text{InBeta}(z_1, z_2, \delta)$ is the incomplete beta function defined by

$$\text{InBeta}(z_1, z_2, \delta) = \int_0^{\delta} \frac{t^{z_1-1}}{(1+t)^{z_1+z_2}} dt.$$

The lower and upper 100 τ % prediction bounds for Y could be found numerically by finding λ from (4.5), using

$$\Pr[LL(x_n) < Y < UL(x_n)] = \tau,$$

where $LL(x)$ and $UL(x)$ are the lower and upper limits, respectively, satisfying

$$\Pr[Y > LL(x_n) | x_n] = \frac{1+\tau}{2} \text{ and } \Pr[Y > UL(x_n) | x_n] = \frac{1-\tau}{2}. \tag{4.6}$$

As a special important case from (4.5), we predict the first unobserved record value $X_{L(n+1)}$ by putting $s = n + 1$, then we get

$$f(x_{n+1} \geq \lambda | x_n) = 1 - (1 + \delta)^{-A}. \tag{4.7}$$

From (4.6) and (4.7), the lower and upper 100 τ % prediction bounds are given, respectively, by

$$LL(x_n) = \frac{x_n}{\{1 + (b x_n^2 + 1) \left[\left(\frac{1+\tau}{2} \right)^{-\frac{1}{A}} - 1 \right] \}^{\frac{1}{2}}},$$

$$\text{and } UL(x_n) = \frac{x_n}{\{1 + (b x_n^2 + 1) \left[\left(\frac{1+\tau}{2} \right)^{-\frac{1}{A}} - 1 \right] \}^{\frac{1}{2}}}.$$

5. Illustrative Examples and Simulation Study

To illustrate the estimation and prediction techniques that were shown in the previous sections, we present two data sets.

Example 1 (Real Life Data Set)

This data set is obtained from Proschan (1963) and represents times between successive failures of air conditioning (AC) equipment in a Boeing 720 airplane and they are as follows: 502, 386, 326, 153, 74, 70, 59, 57, 48, 29, 29, 27, 26, 21, 12, we fit the inverse Rayleigh distribution by used Kolmogorov-Smirnov (K-S) test. It is observed that, the K-S distance is 0.21378 with the corresponding P value is 0.43879. For this data set, the Chi- square value is 2.6383. Therefore, it is clear that inverse Rayleigh model fits quite well to the data set. Using our results in Sections 2 and 3, the MLEs $(\cdot)_{ML}$ and the Byes estimators $(\cdot)_{BS}$, $(\cdot)_{BL}$ of θ , $R(t)$ and $H(t)$ have been computed and the results are given in Tables 1 and 2. Using the prediction procedure described in Section 4, the 90%, 95% and 99% prediction intervals for the next lower record x_{16} are computed respectively, as

follows $(LL(x_{16}), UL(x_{16})) = (3.11395, 3.56848), (3.21959, 3.44610)$ and $(3.30755, 3.35281)$.

Table 1: Estimated values of $\theta, R(t)$ and $H(t)$ with actual Values ($\theta = 2.005, a = 2, b = 2, t = 0.75, R(0.75) = 0.97169$ and $H(0.75) = 0.02872$).

Parameters	(.) _{ML}	(.) _{BS}	(.) _{BL}			
			c= - 0.5	c= 0.001	c= 2	c = 3
θ	2160	8.47059	9.74200	8.46848	5.87703	5.18054
$R(t)$	1	0.99998	0.99998	0.99998	0.99998	0.99998
$H(t)$	0	0.00016	0.00002	0.00002	0.00002	0.00002

Table 2: MSEs of the estimates $\theta, R(t)$ and $H(t)$ when ($\theta = 2.005, a = 2, b = 2, t = 0.75, R(0.75) = 0.97169$ and $H(0.75) = 0.02872$).

Parameters	(.) _{ML}	(.) _{BS}	(.) _{BL}			
			c= - 0.5	c=0.001	c= 2	c = 3
θ	4.65694×10^6	41.80380	59.86110	41.77660	14.9926	10.08400
$R(t)$	0.00080	0.00080	0.00080	0.00080	0.00080	0.00080
$H(t)$	0.00082	0.00082	0.00082	0.00082	0.00082	0.00082

As shown from Table 2 that the Bayes estimates for all parameters are better than the MLE's estimates.

Example 2 (Simulated Data):

In order to assess the statistical performances of these estimates, a simulation study is conducted. The estimated mean and the mean square errors (MSE's) are computed for each estimator. The random samples are generated as follows:

1. For $\theta = 2.05$, we generate a random samples of sizes $n=3, 5, 7, 10$ and 15 .
2. Using θ , obtained in step (1), with

$$a = 1.2, b = 1, t = 5, R(5) = 0.078728$$

and $H(5) = 2.54176$, the MLEs and the Bayes estimates relative to squared error loss and LINEX loss are computed.

3. Using the prediction procedure described in Section 4, the 95% prediction interval for the next lower records are computed.
4. The above steps are repeated 1000 times and the mean square errors are computed for each method.

Our computational results were computed by using Mathematica 8.0. Estimates, MSE's and prediction intervals are displayed in Tables 3, 4 and 5.

Table 3: Estimated mean values of $\theta, R(t)$ and $H(t)$ with actual Values ($\theta = 2.05, a = 1.2, b = 1, t = 5, R(5) = 0.078728$ and $H(5) = 2.54176$).

n	Parameters	(.) _{ML}	(.) _{BS}	(.) _{BL}			
				c= - 0.5	c= 0.001	c= 2	c = 3
3	θ	2.99361	1.88553	2.17131	1.88507	1.32291	0.17202
	$R(t)$	0.10878	0.07179	0.07202	0.07172	0.07055	0.06998
	$H(t)$	2.39402	2.79622	2.86222	2.79606	2.58279	2.49696
5	θ	2.59565	2.00985	2.21562	2.00949	1.52737	1.38104
	$R(t)$	0.09729	0.07645	0.07668	0.07645	0.07555	0.07511
	$H(t)$	2.42947	2.68977	2.73172	2.68968	2.54457	2.48240
7	θ	2.48476	2.08618	2.24524	2.08589	1.66907	1.53126
	$R(t)$	0.09387	0.07940	0.07959	0.79940	0.07867	0.07831
	$H(t)$	2.42921	2.62383	2.65452	2.62377	2.51381	2.46505
10	θ	2.42726	2.14358	2.26337	2.14336	1.79879	1.67545
	$R(t)$	0.09193	0.08159	0.08173	0.08159	0.08102	0.08074
	$H(t)$	2.43880	2.57999	2.60186	2.57994	2.49924	2.46236
15	θ	1.95219	1.86557	1.92143	1.86546	1.67890	1.60244
	$R(t)$	0.07512	0.07175	0.07182	0.07175	0.07146	0.07131
	$H(t)$	2.38871	2.54430	2.59060	2.56889	2.45236	2.35960

Table 4: MSE of θ , $R(t)$ and $H(t)$ when $(\theta = 2.05, a = 1.2, b = 1, t = 5, R(5) = 0.078728$ and $H(5) = 2.54176)$.

n	Parameters	$(\cdot)_{ML}$	$(\cdot)_{BS}$	$(\cdot)_{BL}$			
				$c = -0.5$	$c = 0.001$	$c = 2$	$c = 3$
3	θ	2.50424	0.37600	0.63278	0.37581	0.62311	0.83300
	$R(t)$	0.00662	0.00051	0.00051	0.00051	0.00050	0.00050
	$H(t)$	0.35006	0.16169	0.20043	0.16163	0.09553	0.09437
5	θ	2.19838	0.36540	0.60434	0.36515	0.40672	0.54038
	$R(t)$	0.00258	0.00051	0.00051	0.00051	0.00049	0.00048
	$H(t)$	0.20315	0.10777	0.12248	0.10774	0.08379	0.08630
7	θ	1.17017	0.32847	0.46853	0.32830	0.30776	0.39843
	$R(t)$	0.00142	0.00043	0.00043	0.00043	0.00042	0.00041
	$H(t)$	0.12735	0.07820	0.08598	0.07819	0.06574	0.07793
10	θ	1.14605	0.30293	0.40466	0.30269	0.25988	0.28392
	$R(t)$	0.00141	0.00037	0.00037	0.00037	0.00040	0.00038
	$H(t)$	0.12629	0.07250	0.07452	0.07250	0.06325	0.07788
15	θ	0.19042	0.06424	0.11522	0.06416	0.00052	0.01730
	$R(t)$	0.00025	0.00008	0.00008	0.00008	0.00007	0.00007
	$H(t)$	0.03400	0.00647	0.00432	0.00647	0.01852	0.02631

Table 5: The lower (LL), the upper (UL) and the width of the 95% prediction intervals for the future lower record $X_{L(n+1)}$, $n = 3, 5, 7, 10$ and 15 .

n	Previous Record Values	LL	UL	Width
3	{0.93449, 0.77995, 0.75574}	0.69472	0.70256	0.00784
5	{0.67174, 0.51245, 0.48736, 0.48069, 0.44281}	0.43337	0.43466	0.00129
7	{1.23761, 0.925816, 0.72417, 0.59770, 0.56927, 0.56716, 0.52462}	0.51260	0.51352	0.00092
10	{0.916405, 0.85255, 0.58738, 0.58253, 0.56952, 0.54601, 0.49919, 0.48114, 0.43236, 0.41092}	0.40655	0.40714	0.00059
15	{1.50770, 1.29072, 0.75962, 0.72922, 0.61196, 0.59017, 0.57244, 0.53403, 0.51315, 0.50736, 0.49481, 0.48884, 0.46894, 0.36701, 0.35995}	0.35788	0.35817	0.00029

Tables 1 and 3 show the mean estimates. From Tables 2 and 4, we see that the Bayes estimates for all parameters are better than the MLEs estimates. Table 5 shows the lower and the upper 95% prediction bounds for the next record values ($X_{L(n+1)}$), when $n = 3, 5, 7, 10$ and 15 .

6. Conclusion

In this paper we have presented the Bayesian and non-Bayesian estimates of the parameter, reliability function $R(t)$ and cumulative failure rate function $H(t)$ for the lifetime follow the inverse Rayleigh distribution. The estimations are conducted on the MSE of estimated parameters. The MLEs are obtained based on record values. Bayes estimators, under squared error loss and LINEX loss functions, are also derived.

Our observations concerning the results are stated in the following points:

1- Estimation: Tables 1 and 3 show the mean estimates. From Tables 2 and 4, we observe that the Bayes estimates perform better than the MLEs, we also observe that the MSEs decreases as n increases.

2- Prediction: We conclude, from Table 5, that the width of the predictive decreases as n increases.

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'Synthesis and Biological Assessment of Some New Acrylonitrile Derivatives as Potential Antitumor and Antimicrobial Agents'

Azza Taher Taher

Pharmaceutical Organic Chemistry Department, Faculty of Pharmacy, Cairo University, Kasr El-Aini street, Cairo, P.O. Box, 11562, Egypt. azzataher2005@yahoo.com

Abstract: A series of novel acrylonitrile derivatives **4-10** have been synthesized and characterized by spectral data. The *in-vitro* antitumor activity of all compounds was assessed in the MCF-7 human breast cancer cell line. The results showed that compound **8a** exhibited promising anticancer activity with $IC_{50} = 9.92 \mu\text{g/mL}$ while, compounds **4b**, **4c**, **7b**, **8c** and **10b** possessed moderate cytotoxic effect with IC_{50} ranging 15.64-20.76 $\mu\text{g/mL}$. The final targets were also tested for their antimicrobial activity. The results revealed that compounds **4a**, **5b**, **8a** and **8c** showed remarkable broad spectrum antimicrobial activity, while compounds **5c**, **8a** and **8c** displayed high antifungal activity against *candida albicans* compared to amphotericin B reference drug with $IC_{50} = 9.30$, 6.25 and 2.30 $\mu\text{g/mL}$, respectively.

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Keywords: Acryloylnitrile; Synthesis; Antitumor; antimicrobial activities.

1. Introduction

Cancer is a disease, in which the control of growth is lost in one or more cells, leading to a solid mass of cells known as a tumor. The initial tumor often becomes life-threatening by obstructing vessels or organs. However, death is most commonly caused by spread of the primary tumor to one or more other sites in the body. On the other hand, the most common cancer in women is breast cancer which remains the most frequent cause of malignancy-associated death among women [1]. Although the use of available chemotherapeutics is often limited due to undesirable side effects and the interest in novel anti-cancer agents is stimulated by growing incidence of drug resistance to cancer chemotherapeutic agents [2], more efforts should be developed in this field.

Literature survey, many investigators reported that cyanoacrylate and acrylamide derivatives [3-7] either substituted aromatic or heteroaromatic compounds were found to display potent anti-proliferative activity against MCF-7 cell lines [8]. They have attracted considerable attention on the part of synthetic chemists and pharmacists because some representatives exhibit anticancer activity [9-12].

The present work comprises the combination between 2-cyanoacrylate pharmacophore with either aminoguanidine moiety as in compounds (**4a-c**) or thiosemicarbazide as in compounds (**5a-c**) which possessed potent anticancer activity [13-16] (scheme 1). Additionally, in light of anticancer activity observed by imidazole moiety [17-19] and 1,2,4-triazole moiety [20-24], it was interesting to synthesize conjugates of cyanoacrylamide hydrazide and each moiety (compounds **6-10**) (schemes 2 and 3), to study the potential additive effect of the combined molecule towards cytotoxic activities [25,26] hoping the new

combination may enhance the potential anticancer profile.

From the view point of molecular design, the combination of two biologically active molecules or pharmacophores is a well-known approach for the build-up of drug-like molecules, which allows us to find more potent agents. It was thought that it would be of interest to synthesize a single molecule containing more than one pharmacophore conjugates.

On the other hand, the development of effective antibacterial is essential to avoid the major cause of death by bacterial infection. During the past decades, the human population had been affected with life-threatening infectious diseases caused by multidrug-resistant Gram-positive and Gram-negative pathogen bacteria [27]. Moreover, the long term use of several drugs to treat microbial infections may cause serious health problems, especially in patients with impaired liver or kidney functions [28]. Therefore, there is an increasing need to design new antibacterial and antifungal agents with better activity and higher safety profile. Additionally, we are facing now with the major problem of increasing bacterial resistance to antibacterial drugs. Since, there is unmet need for the synthesis of new antibacterial agents to overcome this increase in bacterial resistance.

Furthermore, in light of the antimicrobial and antifungal importance of cyanoacrylamide, hydrazine moiety and /or aromatic, heterocyclic compounds, hybridization of the different bioactive molecules with complementary pharmacophoric functions or with different mechanisms of action often showed synergistic effects. The biological relevance of these heteroaromatic moieties is due to their being good bioisosteres of biomolecules. Based on these prior observations, we postulated that compounds containing

both acrylamide hydrazide [29-32] and imidazole [33-35] or triazole [36-38] pharmacophores could be effective as antibacterial agents. These merged pharmacophores, may be addressing the active site of different targets for the purpose to overcome drug resistance, as well as reducing side effects. In the meanwhile, multi-targets drug strategies have emerged as a therapeutic approach to treat diseases that stem from a combination of factors and leading to the final pathology such as cancer. Using this strategy, a single molecule hits multiple targets, which participate in pathways implicated to a given disease, leading to more efficacious therapy and minimizing the emergence of resistance.

2. Experimental Protocols

2.1 General Remarks:

Melting points are uncorrected and determined in one end open capillary tubes using Gallen Kamp melting point apparatus MFB-595-010M (Gallen Kamp, London, England). Microanalysis was carried out at Micro-analytical Unit, the regional centre for microbiology and biotechnology, Al-Azhar University. Infrared Spectra were recorded on Shimadzu FT-IR 8400S spectrophotometer (Shimadzu, Kyoto, Japan), and expressed in wave number (cm^{-1}), using potassium bromide discs. The NMR spectra were recorded on a Varian Mercury VX-300 NMR spectrometer. ^1H spectra were run at 300 MHz and ^{13}C spectra were run at 75.46 MHz in dimethylsulphoxide ($\text{DMSO}-d_6$). Chemical Shifts are quoted in δ and were related to that of the solvents. Mass spectra were recorded using Hewlett Packard Varian (Varian, Palo, USA) and Shimadzu Gas Chromatograph Mass spectrometer-QP 1000 EX (Shimadzu, Kyoto, Japan). TLC were carried out using Art.DC-Plastikfolien, Kieselgel 60 F254 sheets (Merck, Darmstadt, Germany), the developing solvents were chloroform/methanol (9:1) or benzene/acetone (8:2) and the spots were visualized at 366 and 254 nm by UV Vilber Lourmat 77202 (Vilber, Marne La Vallee, France). Compound **4a** was reported with no data [39,40].

2.2. Chemistry

2-(2-Cyano-3-substitutedphenylacryloyl)hydrazinecarboximidamide (4a-c)

Method A: a mixture of aminoguanidine bicarbonate (1.36 g, 0.01 mol), ethyl cyanoacetate (1.13 g, 1.07 mL, 0.01 mol), anhydrous potassium carbonate (2.76 g, 0.02 mol) and the appropriate aromatic aldehyde (0.01mol) in absolute ethanol (25 mL) was heated under reflux for 12 hours. The formed precipitate was filtered while hot, washed twice with water (20 mL), dried and crystallized from ethanol.

Method B: a mixture of aminoguanidine bicarbonate (1.36 g, 0.01 mol), ethyl cyanoacetate (1.13 g, 1.07 mL, 0.01mol), and the appropriate aromatic aldehyde (0.01 mol) was added to sodium ethoxide solution (sodium metal 2.3 g in absolute

ethanol (25 mL) with continuous stirring for 30 min. The reaction mixture was heated under reflux for 5 hours. The solvent was evaporated under vacuum and after cooling the residue was extracted twice with ethyl acetate (20 mL). The ethyl acetate layer was washed twice with 10% hydrochloric acid (15 mL), and then washed twice with 5% sodium hydroxide (15 mL). The organic layer dried over anhydrous sodium sulphate, filtered and the filtrate was evaporated under reduced pressure and cooled. The crystalline solid was separated, collected and recrystallized from methanol.

2-(2-Cyano-3-substitutedphenylacryloyl)hydrazinecarboximidamid (4a-c) and 2-(2-Cyano-3-substitutedphenylacryloyl)hydrazinecarboxthioamide (5a-c)

Method C: a mixture of ethyl cyanoacetate (1.13 g, 1.07 mL, 0.01mol), and the appropriate aromatic aldehyde (0.01 mol) was added to potassium hydroxide solution (potassium hydroxide (0.56 g, 0.01 mol) in dry dimethylformamide (20 mL) with continuous stirring for 30 min. A solution of aminoguanidine bicarbonate (1.36 g, 0.01 mol) or thiosemicardazide (0.91 g, 0.01 mol) in dry dimethylformamide (10 mL) was added with continuous stirring for 24 hours at room temperature. The formed precipitate was filtered and suspended in a solution of acetic acid/water (15 mL)(1:1) and filtered. The precipitate was dried and crystallized from ethanol.

2-(2-Cyano-3-phenylacryloyl)hydrazine carboximidamide (4a)

Faint yellow cubic crystals; yield 21% [method A], yield 30% [method B], yield 81% [method C]; mp: 166-167°C; IR (KBr, cm^{-1}): 3365, 3256 (NH_2 , NH), 3171 (CH aromatic), 2214 (CN), 1686 (CO); ^1H -NMR ($\text{DMSO}-d_6$): δ 3.61 (br, 2H, NH_2 exchangeable by D_2O), 4.64 (s, 1H, NH exchangeable by D_2O), 7.35-7.81 (m, 5H, ArH), 7.56 (br, 1H, NH exchangeable by D_2O), 8.05 (s, 1H, =CH- C_6H_5), 9.51 (s, 1H, NH exchangeable by D_2O); MS (EI) m/z: 227 (M-2), 228 (M-1); Anal. Calcd for $\text{C}_{11}\text{H}_{11}\text{N}_5\text{O}$ (229.24): C, 57.63; H, 4.84; N, 30.55; Found: C, 57.49; H, 4.81; N, 30.56.

2-(2-Cyano-3-(4-fluorophenyl)acryloyl)hydrazinecarboximidamide (4b)

yellow needle crystals; yield 20% [method A], yield 33% [method B], yield 76% [method C]; mp: 140-141°C; IR (KBr, cm^{-1}): 3362, 3255 (NH_2 , NH), 3144 (CH aromatic), 2218 (CN), 1682 (CO); ^1H -NMR ($\text{DMSO}-d_6$): δ 3.20 (br, 2H, NH_2 exchangeable by D_2O), 4.25 (s, 1H, NH exchangeable by D_2O), 7.33, 7.96 (2d, 4H, ArH), 8.05 (br, 1H, NH exchangeable by D_2O), 8.19 (s, 1H, =CH- C_6H_5), 9.01 (s, 1H, NH exchangeable by D_2O); ^{13}C -NMR (DMSO): 168.66, 165.44, 161.57, 156.34, 144.32, 130.67, 130.15, 116.62, 116.37, 114.90, 114.61; MS (EI) m/z: 248 (M+1); Anal. Calcd for $\text{C}_{11}\text{H}_{10}\text{FN}_5\text{O}$ (247.23): C, 53.44; H, 4.08; N, 28.33; Found: C, 53.52; H, 4.15; N, 28.11.

2-(3-(4-Bromophenyl)-2-cyanoacryloyl)hydrazinecarboximidamide (4c)

Dark yellow micro crystals; yield 19% [method A], yield 28% [method B], yield 74% [method C]; mp: 146-147°C; IR (KBr, cm^{-1}): 3353, 3256 (NH_2 , NH), 3144 (CH aromatic), 2214 (CN), 1682 (CO); $^1\text{H-NMR}$ (DMSO-*d*6): δ 3.42 (br, 2H, NH_2 exchangeable by D_2O), 5.25 (s, 1H, NH exchangeable by D_2O), 6.06 (br, 1H, NH exchangeable by D_2O), 7.28, 7.72 (2d, 4H, ArH), 7.61 (br, 1H, NH exchangeable by D_2O), 8.18 (s, 1H, =CH- C_6H_5); MS (EI) m/z: 305 (M-2), 307 (M+); Anal. Calcd for $\text{C}_{11}\text{H}_{10}\text{BrN}_5\text{O}$ (308.13): C, 42.88; H, 3.27; N, 22.73; Found: C, 42.59; H, 3.21; N, 22.93.

2-(2-Cyano-3-phenylacryloyl)hydrazine carbothioamide (5a)

Dark yellow crystals; yield 68% [method C]; mp: 158-160°C; IR (KBr, cm^{-1}): 3387, 3264 (NH_2 , NH), 3174 (CH aromatic), 2261 (CN), 1662 (CO), 1372 (CS); $^1\text{H-NMR}$ (DMSO-*d*6): δ 4.50 (s, 2H, NH_2 exchangeable by D_2O), 7.20 (s, 1H, NH exchangeable by D_2O), 7.37 (m, 3H, C_3 , C_4 , C_5 ArH), 7.78 (m, 2H, C_2 , C_6 ArH), 8.21 (s, 1H, =CH- C_6H_5), 11.41 (s, 1H, NH exchangeable by D_2O); MS (EI) m/z: 247 (M+H); Anal. Calcd for $\text{C}_{11}\text{H}_{10}\text{N}_4\text{OS}$ (246.29): C, 53.64; H, 4.09; N, 22.75; Found: C, 53.44; H, 4.12; N, 22.87.

2-(2-Cyano-3-(4-fluorophenyl)acryloyl)hydrazine carbothioamide (5b)

Faint brown crystals; yield 61% [method C]; mp: 125-127°C; IR (KBr, cm^{-1}): 3379, 3260 (NH_2 , NH), 3175 (CH aromatic), 2241 (CN), 1644 (CO), 1365 (CS); $^1\text{H-NMR}$ (DMSO-*d*6): δ 4.51 (s, 2H, NH_2 exchangeable by D_2O), 7.21 (m, 2H, C_3 , C_5 ArH), 7.57 (s, 1H, NH exchangeable by D_2O), 7.87 (m, 2H, C_2 , C_6 ArH), 8.02 (s, 1H, =CH- C_6H_5), 11.44 (s, 1H, NH exchangeable by D_2O); MS (EI) m/z: 262 (M-2), 263 (M-1); Anal. Calcd for $\text{C}_{11}\text{H}_9\text{FN}_4\text{OS}$ (264.28): C, 49.99; H, 3.43; N, 21.20; Found: C, 49.91; H, 3.21; N, 21.11.

2-(3-(4-Bromophenyl)-2-cyanoacryloyl)hydrazinecarbothioamide (5c)

Brownish yellow crystals; yield 59% [method C]; mp: 230-231°C; IR (KBr, cm^{-1}): 3433, 3287 (NH_2 , NH), 3167 (CH aromatic), 2245 (CN), 1665 (CO), 1388 (CS); $^1\text{H-NMR}$ (DMSO-*d*6): δ 4.52 (s br, 2H, NH_2 exchangeable by D_2O), 7.17 (m, 2H, C_3 , C_5 ArH), 7.83 (m, 2H, C_2 , C_6 ArH), 8.04 (s, 1H, =CH- C_6H_5), 8.61 (s, 1H, NH exchangeable by D_2O), 11.40 (s, 1H, NH exchangeable by D_2O); $^{13}\text{C-NMR}$ (DMSO): 181.19, 177.99, 164.59, 161.31, 141.13, 130.76, 130.73, 129.48, 129.37, 115.77, 115.48; Anal. Calcd for $\text{C}_{11}\text{H}_9\text{BrN}_4\text{OS}$ (325.18): C, 40.63; H, 2.79; N, 17.23; Found: C, 40.41; H, 2.32; N, 17.00.

General procedure for synthesis compounds 6a-c

A mixture of **4a-c** (0.01 mol) and ethyl chloroacetate (1.65 g, 1.38 mL, 0.01 mol) in absolute ethanol (25 mL) was heated under reflux for 15 hours with continuous stirring. The solvent was evaporated

under reduced pressure and cooled. The formed precipitate was collected, dried and crystallized from ethanol.

2-Cyano-*N'*-(4-oxo-4,5-dihydro-1*H*-imidazol-2-yl)-3-phenylacryloylhydrazide (6a)

Buff micro crystals; yield 44%; mp: 266-267°C; IR (KBr, cm^{-1}): 3402, 3236 (NH), 3170 (CH aromatic), 2224 (CN), 1701, 1662 (2CO); $^1\text{H-NMR}$ (DMSO-*d*6): δ 4.69 (s, 2H, CH_2CO), 4.64 (s, 1H, NH exchangeable by D_2O), 7.11-7.82 (m, 5H, ArH), 7.20 (br, 1H, NH exchangeable by D_2O), 8.90 (s, 1H, =CH- C_6H_5), 10.21 (s, 1H, NH exchangeable by D_2O); MS (EI) m/z: 267 (M-2); Anal. Calcd for $\text{C}_{13}\text{H}_{11}\text{N}_5\text{O}_2$ (269.26): C, 57.99; H, 4.12; N, 26.01; Found: C, 57.69; H, 4.41; N, 26.16.

2-Cyano-3-(4-fluorophenyl)-*N'*-(4-oxo-4,5-dihydro-1*H*-imidazol-2-yl)acryloylhydrazide (6b)

Faint yellow micro crystals; yield 44%; mp: >300°C; IR (KBr, cm^{-1}): 3412, 3294 (NH), 3132 (CH aromatic), 2199 (CN), 1700, 1659 (2CO); $^1\text{H-NMR}$ (DMSO-*d*6): δ 3.53 (s, 2H, CH_2CO), 5.01 (s, 1H, NH exchangeable by D_2O), 7.29 (s, 1H, NH exchangeable by D_2O), 7.34 (d, 2H, ArH), 7.84 (d, 2H, ArH), 8.17 (s, 1H, =CH- C_6H_5); MS (EI) m/z: 285 (M-2), 286 (M-1), 287 (M+); Anal. Calcd for $\text{C}_{13}\text{H}_9\text{FN}_5\text{O}_2$ (287.25): C, 54.36; H, 3.51; N, 24.38; Found: C, 54.41; H, 3.54; N, 24.53.

3-(4-Bromophenyl)-2-cyano-*N'*-(4-oxo-4,5-dihydro-1*H*-imidazol-yl)acryloylhydrazide (6c)

yellow micro crystals; yield 44%; mp: 266-267°C; IR (KBr, cm^{-1}): 3452, 3325 (NH), 3150 (CH aromatic), 2227 (CN), 1710, 1667 (2CO); $^1\text{H-NMR}$ (DMSO-*d*6): δ 3.71 (s, 2H, CH_2CO), 4.61 (s, 1H, NH exchangeable by D_2O), 7.53-7.74 (m, 4H, ArH), 7.66 (s, 1H, NH exchangeable by D_2O), 8.09 (s, 1H, =CH- C_6H_5), 8.61 (s, 1H, NH exchangeable by D_2O); MS (EI) m/z: 348 (M+H), 349 (M+2); Anal. Calcd for $\text{C}_{13}\text{H}_{10}\text{BrN}_5\text{O}_2$ (348.15): C, 44.85; H, 2.90; N, 20.12; Found: C, 44.69; H, 2.87; N, 20.16.

General procedure for synthesis compounds 7a-c

A mixture of **4a-c** (0.01 mol) and ethyl orthoformate (1.48 g, 1.66 mL, 0.01 mol) in acetic anhydride (10 mL) was heated under reflux with stirring for 8 hours. The solvent was removed under reduced pressure and the product was left overnight. The formed solid was collected, dried and crystallized from methanol.

***N*-(1-(2-Cyano-3-phenylacryloyl)-1*H*-1,2,4-triazol-3-yl)acetamide (7a)**

Brownish yellow micro crystals; yield 45%; mp: 302-303°C; IR (KBr, cm^{-1}): 3433, 3267 (NH), 3179 (CH aromatic), 2936 (CH aliphatic), 2222 (CN), 1708, 1641 (2CO); $^1\text{H-NMR}$ (DMSO-*d*6): δ 2.05, 2.20 (2s, 3H, CH_3CO), 7.30-7.47 (m, 5H, ArH), 7.80 (s, 1H, triazole), 7.49 (s, 1H, NH exchangeable by D_2O), 8.01 (s, 1H, =CH- C_6H_5); $^{13}\text{C-NMR}$ (DMSO): 179.11, 175.07, 149.97, 140.87, 134.57, 128.12 (2C), 126.99 (2C), 111.62, 115.80, 24.73, 22.88; MS (EI) m/z: 282

(M+H); Anal. Calcd for C₁₄H₁₁N₅O₂ (281.27): C, 59.78; H, 3.94; N, 24.90; Found: C, 59.90; H, 3.74; N, 24.61.

***N*-(1-(2-Cyano-3-(4-fluorophenyl)acryloyl)-1*H*-1,2,4-triazol-3-yl)acetamide (7b)**

Brownish yellow micro crystals; yield 39%; mp: 217-218°C; IR (KBr, cm⁻¹): 3437, 3244 (NH), 3082 (CH aromatic), 2890 (CH aliphatic), 2214 (CN), 1713, 1681 (CO); ¹H-NMR (DMSO-*d*₆): δ 2.05, 2.22 (2s, 3H, CH₃CO), 7.19 (m, 2H, ArH), 7.86 (m, 2H, ArH), 8.16 (s, 1H, triazole), 8.21 (s, 1H, =CH-C₆H₅), 11.09 (s, 1H, NH exchangeable by D₂O); MS (EI) m/z: 299 (M+), 300 (M+1); Anal. Calcd for C₁₄H₁₀FN₅O₂ (299.26): C, 56.19; H, 3.37; N, 23.40; Found: C, 56.22; H, 3.42; N, 23.68.

***N*-(1-(3-(4-Bromophenyl)-2-cyanoacryloyl)-1*H*-1,2,4-triazol-3-yl)acetamide (7c)**

Brownish yellow micro crystals; yield 39%; mp: 217-218°C; IR (KBr, cm⁻¹): 3431, 3228 (NH), 3075 (CH aromatic), 2936 (CH aliphatic), 2212 (CN), 1712, 1685 (CO); ¹H-NMR (DMSO-*d*₆): δ 2.04, 2.17 (2s, 3H, CH₃CO), 7.62-7.77 (m, 4H, ArH), 7.80 (s, 1H, triazole), 7.91 (s, 1H, =CH-C₆H₅), 11.35 (s, 1H, NH exchangeable by D₂O); MS (EI) m/z: 357 (M-2), 359 (M+); Anal. Calcd for C₁₄H₁₀BrN₅O₂ (360.17): C, 46.69; H, 2.80; N, 19.44; Found: C, 46.77; H, 2.87; N, 19.61.

General procedure for synthesis compounds 8a-c

A suspension of equimolar amounts of **4a-c** and an appropriate aromatic aldehyde (0.01 mol each) in absolute ethanol (20 mL) and glacial acetic acid (2 mL) was heated under reflux for 11 hours. After cooling, the obtained product was filtered and recrystallized from ethanol.

***N*-(4-Chlorobenzylidene)-2-(2-cyano-3-phenylacryloyl)hydrazine carboximidamide (8a)**

Buff needle crystals; yield 66%; mp: 164-165°C; IR (KBr, cm⁻¹): 3444, 3256 (NH), 3140 (CH aromatic), 2214 (CN), 1686 (CO); ¹H-NMR (DMSO-*d*₆): δ 7.44 (m, 3H, C₃, C₄, C₅ ArH), 7.62 (d, 2H, *p*-Cl-C₆H₅), 7.85 (m, 2H, C₂, C₆ ArH), 7.87 (s, 1H, NH exchangeable by D₂O); 7.96 (d, 2H, *p*-Cl-C₆H₅), 8.12 (s, 1H, =CH-C₆H₄-Cl), 8.18 (s, 1H, =CH-C₆H₅), 12.62 (br.s, 2H, NH exchangeable by D₂O); Anal. Calcd for C₁₈H₁₄ClN₅O (351.79): C, 61.46; H, 4.01; N, 19.91; Found: C, 61.29; H, 4.11; N, 19.88.

***N*-(4-Chlorobenzylidene)-2-(2-cyano-3-(4-fluorophenyl)acryloyl) hydrazinecarboximidamide (8b)**

Buff scales crystals; yield 60%; mp: 282-283°C; IR (KBr, cm⁻¹): 3424, 3395 (NH), 3140 (CH aromatic), 2214 (CN), 1681 (CO); ¹H-NMR (DMSO-*d*₆): δ 7.25 (d, 2H, ArH), 7.37 (m, 2H, ArH), 7.91 (d, 2H, ArH), 7.94 (s, 1H, NH exchangeable by D₂O); 7.95 (m, 2H, ArH), 8.13 (s, 1H, =CH-C₆H₄-Cl), 8.17 (s, 1H, =CH-C₆H₅), 12.55 (br.s, 2H, NH exchangeable by D₂O); MS (EI) m/z: 367 (M-2), 369 (M+), 370 (M+1); Anal.

Calcd for C₁₈H₁₃ClFN₅O (369.78): C, 58.47; H, 3.54; N, 18.94; Found: C, 58.29; H, 3.41; N, 18.82.

2-(3-(4-Bromophenyl)-2-cyanoacryloyl)-*N*-(4-chlorobenzylidene) hydrazinecarboximidamide (8c)

Yellow micro crystals; yield 60%; mp: 298-299°C; IR (KBr, cm⁻¹): 3472, 3352 (NH), 3163 (CH aromatic), 2218 (CN), 1678 (CO); ¹H-NMR (DMSO-*d*₆): δ 7.15 (s, 1H, NH exchangeable by D₂O), 7.41 (d, 2H, ArH), 7.43 (d, 2H, ArH), 7.70 (d, 2H, ArH); 7.75 (d, 2H, ArH), 7.78 (s, 1H, =CH-C₆H₄-Cl), 8.03 (s, 1H, =CH-C₆H₅), 12.52 (br.s, 2H, NH exchangeable by D₂O); MS (EI) m/z: 429 (M+), 430 (M+H); Anal. Calcd for C₁₈H₁₃BrClN₅O (430.69): C, 50.20; H, 3.04; N, 16.26; Found: C, 50.29; H, 3.01; N, 16.01.

General procedure for synthesis compounds 9a,b

A mixture of **4b,c** (0.01 mol) and dry dimethylformamide (10 mL) was heated under reflux with stirring for 13 hours. The solvent was reduced to half its volume under vacuum and the product was poured into ice-cold water (20 mL). The formed solid was collected, dried and crystallized from methanol.

***N'*-(5-(1-cyano-2-(4-fluorophenyl)vinyl)-1*H*-1,2,4-triazol-3-yl)-*N,N*-dimethylformimidamide (9a)**

faint yellow crystals; yield 73%; mp: >300°C; IR (KBr, cm⁻¹): 3367 (NH), 3086 (CH aromatic), 2939, 2831 (CH aliphatic), 2218 (CN), 1631 (C=N); ¹H-NMR (DMSO-*d*₆): δ 2.72, 2.88 (2s, 6H, 2CH₃), 6.02 (s, 1H, NH exchangeable by D₂O), 7.07 (d, 2H, ArH), 7.38 (s, 1H, N=CH), 7.95 (d, 2H, ArH), 8.50 (s, 1H, =CH-C₆H₄), MS (EI) m/z: 284 (M+), 285 (M+1); Anal. Calcd for C₁₄H₁₃FN₆ (284.29): C, 59.15; H, 4.61; N, 29.56; Found: C, 59.04; H, 4.72; N, 29.47.

***N'*-(5-(2-(4-bromophenyl)-1-cyanovinyl)-1*H*-1,2,4-triazol-3-yl)-*N,N*-dimethylformimidamide (9b)**

Faint brownish yellow crystals; yield 67%; mp: 212°C; IR (KBr, cm⁻¹): 3325 (NH), 3089 (CH aromatic), 2958, 2812 (CH aliphatic), 2210 (CN), 1632 (C=N); ¹H-NMR (DMSO-*d*₆): δ 2.73, 2.91 (2s, 6H, 2CH₃), 6.23 (s, 1H, NH exchangeable by D₂O), 7.13 (d, 2H, ArH), 7.34 (s, 1H, N=CH), 7.93 (d, 2H, ArH), 8.51 (s, 1H, =CH-C₆H₄); Anal. Calcd for C₁₄H₁₃BrN₆ (345.2): C, 48.71; H, 3.80; N, 24.35; Found: C, 48.76; H, 3.78; N, 24.44.

General procedure for synthesis compounds 10a,b

A mixture of **4b,c** (0.01 mol) and ethyl orthoformate (4.44 g, 5 mL, 0.03 mol) in absolute ethanol (5 mL) was heated under reflux with stirring for 6 hours. The solvent was removed under reduced pressure and the product was left overnight. The formed solid was collected, dried and crystallized from methanol.

Ethyl *N*-(1-(2-cyano-3-(4-fluorophenyl)acryloyl)-1*H*-1,2,4-triazol-3-yl)formimidate (10a)

Yellow fine crystals; yield 63%; mp: 193-194°C; IR (KBr, cm⁻¹): 3089 (CH aromatic), 2931, 2851 (CH aliphatic), 2212 (CN), 1690 (C=N); ¹H-NMR (DMSO-*d*₆): δ 1.11 (t, 3H, CH₃), 3.53 (q, 2H, CH₂), 7.31 (d,

2H, ArH), 7.60 (s, 1H, N=CH-O), 7.91 (d, 2H, ArH), 7.96 (s, 1H, triazole), 8.52 (s, 1H, =CH-C₆H₄); MS (EI) m/z: 312 (M-1), 313 (M+); Anal. Calcd for C₁₅H₁₂FN₅O₂ (313.29): C, 57.51; H, 3.86; N, 22.35; Found: C, 57.58; H, 3.93; N, 22.43.

Ethyl N-1-(3-(4-bromophenyl)-2-cyanoacryloyl)-1H-1,2,4-triazol-3-ylformimidate (10b)

Dark yellow crystals; yield 56%; mp: >300°C; IR (KBr, cm⁻¹): 3113 (CH aromatic), 2920, 2843 (CH aliphatic), 2210 (CN), 1694 (CO); ¹H-NMR (DMSO-d₆): δ 1.07 (t, 3H, CH₃), 3.47 (q, 2H, CH₂), 7.29-7.62 (m, 4H, ArH), 7.58 (s, 1H, N=CH-O), 7.94 (s, 1H, triazole), 8.51 (s, 1H, =CH-C₆H₄); ¹³C-NMR (DMSO): 158.83, 146.64, 138.88, 131.57, 131.38, 130.92, 130.66, 130.12, 129.69, 128.38, 121.08, 120.60, 118.37, 48.20, 22.52; Anal. Calcd for C₁₅H₁₂BrN₅O₂ (374.19): C, 48.15; H, 3.23; N, 18.72; Found: C, 48.23; H, 3.21; N, 18.89.

2.3. Biological Evaluation

The antitumor screening of the novel synthesized compounds was carried-out at the National Cancer Institute (NCI), Biology Department, Pharmacology Unit, Cairo, Egypt. However, the antimicrobial testing was carried-out at Biotechnology Center, Faculty of Pharmacy, Cairo, Egypt.

2.3.1. Preliminary *in-vitro* antitumor screening

The novel synthesized compounds were subjected to SulfoRhodamine-B (SRB) assay for cytotoxic activity against human breast adenocarcinoma cell line (MCF7), at concentrations between 1 and 10 mg/mL according to Shehan method [41]. The data was represented in Table 1 and Figs. 1 & 2.

2.3.1.1. Measurement of cytotoxicity by SRB assay

The cytotoxic activity of some of the newly synthesized compounds was measured *in vitro* using the Sulfo-Rhodamine-B stain (SRB) assay according to the method of Skehan. Cells were plated in 96-multiwell microtiter plate (10⁴ cells/well) for 24 hours before treatment with the compound(s) to allow attachment of cell to the wall of the plate. Test compounds were dissolved in DMSO and diluted with saline to the appropriate volume. Different concentrations of the compounds under test (0, 1, 2.5, 5 and 10 µg/mL) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compound(s) for 48 hours at 37°C and in atmosphere of 5% CO₂. After 48 hours, cells were fixed, washed and stained for 30 minutes with 0.4% (wt/vol) with SRB dissolved in 1% acetic acid. Unbound dye was removed by four washes with 1% acetic acid and attached stain was recovered with Tris EDTA buffer. Color intensity was measured in an ELISA reader. The relation between surviving fraction and drug concentration was plotted to get the survival curve. The

concentration required for 50% inhibition of cell viability (IC₅₀) was calculated.

Data were collected, checked, revised and entered the computer. Data were analyzed by SPSS statistical package version 17. Excel computer program was used to tabulate the results, and represent it graphically.

Probit regression analysis procedure will be introduced to select the best model that describes the relationship among the Probit (IC) (as a dependent variable) in order to be used for prediction of the concentration of the drug that cause inhibition of 50% or 90% of cancer cells. The probity (P) = intercept + (regression coefficient x conc.).

The results are given in Table 1, and presented graphically in Figs. 1 & 2.

2.3.2. Antimicrobial activity screening

The newly synthesized compounds were evaluated for their *in vitro* antibacterial activity against *Staphylococcus aureus* ATCC 6538P, *Bacillus subtilis* ATCC CC33, *Escherichia coli* ATCC 5087 and *Pseudomonas aeruginosa* ATCC 9027, as well as for their antifungal activity against *Candida albicans* ATCC 60193 and *Aspergillus niger* ATCC 1718109 using the microbroth dilution method [42].

The Gram-positive antibacterial agent, amoxicillin, the Gram-negative antibacterial agent, gentamycin, and the anti-fungal agent, amphotericin B, were used as controls. In addition to, MICs (minimum inhibitory concentration, MBCs (minimum bactericidal concentration and IC₅₀ (the concentration which inhibits 50% of microorganisms) of all compounds were determined according to reported method [42,43]. The *in vitro* antimicrobial properties against a number of Gram-negative and Gram-positive bacteria, and yeasts are presented in Tables 2 & 3 and Fig. 3, respectively.

2.3.2.1. Determination of the Minimum Inhibitory Concentration (MIC)

The preliminary MICs were firstly determined by the microbroth dilution method [42]. Briefly, 100 µL of double strength DMSO (Sigma-Aldrich, Germany) were placed in each well of a 96-well microtiter plate. Aliquot of 100 µL of the solutions to be tested were added to the first column. Then two fold dilutions were carried out from one well to the next up to final well in each row for each tested compound.

MICs were then determined using agar streaking technique as per Clinical Laboratory Standard Institute guidelines [42]. A total of 15 mL molten (45°C) Nutrient agar (Sigma-Aldrich, Germany) were supplemented with the required concentration then were added into sterilized Petri dishes, allowed to solidify. Then 10 µL of each bacterial or fungal suspension (10⁵ CFU mL⁻¹) were streaked onto the surface. Finally all plates were incubated at 37 °C for 24 hours for bacterial strains and 25 °C for 48 hours fungal strains under aerobic conditions. MIC was

determined as the average between the last plate had growth and the first plate with no growth.

2.3.2.2. Determination of the MBC and IC₅₀

MBC and IC₅₀ were determined in 96 well microtiter plate where a 100 μ L of tryptic soya broth (Oxoid, USA) for bacterial isolates or sabaroud's dextrose broth for fungal strains were placed in each well. A proper amount of the stock solution of the tested compounds was added to reach the desired concentration. All columns were then inoculated with 20 μ L of bacterial suspension (10^6 CFU mL⁻¹) and incubated for 5-6 hours. An aliquot of 100 μ L from each well was transferred into another pre-supplemented with 100 μ L of Dey-Engley broth medium (Fluka, USA) and allowed to stand for 10-20 minutes to neutralize any antimicrobial activities. Then these neutralized solutions were subjected to proper dilutions and streaked onto tryptic soya agar or sabaroud's dextrose agar plates to determine the viable count [43]. Controls were done for sterility and growth and subjected to the same regimen of treatment. MBC was determined as the lowest concentration which decreased the number of viable bacteria by 3 log units. IC₅₀ was determined as the lowest concentration reduced the viable count by about 50 %.

3. Results and Discussion

3.1. Chemistry

The synthetic approaches adopted to obtain the target compounds **4-10** are depicted in Schemes 1-3. The structures of the newly synthesized compounds were established on the basis of their elemental analyses and spectral data.

The starting compounds **4a-c** was synthesized by three methods varying in the yield percentage. Firstly, it was prepared in 19-21% yield through heating ternary component (ethyl cyanoacetate, appropriate aldehyde and aminoguanidine) in boiling ethanol according to the reported method [44]. The second procedure is one pot reaction by refluxing the reactants in strong alkaline medium for five hours to give compounds **4a-c** in yield 28-33% following Fadda procedure [45]. Method C was adopted by stirring the reactants in alkaline polar aprotic solvent DMF (dimethyl formamide) to afford **4a-c** in yield 74-81%. On the other hand, the target compounds **5a-c** was prepared in 59-61% following method C by using thiosemicarbazide instead of aminoguanidine. However, the three procedures failed to obtain cyclic aminotriazepine compound **3**. IR spectra of compounds **4a-c** & **5a-c** showed absorption bands in the range 3433-3365 cm⁻¹ of (NH₂, NH) and bands at 1645-1683 cm⁻¹ which confirmed the presence of carbonyl function, additionally characteristic band at 1365, 1388 due to C=S (compounds **5a-c**). Furthermore, ¹H-NMR spectra of **4a-c** and **5a-c** showed sharp singlet signal for CH protons at 8.22 ppm [open structure]. ¹³C-NMR

depicted spectra at 168.66 (C=NH) in **4b** and 181.19 (C=S) in **5c**, respectively.

In scheme 2, the reaction of ethyl chloroacetate and compounds **4a-c** was adopted according to the literature method [46] to obtain imidazoacrylohydrazide **6a-c**. The structures of all synthesized compounds were determined by spectral and microanalytical analyses. The ¹H-NMR spectra of **6a-c** have shown new singlet signals around δ 3.53 and 4.69 ppm corresponding to the CH₂CO protons. All the other aromatic protons were observed in the expected regions. The title compounds were further confirmed by mass spectral data which showed the molecular ion peak. Moreover, compounds **7a-c** was achieved by cyclization of 2-(2-Cyano-3-substitutedphenyl acryloyl)hydrazinecarboximidamide (**4a-c**) using ethyl orthoformate [47] followed by acetylation of amino (imino) group by acetic anhydride. IR spectra exhibited very similar features and showed the expected bands for the characteristic groups which are present in the compounds such as NH stretching vibrations, amide C=O stretching, and another aliphatic band for CH₃ vibrations. ¹H-NMR spectra were consistent with the proposed structures which showed two singlet peaks around δ 2.04 and 2.22 ppm corresponding to the acetyl group, in addition to the aromatic protons observed in the expected region. ¹³C-NMR depicted spectra at 24.73 (CH₃) of acetyl amino and at 22.88 (CH₃) of acetyl imino, respectively. 4-Chlorobenzylidene derivatives **8a-c** was obtained by refluxing of **4a-c** with an equimolar amount of 4-chlorobenzaldehyde in acidified absolute ethanol [48]. The structures of new compounds were elucidated by analytical and spectroscopic measurements, ¹H-NMR spectra showed benzylidene CH around 7.78 and 8.18 ppm.

Scheme 3 deals with the preparation of the target 1,2,4-triazole derivatives **9a,b** and **10a,b**. Refluxing of **4b,c** in dimethylformamide (DMF) yielded the corresponding *N,N*-dimethylformimidamide derivatives **9a,b**. The reaction proceeds through cyclodehydration followed by condensation with DMF, the experimental procedure was similar to the literature [49,50]. IR spectra showed appearance of absorption bands for methyl group in the range of 2958-2830 cm⁻¹ and disappearance of carbonyl group. ¹H-NMR spectra revealed two equivalent peaks in the region 2.72-2.91 ppm due to methyl group and additional sharp peak at 7.34 and 7.38 for CH=N proton corresponding to **9a** and **9b**, respectively. In addition to, the mass spectrum data of compound **9a** showed the molecular ion peak. On the other hand, compounds **10a,b** obtained in 56-63% yield through heating **4b,c** in excess ethyl orthoformate [51]. ¹H-NMR spectra showed the characteristic triplet and quartet peaks which were informative to the ethyl group and singlet signal of triazole at 7.96 and 7.94 ppm corresponding to **10a** and

10b, respectively. Moreover, ^{13}C -NMR depicted spectra at 48.20 and 22.52 pointed to ethyl group in compound **10b** (c.f. experimental part).

Table 1: *In vitro* cytotoxic activity of some of the synthesized compounds against the human breast cancer cell line (MCF-7).

Compds.no.	Cell line	
	IC ₅₀	IC ₉₀
4a	46.62 ^{jk} ±2.11	89.29 ⁱ ±0.73
4b	19.52^c ±1.29	65.41 ^b ±1.38
4c	16.45^b ±1.45	71.79 ^f ±2.59
5a	26.59 ^{de} ±0.03	71.04 ^f ±2.59
5b	47.93 ^k ±0.57	82.55 ^h ±0.85
5c	26.76 ^{de} ±1.39	71.36 ^f ±0.57
6a	41.39 ^h ±1.53	78.13 ^g ±2.46
6b	28.05 ^f ±2.38	74.11 ^f ±1.53
6c	44.78 ^{ij} ±1.57	82.96 ^h ±1.79
7a	27.5f±1.19	80.36 ^g ±1.24
7b	16.19^b ±2.57	64.33 ^a ±0.99
7c	23.77^d ±0.52	69.23 ^{cd} ±2.28
8a	9.92^a ±2.62	68.49 ^{bc} ±0.95
8b	30.56 ^{fg} ±2.25	72.94 ^f ±0.72
8c	20.76^e ±1.61	67.71 ^{bc} ±2.7
9a	26.36 ^{de} ±2.05	66.80 ^{bc} ±1.86
9b	42.27 ⁱ ±1.58	79.18 ^g ±0.62
10a	32.92 ^g ±0.57	68.32 ^{cd} ±2.73
10b	15.64^b ±2.78	69.67 ^{cd} ±0.64
F-value	126.30	47.46
p-value	0.000*	0.000*

All values are represented as Mean ± S.D

*= There is a significant difference between the com. no. by using One Way ANOVA at $p < 0.05$

The different letters means that there is a significant difference between the two compounds by using Duncun multiple comparison test at $p < 0.05$

Values were calculated from dose-response curves done in triplicate for each compound.

IC₅₀µg/mL : dose of the compound which caused 50% reduction of survival.

IC₉₀µg/mL: dose of the compound which caused 90% reduction of survival.

3. 2. Results of *in-vitro* antitumor screening

All compounds were evaluated for their *in-vitro* antitumor activity against human breast adenocarcinoma cell line (MCF-7). The IC₅₀ and IC₉₀ (the concentration required for 50% and/or 90%

inhibition of cell viability) were calculated for each compound and the results are given in Table 1 and Figs. 1 & 2.

All the newly tested compounds were found to possess moderate to weak antitumor activities against breast adenocarcinoma cell line (MCF-7) with IC₅₀ range from 9.92 to 47.93 µg/mL). Nevertheless, compound **8a** displayed the highest anti-breast cancer activity with IC₅₀=9.92 µg/mL. and IC₉₀=68.49 µg/mL (the concentration required for 90% inhibition of cell viability). On the other hand, compounds **4b,c**, **7b**, **7c**, **8c** and **10b** possessed moderate activity with IC₅₀ ranged from 15.64 to 23.77 µg/mL and IC₉₀=64.33-71.79 µg/mL.

Regarding the structure activity relationship of 2-(2-Cyano-3-substituted phenylacryloyl) hydrazine carboximidamide (**4a-c**) toward MCF-7 breast cancer cell lines: Compounds **4b** and **4c** showed significant activity more than the unsubstituted congener **4a**. This may attributed to the lipophilicity of halogen substitution which enhances the cytotoxic activity [52]. On the other hand, the thioxo analogues **5a-c** found to be moderate to weak anticancer effect with IC₅₀=26.59-47.93 µg/mL. Whereas, compound **4b** displayed promising activity against MCF-7 cell panel compared to **5b**, this could be assigned to the presence of the amino group which favors the potency than that thioxo moiety [53]. The cyclized imidazolidinone acrylohydrazide derivatives **6a-c** led to compounds that had slightly inhibitory efficacy compared to the precursor hydrazine carboximidamide **4a-c**. For instance, the analogue p- Fluoro **6b** exhibited the best member in this series with IC₅₀=28.05 µg/mL. All acetamidotriazole derivatives **7a-c** showed moderate anticancer activity [54] with IC₅₀ around 16.19 to 27.50 µg/mL. Interestingly, within Schiff's bases derivatives **8a-c** displayed potent to moderate antitumor activity against MCF-7 breast cancer, which is well known to have potential anticancer activity [55,56]. It was envisioned that compound **8a** possessed remarkable cytotoxic activity than other congeners **8b** and **8c**. On the other hand, triazole derivatives compound **9a** recorded fair antitumor activity with IC₅₀=26.36 µg/mL, while compound **9b** showed weak activity with IC₅₀ value 42.27 µg/mL although the *N,N*-dimethylformimidamide derivatives proved recently to be have potential antitumor activity [57]. Moreover, compounds **10a** and **10b** displayed moderate to weak anticancer activity with IC₅₀=15.64 and 32.92 µg/mL, respectively.

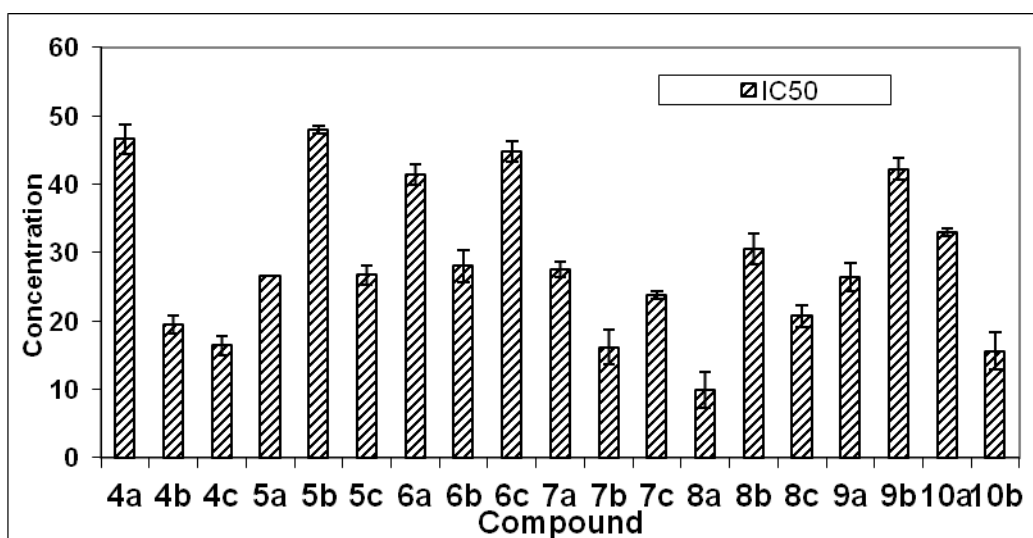


Fig. (1): IC₅₀ values of the new synthesized compounds against the human breast cancer cell line (MCF-7).

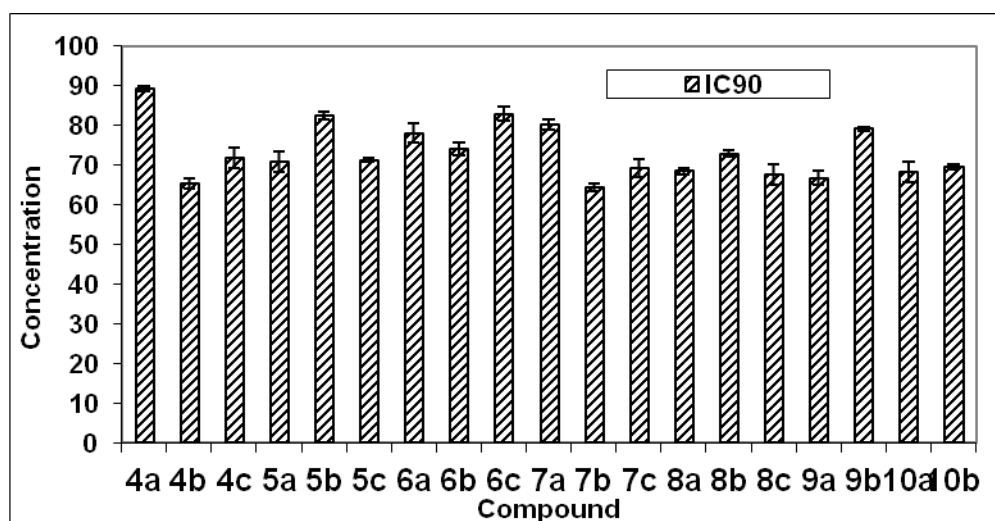


Fig. (2): IC₉₀ values of the new synthesized compounds against the human breast cancer cell line (MCF-7).

3.3. Results of antimicrobial activity

The newly synthesized compounds were subjected for evaluation their antimicrobial activities using microbroth dilution method [42]. The data presented in table 2 which revealed that compounds **4a**, **4c**, **5b** and **8a** and **8c** showed broad spectrum antibacterial and antifungal activities, while compounds **7a** and **9a** were only active against Gram negative strains. Moreover, compounds **7b** and **10b** showed antibacterial activity against Gram positive bacteria. The remaining compounds **4b**, **6a-c**, **7c**, **8b** and **9b** had no significant activity against any of the tested strains at concentration up to 50 µg/mL. (Fig. 3).

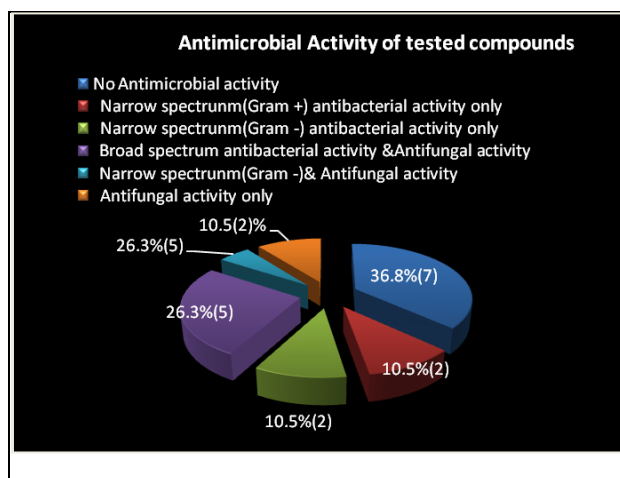


Fig. 3: Pie Chart of the % antimicrobial activity of tested compounds

Table 2: Antimicrobial activity of the synthesized compounds expressed as minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and concentration that inhibit 50% of microorganisms (IC₅₀) in µg /mL against the pathological strains based on two fold serial dilution technique.

Compds. no.		<i>S. aureus</i>	<i>B.subtilis</i>	<i>E.coli</i>	<i>P.aeruginosa</i>	<i>C.albicans</i>	<i>A.niger</i>
4a	MIC	18.75	18.75	18.75	18.75	18.75	37.50
	MBC	18.37	18.75	18.75	18.75	18.75	37.50
	IC ₅₀	12.50	12.50	9.30	12.50	12.50	12.50
4b	MIC	>50	>50	>50	>50	>50	>50
	MBC	>50	>50	>50	>50	>50	>50
	IC ₅₀	>50	>50	>50	>50	>50	>50
4c	MIC	37.50	37.50	37.50	37.50	37.50	>50
	MBC	37.50	37.50	37.50	37.50	37.50	>50
	IC ₅₀	12.50	25	25	25	12.50	>50
5a	MIC	>50	>50	18.75	37.50	37.50	>50
	MBC	>50	>50	18.75	50	37.50	>50
	IC ₅₀	>50	>50	9.30	37.50	25	>50
5b	MIC	18.75	18.75	9.38	9.38	18.75	18.75
	MBC	18.75	18.75	9.30	9.30	18.75	18.75
	IC ₅₀	9.30	12.50	6.25	6.25	9.30	12.50
5c	MIC	>50	>50	>50	>50	9.38	18.75
	MBC	>50	>50	>50	>50	12.50	18.75
	IC ₅₀	>50	>50	>50	>50	9.30	12.50
6a	MIC	>50	>50	>50	>50	>50	>50
	MBC	>50	>50	>50	>50	>50	>50
	IC ₅₀	>50	>50	>50	>50	>50	>50
6b	MIC	>50	>50	>50	>50	>50	>50
	MBC	>50	>50	>50	>50	>50	>50
	IC ₅₀	>50	>50	>50	>50	>50	>50
6c	MIC	>50	>50	>50	>50	>50	>50
	MBC	>50	>50	>50	>50	>50	>50
	IC ₅₀	>50	>50	>50	>50	>50	>50
7a	MIC	>50	>50	18.75	18.75	>50	>50
	MBC	>50	>50	18.75	25	>50	>50
	IC ₅₀	>50	>50	9.30	12.50	>50	>50
7b	MIC	37.50	37.50	>50	>50	>50	>50
	MBC	37.50	42.50	>50	>50	>50	>50
	IC ₅₀	12.50	28.75	>50	>50	>50	>50
7c	MIC	>50	>50	>50	>50	>50	>50
	MBC	>50	>50	>50	>50	>50	>50
	IC ₅₀	>50	>50	>50	>50	>50	>50
8a	MIC	4.69	2.34	4.69	2.34	9.38	18.75
	MBC	4.70	2.30	4.70	2.30	12.50	25
	IC ₅₀	3.13	0.78	1.17	0.78	6.25	9.30
8b	MIC	>50	>50	>50	>50	>50	>50
	MBC	>50	>50	>50	>50	>50	>50
	IC ₅₀	>50	>50	>50	>50	>50	>50
8c	MIC	4.69	4.69	9.38	9.38	4.69	18.75
	MBC	4.70	4.70	9.30	9.30	4.70	18.75
	IC ₅₀	2.30	2.30	3.13	3.13	2.30	9.30
9a	MIC	>50	>50	>50	18.75	18.75	>50
	MBC	>50	>50	>50	25	18.75	>50
	IC ₅₀	>50	>50	>50	18.75	9.30	>50

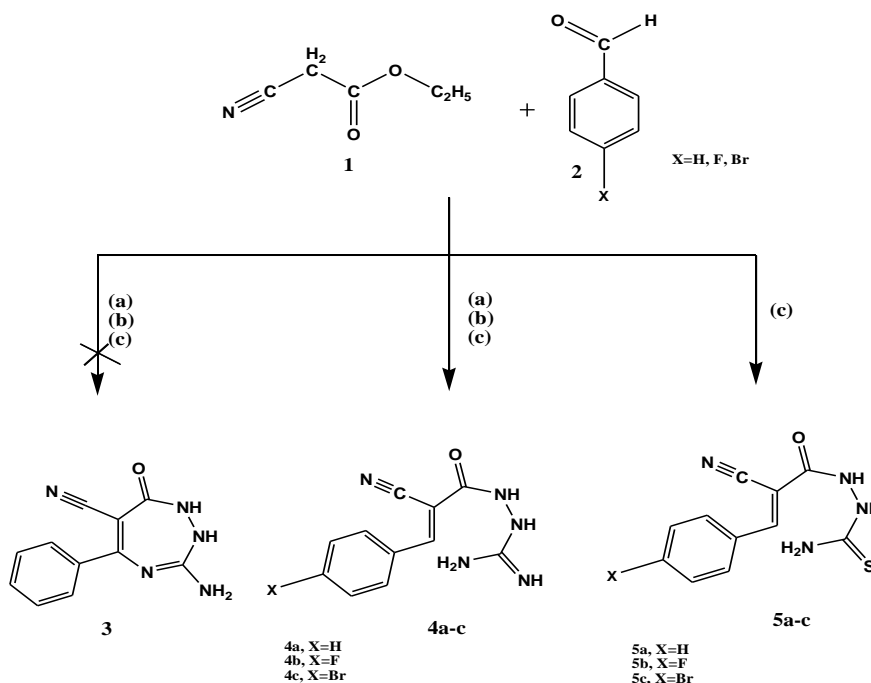
9b	MIC	>50	>50	>50	>50	>50	>50
	MBC	>50	>50	>50	>50	>50	>50
	IC ₅₀	>50	>50	>50	>50	>50	>50
10a	MIC	>50	>50	>50	>50	>50	37.50
	MBC	>50	>50	>50	>50	>50	>50
	IC ₅₀	>50	>50	>50	>50	>50	>50
10b	MIC	9.38	2.34	>50	>50	>50	>50
	MBC	12.50	3.13	>50	>50	>50	>50
	IC ₅₀	6.25	2.30	>50	>50	>50	>50
Amoxicillin	MIC	10	100	NA	NA	NA	NA
Gentamicin	MIC	NA	NA	10	25	NA	NA
Amphotericin B	MIC	NA	NA	NA	NA	10	15

NA: no action.

Table 3: Relative potency (%) of tested compounds against six microorganisms at concentration 50 µg/mL

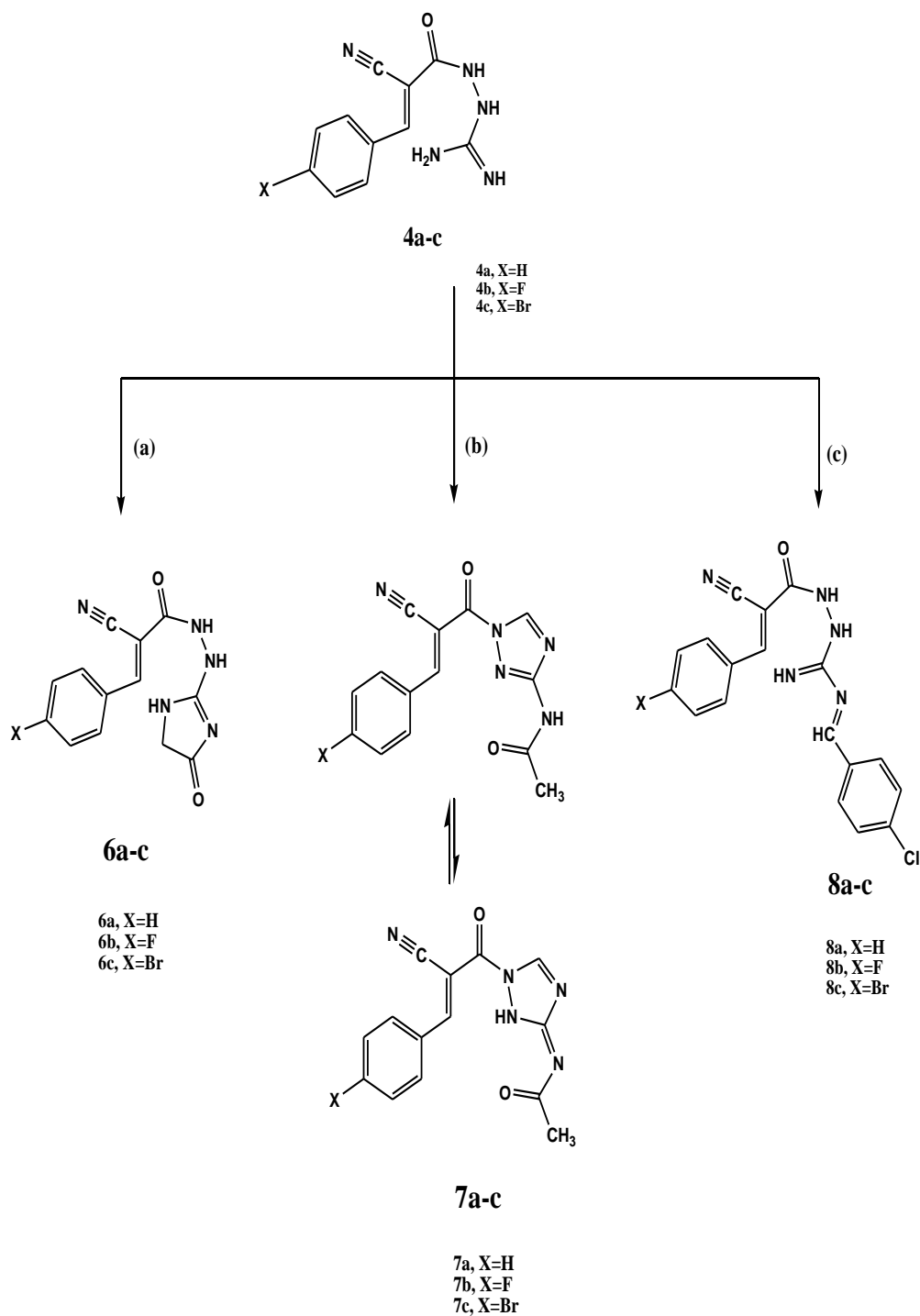
M. O.	4a	4c	5a	5b	5c	7a	7b	8a	8c	9a	10a	10b	Reference
<i>S. aureu</i>	54.45	40.9	0	45.45	0	0	40.91	131.82	100	0	0	86.36	Amoxicillin
<i>B. subtilis</i>	57.89	36.84	0	47.37	0	0	31.58	200	147	0	0	89.47	Amoxicillin
<i>E. coli</i>	50	40.9	68.81	81.81	0	63.63	0	109.09	111.11	50	0	0	Gentamicin
<i>P.aeruginosa</i>	72.22	44.44	83.33	100	0	83.33	0	172.22	111.11	55.56	0	0	Gentamicin
<i>C. albicans</i>	133	38.88	0	138.88	122	0	0	127.77	86	0	0	0	Amphotericin B
<i>A. niger</i>	62.5	0	0	77.71	119	0	0	93.38	75	0	56.25	0	Amphotericin B

Scheme 1

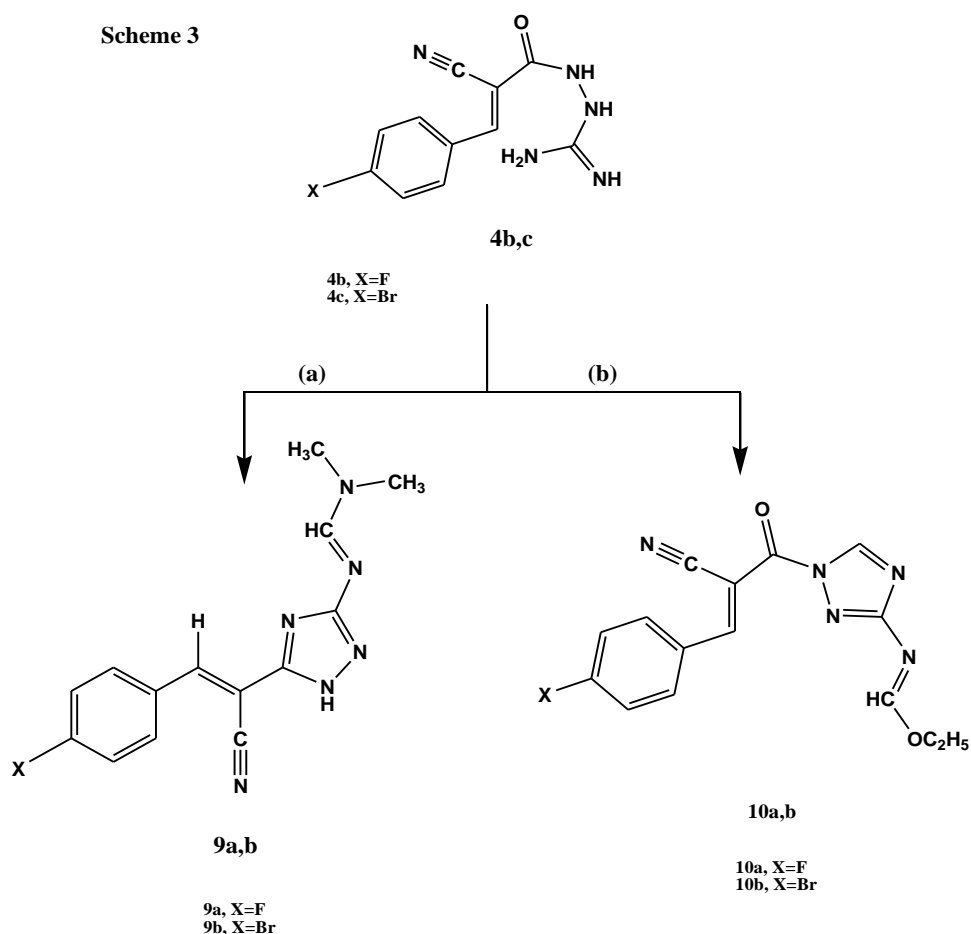


Scheme1: Reagents and condition; (a) Aminoguanidine bicarb/reflux in absolute ethanol/anhyd. /12 hrs. (b) Aminoguanidine bicarb/reflux in sod.ethoxide/ 5hrs.; (c) Aminoguanidine bicarb. or thiosemicarbazide/stirring in dry DMF/KOH/at R.T/24hrs.

Scheme 2



Scheme 2: Reagents and condition; (a) Ethyl chloroacetate/reflux in absolute ethanol/anhyd. K_2CO_3 /15 hrs. (b) Ethyl orthoformate/ AC_2O /8hrs. (c) p-Chlorobenzaldehyde/reflux in absolute ethanol/HAC/12hrs.



Scheme 3: Reagents and condition; (a) Reflux in dry DMF/13 hrs. (b) Reflux in ethyl orthoformate excess/ 7hrs.

The structure activity correlation of the tested compounds showed that the starting compound **4a** possessed broad antibacterial spectrum against *S. aureus*, *B. subtilis* (G⁺) and *E. coli*, *P. aeruginosa* (G⁻) bacteria. Also, it recorded superior antifungal activity against *C. albicans* than amphotericin B reference drug at concentration 18.75-50 µg/mL. The percentage of relative potency of compound **4c** against (G⁺) bacteria ranged 40.9-36.8% compared to amoxicillin reference drug at concentration 37.50-50 µg/mL. However, it exhibited an inhibitory activity against (G⁻) bacteria around 44.44 - 40.90% compared to gentamicin standard drug and it showed antifungal activity against *Candida albicans* with relative potency 38.88% at the same concentration. Compound **5a** displayed decent inhibitory activity against *E. coli* and *P. aeruginosa* (G⁻) bacteria with % of potency

68.81 and 83.33, respectively at concentration 18.75-50 µg/mL. Moreover, compound **5b** showed promising broad spectrum antimicrobial activities, this may be attributed to the combination of p-fluorophenyl and thiosemicarbazide enhances the antimicrobial activity [58]. It exhibited comparable activity against *P.aeruginosa* as gentamicin reference drug at concentration 9.38 µg/mL, while it showed higher antifungal activity against *C. albicans* compared to the standard at concentration 18.75 µg/mL. Additionally, compound **5c** showed better antifungal activity more than reference drug against *C. albicans* in concentration 9.38-50 µg/mL and against *A. niger* in concentration 18.75-50 µg/mL, respectively. The percentage of relative potency of compound **7a** against *E. coli* and *P. aeruginosa* (G⁻) bacteria is 63.63 and 83.33%, respectively at concentration 18.75 µg/mL for

each. On the other hand, compound **7b** showed moderate activity against Gram positive bacteria only at concentration 37.50 µg/mL. Compound **8a** recorded the best antimicrobial activities derivative in this work. It displayed about two folded antibacterial activity against *B. subtilis* (G+) and *P. aeruginosa* (G-) bacteria compared to the reference drug in concentration 2.34 µg/mL. In additions to, it exhibited superior antimicrobial activity against *S. aureus* (G+), *E. coli* (G-) and *C. albicans* compared to the amphotericin B standard drug. This is may be referred to the combined factors of the unsubstituted phenyl residue and shiff's base with two atom spacer may be potentiate the activity [59].

Moreover, compound **8c** registered an excellent antibacterial activities against both (G+ and G-) bacteria more than the reference drugs at concentration 4.69 and 9.38 µg/mL, respectively, while it exhibited good antifungal activity with percentage relative potency ranged 86 to 75% at concentration 4.69 and 18.75 µg/mL. On the other hand, compound **9a** showed narrow moderate inhibitory activity against *E. coli* and *P. aeruginosa* (G-) bacteria with percentage relative potency 50 and 55.56 %, respectively. Furthermore, compound **10a** showed moderate antifungal activity against *A. niger* at concentration 37.50-50 µg/mL. On the other hand, compound **10b** displayed promising antibacterial activity against *S. aureus*, *B. subtilis* (G+) bacteria with percentage relative potency values 86.36 and 89.47%, respectively.

4. Conclusion

This study reports the synthesis of acrylonitrile based compounds **4-10** as potential antitumor and antimicrobial agents. According to the results of bioactivity; the *in vitro* cytotoxic screening of novel derivatives revealed that most of the compounds had moderate to limited anticancer activity against MCF-7 human breast cancer cell line. However, compound **8a** exhibited potent inhibitory effect against human breast adenocarcinoma cell line with IC₅₀ = 9.92 µg/mL. Considering the imidazolone derivatives **6a-c**, they proved to have negative impact on the anticancer activity. On the other hand, several of the newly compounds displayed promising antimicrobial activity compared to the reference drugs amoxicillin, gentamicin and amphotericin B. It can stated that, the starting amino derivatives **4a**, **4c**, thioxo analogue **5b** and shiff's bases compounds **8a,c** were found to be broad spectrum than the remaining compounds. These findings demonstrated a new potential for acrylonitrile derivatives which could be useful templates for further derivatives to obtain more potent antitumor and antimicrobial agent(s).

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Avoiding Pitfalls in Trauma Triage: Effect of Nursing Staff Development

Soad M. Hegazy^{*1}, Lamiaa A. El-Sayed², Tarek Y. Ahmed³, Mohamed Rady³

Medical–Surgical Nursing Department¹, Pediatric Nursing Department², Faculty of Nursing, Ain shams University
General Surgery Department³, Faculty of Medicine, Ain shams University
Soadmahmoud43@yahoo.com

Abstract: Aim: This study aims to evaluate the effect of nursing staff development on avoiding pitfalls in trauma triage Methods: A quasi experimental design was used. This study was carried out at emergency department of surgery at El-Demerdash Hospital and Children Hospital, affiliated to Ain Shams University. Sample: A purposive sample composed of 40 emergency nurses, 20 were dealing with children and rest of them 20 were dealing with adults, add to 200 trauma patients (100 adults and 100 children with school age) from the above mentioned settings. Tools: 1) Self administered questionnaire to assess nurses' knowledge (pre/post tests), 2) Factors affecting triage process assessment sheet (pre- test) 3) An observation checklist to assess: nurses practice and potential errors in trauma triage (pre/post tests), 4) Nurses opinionnaire sheet (pre- test) and 5) Clinical data sheet for trauma patients (pre/post tests) Results: There are insignificant differences between studied nurses reports regarding sources of information in triage decision making and factors affecting triage process, added to trauma triage errors reduction among them in post tests .Moreover, nurses knowledge and practice were improved post training . Conclusion: Nursing staff development through training sessions was helpful on avoiding pitfalls in trauma triage. *Recommendations:* The study reinforces the need for sustained training on triage intervention. Further studies should be done to assess the long term effects of such study on trauma patient's outcome at the emergency department. [Soad M. Hegazy, Lamiaa A. El-Sayed, Tarek Y. Ahmed, Mohamed Rady. **Avoiding Pitfalls in Trauma Triage: Effect of Nursing Staff Development.** *Life Sci J* 2012;9(1):1006-1014] (ISSN:1097-8135). <http://www.lifesciencesite.com>. 144

Key words: Trauma triage pitfalls, nursing staff development.

1. Introduction:

Triage is the classification of patients according to medical need and it was developed to categorize the injuries into emergent, urgent and non-urgent. It starts at the front door of the emergency department and repeatedly performed as management of patient evolves. In general, the principles of triage are the same for children and adults, though the priority of children over adults within the same categories is controversial. In recent years, trauma and triage have become more prominent. Development of triage, which to an extent has arisen as a result of trauma has gone some way to overcoming this problem .Researches suggests that, triage has a positive effect on patient waiting times and patient / nurse satisfaction (*Derlet & Richards, 2008 and Johansen & Forberg, 2011*).

Triage is a central task in an emergency department, viewed as rating of patients' clinical urgency to identify the order in which patients should be given care and is not needed if there is no queue for care. Triage scales aim to optimize waiting time of patients according to severity of their medical condition and to treat as fast as necessary the most intense symptoms added to reducing negative impact on prognosis of a prolonged delay before treatment. Moreover, Triage decisions may be based on both, patients' vital signs (respiratory rate, oxygen

saturation in blood, heart rate, blood pressure, level of consciousness and body temperature) and their chief complaints. In children, triage poses a greater challenge, as measurements of vital signs, particularly of blood pressure, are difficult to obtain and cooperation is limited. Triage is often a major determinant of outcome (*Gautschi et al., 2008 and Durand et al., 2011*).

In children special considerations were found regarding mechanisms of injury compared to adults as follows: Head injuries account for approximately 60% of all injuries, which can be explained by large and heavy heads relative to the bodies. Furthermore, unconsciousness, children with upper airways tend to get obstructed by their relatively large, flaccid tongue or kinked because of large head flexion induced by short occiput. The less mature thermoregulatory mechanism and higher surface area-to-mass ratio which make heat loss and hypothermia more common in them, particularly during exposure to extreme conditions, such as cold weather, decontamination with cold water during biochemical events, or when undressed at triage. As children have relatively small amounts of blood (80 ml/kg), so minor bleeding represent a significant volume loss and severe shock (*Kyle, 2008 and Fuzak & Mahar, 2009*).

Triage is the most fundamental and important aspect of management of emergency department. It cannot be operationalized in such a way that just anyone can safely perform this critical duty. It requires in-depth knowledge skills that nurses have to learn to keep them oriented with the changes in their roles and functions, and modify their attitudes and understanding (Goh, 2009 and Lee, 2010). The complex and skilled nature of triage require a highly competent health care professional. The ability of emergency nurse on triage to assess, intervene and communicate effectively helps establish rapport and trust with patient and significant others. Research suggests that, triage has a positive effect on patient waiting times and patient/nurse satisfaction (Peitzman et al, 2008 and Aacharya, et al, 2011).

Staff development and continuing education are shown to cover a very broad expanse of responsibilities. Health care organizations have put into place staff development departments, sometimes called nursing education . Staff development nurses are responsible for a wide array of duties, beginning with orientation of new staff: from newly graduated nurses, to those returning to nursing or changing areas of practice, to experienced nurses changing location but not areas of expertise. The educational needs of each group are different and should be addressed differently. Some researches into stress and coping factors for nurses gives insight into how important a role staff development can be in assisting nurses cope with the unique problems faced in today's health care milieu. When nurses feel they are doing well, they report satisfaction with their accomplishments, feel challenged and enjoy learning new skills (Budd, 2007 and Daleen, 2010).

Significance of the Study:

Attendance at emergency department has increased in recent years. The majority of trauma deaths occur either before reach the hospital or within four hours of arrival, pre-hospital and emergency department personnel must make rapid triage decisions based on pre-established system standard. The problem of inappropriate attendance remains and traditional methods of patient reception do not adequately deal with differing degrees of serious injuries. This can lead to long waiting times for dangerous injuries and a poor service for minor injuries. Although children may account for 10 to 100% of victims in mass casualty events and disasters, most of the triage studies to date have focused on adult population. Increasing attendance at emergency departments presents a major challenge to staff and demands highest level of knowledge and skills to provide safe and competent care (Hoot &

Aronsky, 2008, Kirkpatrick et al., 2009 and Lerner et al, 2010).

Aim of the study:

This Study aims to evaluate the effect of nursing staff development on avoiding pitfalls in trauma triage.

This aim was achieved through the following:

- Identify the pitfalls in trauma triage among the emergency nurses.
- Identify the factors that influence triage process.
- Assess emergency nurses knowledge and practice regarding trauma triage.
- Develop and implement training sessions about trauma triage and common pitfalls.
- Evaluate the training sessions effects on nurses knowledge and practice, added to patients conditions outcomes.

Research hypothesis :

It was hypothesized that nursing staff development will be helpful on avoiding pitfalls in trauma triage at emergency department.

2. Subjects and Methods:

Operational definition:

Nursing staff development: means training sessions for nursing staff at emergency department.

Design:

A quasi – experiment design was adopted in the following phases: pre-training assessment, training intervention, post intervent and follow-up.

Setting:

This study was conducted in the emergency department of surgery at El-Demerdash Hospital and children Hospital, which are affiliated to Ain shams university.

Subjects:

A purposive sample included two groups, the first one was all available emergency nurses from both Hospitals, 20 from each one. Second group included 100 adult and 100 children (school age) with different injuries from the above mentioned settings.

Tools for data collection:

1- Self-administered questionnaire sheet that was designed by the researchers after reviewing related literature and consulting experts. It was written in simple Arabic language and divided into the following:

- Characteristics of the studied nurses as regards age, qualifications and experiences.
- Nurses knowledge regarding; definition, assessment, site, level of triage, life saving measures, secondary assessment and nursing intervention (pre/post test).

The satisfactory level was from 70% while the unsatisfactory level was less than 70%.

- 2- Assessment sheet to identify the factors affecting trauma triage process as regards: time constraints, formal training and environment (pre-test).
- 3- An observation check list (pre/ post tests), adapted from: *Lewis et al. (2004)*, *Kyle (2008)* and *Peitzman et al (2008)*. It was used to assess the following:
 - Common potential errors in trauma triage by the emergency nurses as regards: assessment, diagnosis, discharge documentation, standard of care and waiting time (pre/post tests).
- Nurses practice on trauma triage process: primary assessment, life saving measures, secondary assessment and nursing intervention (pre/post tests). A correct answer was scored as (1) while the incorrect (zero). It was scored into either inadequately done (less than 75%) or adequately done (75% or more).
- 4- Nurses opinionnaire sheet about sources of information which used in triage decision making (pre – test).
- 5- Trauma patients clinical data sheet. It was used to obtain the following:
 - Characteristics of trauma patients (adult and children) e.g age and gender.
 - Different injuries among studied patients (pre- test)
 - Levels of triage for the studied patients (pre-test)
 - Patients conditions outcomes (discharge home – discharge against medical advice – transferred to ward – death) (post test).

Validity and reliability of study tools :

Content validity was ascertained by a group of experts including medical–surgical nursing, General surgery and nursing administration. Their opinions were elicited regarding to the tools format layout, consistency, scoring system. The tools content were tested regarding to the knowledge accuracy, relevance and competence. Reliability of all items of an observation checklist was done using test – retest that revealed that, all items were significant and has a correlation coefficient above the significance level ($r= 0.9$).

Ethical considerations and human rights:

In the planning stage approval was obtained from authorities in El-Demerdash Hospital and Children Hospital. Verbal consent was then obtained from the directors of the above mentioned settings and the head nurses. All nurses were informed about the procedure and their rights according to medical research ethics, that they were free to decide whether or not they would participate in the study. Then a written informed consent was obtained from each nurse who agreed to participate in the study.

Pilot study:

A pilot trial was carried out on 10% of the total study sample to test the clarity and practicability of the tools, in addition to subjects and settings. Pilot subjects were later included in the study as there were no radical modifications in the study tools.

Procedure of the study:

- Sampling was started and completed within 6 months.
- Purpose of the study was simply explained to studied nurses prior to any data collection.
- The researcher started to collect data from first time on the same day of trauma patients admission to emergency department, using the pre – constructed tools.
- Questionnaire sheet regarding trauma triage knowledge, assessment sheet about the factors effecting triage process, added to opinionnaire sheet about sources of information used in triage decision making were filled in and completed by the studied nurses while they were on duty.
- An observation checklist for trauma triage practice and common pitfalls in triage process were filled in and completed by the researchers while they were observe nurses on duty hours - Clinical data sheet for trauma patients were filled in by the researcher from first moment of patients arrival at Hospital .
- The researchers were available two days / week at morning and afternoon shifts on the emergency department.
- The training was designed based on analysis of the actual educational needs assessment pre training by using the pre-constructed tools and consistent with related literature. In addition, meeting nurses level of understanding.
- Beginning of training included classification of the studied nurses into groups, each group consisted of 5-6 nurses, then orientation of the nurses about training objectives, outline, schedule, expected outcomes and benefits.
- Conduction of theoretical part was preformed through lectures and group discussions using data show and pictures as media. It was taken in 8 hours for 4 sessions, which were covered on two weeks. The sessions covered the following items: definition of triage, primary assessment, site and level of triage, life saving measures, secondary assessment and nursing intervention.
- Conduction of practical part began at previously mentioned settings whereas each group obtained 4 sessions (one session weekly for 3 hours). First session: primary assessment. Second session: life saving measures e.g (CPR, shock, choking, bleeding and wounds trauma). Third session: seconsery assessment. Fourth session: nursing

intervention. It was performed through demonstration, redemonstration, role play, simulator manikin, real objects, first aid kits and orthopedic supplies.

- Evaluation of the training was done through pre / post and 3 months follow -up tests using previous tools to measure the change in emergency nurses knowledge and practice and reduction of common pitfalls in triage procedure among nurses. The researchers were rotated on morning and afternoon shifts to evaluate nurses.

Statistical analysis:

Data were presented using numbers, percentages and chi-square test. Level of significance was threshold at 0.05.

3. Results:

Table (1): Presents demographic characteristics of the studied nurses and patients. Results revealed that more than one third of the studied nurse (adults and pediatric) had the age from 35- < 45 years (35.5% and 31.0% respectively). Moreover, nearly two fifths of them had diploma degree (42.5% and 41.0% respectively). Insignificant differences were indicated between adults and pediatric nurses regarding to their age, qualifications and years of experience with $X^2 = 2.12, 4.4$ and 0.06 respectively, $P > 0.05$. As regards characteristics of the studied trauma patients, less than one fifth of adults (16.0) had the age from 50.0 yrs and more. In addition, more than two third of children and adolescent (71.5) had the age from 12 –< 20 yrs.

Table (2): Reveals frequency distribution of trauma patients with different injuries. As shown, there is insignificant difference between the studied adults and children with different trauma regarding to their triage, with ($P > 0.05$). Meanwhile, for shocking, significant difference was found ($P < 0.01$).

Figure (1): Shows different levels of trauma patients triage. There is insignificant difference between the studied subjects (adults and children) in relation to triage levels, whereas level I ($X^2 = 2.0$), level II ($X^2 = 0.06$), level III ($X^2 = 0.96$), level IV ($X^2 = 0.27$), $P > 0.05$.

Table (3): Presents sources of information used in triage decision making among studied nurses. As shown insignificant differences was found between adults and pediatric nurses regarding to the sources of information, with ($X^2 = 0.02, p > 0.05$).

Table (4): Indicates the factors that influence trauma triage process. As obvious, insignificant difference was found between nurses (adults and pediatric) regarding the factors: interruptions ($X^2 = 0.015, p > 0.05$), time constraints ($X^2 = 0.16, P > 0.05$) and lack of formal training ($X^2 = 0.14, p > 0.05$).

Table (5): Presents potential errors in trauma triage among emergency nurses pre/post training. There is statistically significant difference between nurses errors in all items of triage post training with $X^2 = 24.4, P < 0.001$. Meanwhile insignificant difference was found in follow up test, with $X^2 = 9.0, p > 0.05$.

Table (6): Displays satisfactory nurse's knowledge regarding trauma triage pre/post training. As found the percent of all items was high in immediate post- Test and the differences between scores were significant in all items ($X^2 = 91.7, p < 0.001$). Meanwhile, in follow up test no statistically significant difference was indicated with $X^2 = 2.03, P > 0.05$.

Table (7): Shows satisfactory nurses' practice regarding trauma triage pre/post training. There is statistically significant difference between nurses practice in all items post training with $X^2 = 18.7, P < 0.001$. Regarding follow up test no significant difference was found, with ($X^2 = 0.11, p > 0.05$).

Table (8): Shows satisfactory nurses' intervention regarding assessment indicators pre/post training. As noticed significant improvement was found post training with $X^2 = 50.4, P < 0.001$. Meanwhile, in follow up test no significant difference was indicated with $X^2 = 0.82, p > 0.05$.

Figure (2): presents trauma patient's discharge and outcomes. As observed percentage of home discharge was higher among children (31.0%) than adults (24.0%). Meanwhile percentage of children death was lower (10.0%) than adult (15.0%).

Table (1a): Characteristics of the studied nurses (n = 40).

Items	Adult's nurses (n= 22)	Pediatric nurses (n = 18)	Test
Age/yrs			
20 - < 30	43.0	38.5	$X^2 =$
30 - < 40	35.5	31.0	2.12
40 & more	21.5	30.5	$P >$
			0.05
Qualification			
Diploma	42.5	41.0	$X^2 =$
Diploma with speciality	10.0	19.5	4.4
Bachelor	36.0	27.5	$P >$
Master degree	11.5	12.0	0.05
Years of experience			
5 - < 10	69.5	71.0	$X^2 =$
10 & more	30.5	29.0	0.06
			$P >$
			0.05

Table (1b): Characteristics of the studied trauma patients

Items	Adults (n = 100)	Children (n = 100)
Age / yrs		
6 - < 12		28.5
12 - < 20		71.5
20 < 30	34.5	
30 - < 40	28.0	
40 - < 50	21.5	
50 & more	16.0	
Gender		
Boys		61.5
Girls		38.5
Male	66.5	
Female	33.5	
Ways of transportation		
Ambulance	64.0	57.0
Private	36.0	43.0

Table (2): Distribution of trauma patients according to their injuries (n = 200)

Items	Adults n = 100	Children n = 100	X ² value
- Head injury	25.1	34.2	2.0*
- Shocking	31.0	48.0	6.0**
- Electric shock	9.3	6.0	0.7*
- Bleeding	46.0	44.0	0.3*
- Chest injury	12.2	7.0	1.5*
- Extremity, bone, joint and tissue injury	67.0	75.3	1.7*
- Back pain	23.1	14.2	2.7*
- Abdominal injury/pain	54.0	47.0	1.6*
- Ear injury	11.0	9.0	0.7*
- Ear ringing	8.2	6.1	0.6*
- Wounds	77.0	83.0	1.2*

* Insignificant at P > 0.05

** Significant at P < 0.01

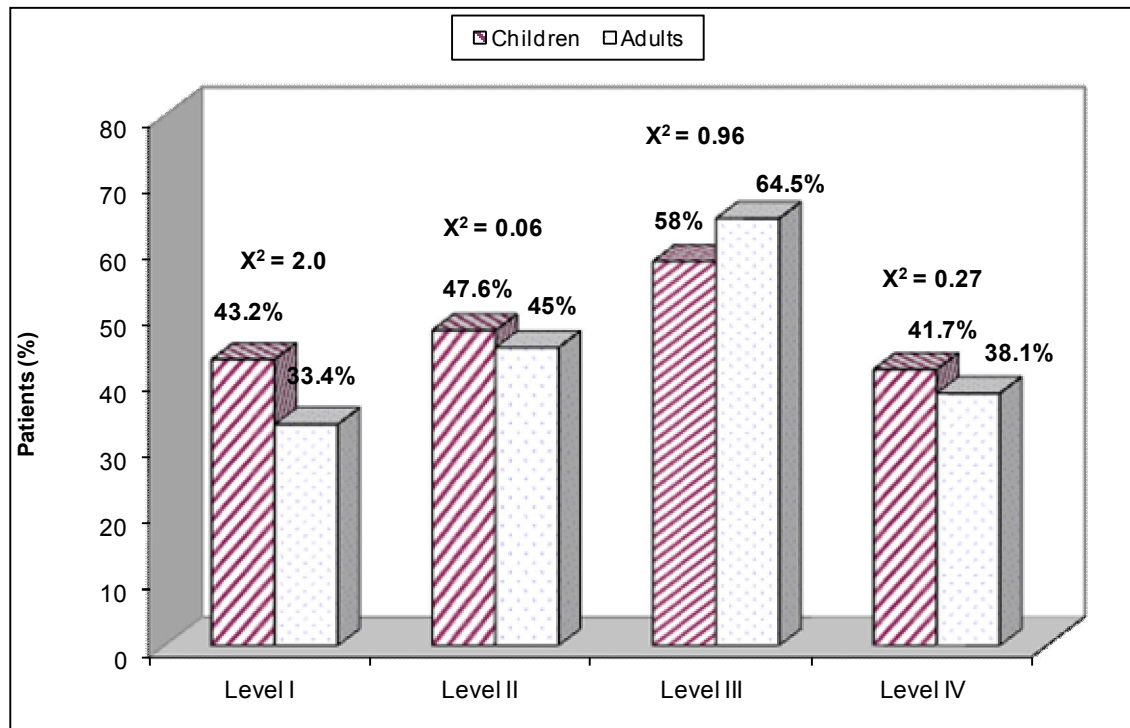


Figure (1): Shows different levels of triage among the studied trauma patients.

* Insignificant, P > 0.05.

Level II (evaluated within 20 minutes).

Level IV (non urgent).

Level I (evaluated immediately).

Level III (evaluated within 2 hours).

Table (3): Sources of information used in triage decision making among studied nurses

Items	Adults' nurses n = 22	Pediatric nurses n = 18	X ² value
- Clinical experience	45.2	41.0	0.52*
-Pre – hospital personnel and patient	9.0	7.5	0.67*
-Intuition	15.4	12.2	0.44*
- Triage guidelines	9.3	8.1	0.05*
- Pre – established triage criteria	12.6	10.4	0.25*
- Physicians verbal instructions	35.1	42.2	1.1*
X ² = 0.02 *, P > 0.05			

* Insignificant at P > 0.05.

Table (4): Factors that influence triage process among the studied nurses

Items	Adults' nurses n = 22	Pediatric nurses n = 18	X ² value
Interruptions	44.0	41.0	0.015*
- Other patients enquiries			
- Sudden case occurring in waiting hall	76.0	74.0	
- Arrival of new patients.	82.0	79.0	0.16*
Time constraints	86.0	84.0	
Lack of formal training	67.0 69.0		0.14*
- Poor assessment skills			
- Medical knowledge not updated	71.0	68.0	
- No constructive feedback and advice from colleagues	84.0	81.0	

* Insignificant at P > 0.05.

Table (5): Potential errors in trauma triage pre/post training among studied nurses (n=40)

Items	Pre %	Post %	Follow-up %
- Incorrect assessment	65.1	59.7	49.0
- Failure to diagnose	46.7	38.3	34.7
- Inappropriate discharge	68.3	65.0	62.3
- Incomplete or poorly documented record	92.4	79.1	68.6
- Failure to comply with standard of care	56.1 74.2	42.4 68.3	38.4 66.1
- Long waiting time			
1X ² = 24.4 P < 0.001		2X ² = 9.0, P > 0.05	

1X² (Between pre and post)

2X² (Between post and follow-up)

Table (6): Satisfactory nurses' knowledge regarding trauma triage pre/post training (n = 40).

Items	Pre %	Post %	Follow-up %
- Definition of triage	42.1	100.0	100.0
- Level and tag of triage	12.3	94.1	89.0
- Site of triage	33.0	100.0	100.0
- Primary assessment	56.1	91.2	88.1
- Life saving measures	36.2	100.0	100.0
- Secondary assessment	18.4	95.1	93.0
- Nursing intervention	14.5	89.1	87.2
1X ² = 91.7 P < 0.001		2X ² = 2.03 P > 0.05	

1X² (Between pre and post).

2X² (Between post and follow-up).

Table (7): Satisfactory nurses' practice regarding trauma triage pre/post training (n = 40).

Items	Pre %	Post %	Follow-up %
- Primary assessment	53.6	84.1	81.7
- Life saving measures	76.1	91.2	89.0
-Secondary assessment	22.3	66.7	65.1
- Nursing intervention	64.7	88.4	87.0
1X ² 18.7 P < 0.001		2X ² = 0.11 P > 0.05	

1X² (Between pre and post).

2X² (Between post and follow-up).

Table (8): Presentation of satisfactory nurses' intervention regarding assessment indicators (n = 40).

Items	Pre %	Post %	Follow-up %
- Airway/cervical spine	42.1	87.2	82.0
- Breathing	51.3	91.3	88.1
- Circulation	46.4	89.5	86.3
- Disability	35.6	92.1	87.4
- Level of Conscious	44.7	93.2	89.1
1X ² = 50.4 P < 0.001		2X ² = 0.82 P > 0.05	

1X² (Between pre and post).

2X² (Between post and follow-up).

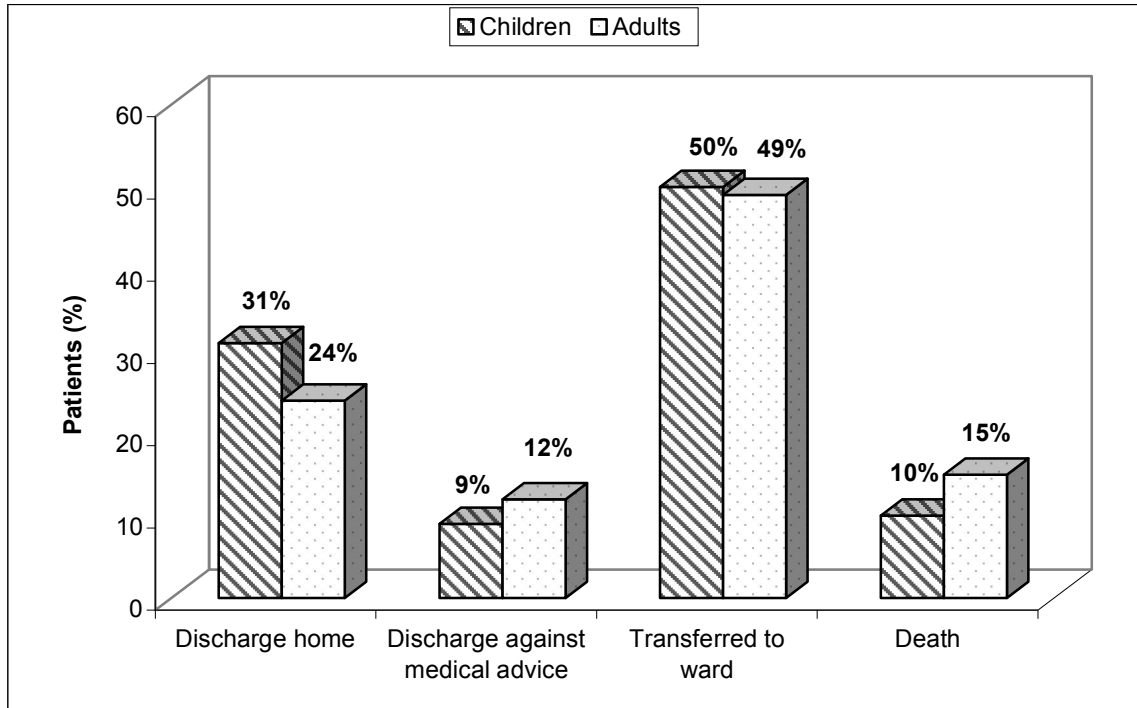


Figure (2): Presentation of studied trauma patients' discharge and outcomes

4. Discussion:

Triage is an important tool to determine and classify the clinical priority of the patients presenting at emergency departments (Lee, 2010 and Lerner et al, 2010). Discussion within this research highlights the effects of nursing staff development on avoiding pitfalls in trauma triage. The current study revealed that, nearly two fifths of the studied emergency nurses had diploma degree. The previous finding indicated that, nurses had inadequate information about trauma triage. Hegazy et al. (2010) reported that education has a vital role in improving nurses' knowledge and consequently improve the quality of care.

Concerning years of experience for studied nurses, results presented that more than two third of them were less than ten years of experience. This finding could be attributed to the fact that emergency department need more effect and healthy personnel to work hard. Moreover, older nurses got the feeling and beliefs that they had enough experience which makes them efficient in their performance, another interpretation is that older nurses had administrative role so, they faraway from practical field. The previous finding was in accordance with Abd El Sattar & Hegazy (2002) and Daleen (2010).

On the same line, more than half of the studied patients were transported by ambulance. According to Gautschi et al (2008) it is important that in the pre-hospital phase and during transportation, trained

paramedical personnel should be able to identify indicators of danger and assess overall status of patient and planning for appropriate mode of transport.

Regarding emergency nurses sources of information used in triage decision making. Results revealed the sources were included clinical experience physicians' verbal instruction. The previous result was in agreement with Durand et al. (2011) who claimed that, all nurses had no knowledge before joining on the emergency department about triage, because they were working after graduation in different units, which led them acquiring general knowledge not specific to trauma triage.

Concerning emergency nurses' knowledge and practices about trauma triage pitfalls, the present study showed significant improvement post training sessions. The previous findings may be due to lack of training and absence of triage nurse. Cone et al. (2009) and Timothy Lant & Megan Jehn (2011) reported that, triage is the most fundamental care and emergency nurse require a broad knowledge base to provide safe and competent care to trauma patients with a variety of injuries.

As regards the follow up tests, slight reduction was noticed in nurses' knowledge and practice. This results may be explained as, presence of the researchers' contact with the nurses for any guidance or clinical demonstrations. The previous results were

in agreement with *Abd El Sattar and Hegazy (2002)*, who recognized that numerous researches and articles concerned with knowledge and skills retention supported the promise that more frequent training was required. In addition, initial review is recommended as early as 2-4 weeks after training, then periodic reviews every 3-6 months until retraining at one year.

In relation to the potential errors in trauma triage among studied nurses. The present study indicated that they were: incorrect assessment, failure to diagnose, inappropriate discharge, poor documentation, failure to comply with standard of care and long waiting time. *Ventolini and Neiger (2003)* stated that, to determine seriousness of each patient's problem, the assessment must be conducted by a suitably educated first level nurse. *Forsgren et al (2009)* and *Simonet (2009)* recognized that, basic principles that run through a good emergency plan, are that nurses should perform best especially under stressful situations.

Considering, long waiting time which may result in serious consequences. *Rutschmann et al. (2005)* and *Lehmann et al. (2009)* concluded that, the number of interruptions and length of time patients wait in pretreated period have been significantly correlated in one study of triage implementation. According to *Thompson & Dowding (2004)* and *Amram et al. (2011)*, trauma triage in the emergency department help to decrease waiting times, improve patient satisfaction, make more efficient use of physician time, concentrate urgent medical care, and streamline traffic.

In the same context, lack of formal training in triage leads to error. It has been shown that experienced emergency nurse misclassify about 10% to 20% of patients. The error rate for untrained nurses is higher. *Johansen and Forberg (2011)* stated that, in the emergency room it is important for nurses to make fast, accurate decisions about the seriousness and urgency of the patient. Moreover, In experienced nursing and medical staff who do not use a formal method for assessing urgency will have error rates somewhere.

Regarding trauma patients' discharge and outcomes, this study revealed that children have a better prognosis than adults. *Lehmann et al. (2009)* reported that children tolerate multiple organ injuries better than adults, have unique physiologic and anatomic characteristics and differ from adults in several ways. *Fuzak and Mahar (2009)* claimed that, children have more pliant and flexible bones, so have fewer bone fractures. In addition, they have less mature regulatory mechanism and higher surface area to mass ratio which make heat loss and hypothermia more common in them according to *Kyle (2008)*,

Children may tolerate hypovolemic stress better than adult, added to their separation from parents which cause emotional trauma .

Conclusion:

Overall, the study has indicated that, nursing staff development through training sessions in the surgical emergency department was helpful on avoiding the pitfalls in trauma triage.

Recommendations:

- The study reinforces the need for sustained education and training for triage implementation with any advance in nursing practice for emergency nurses.
- Revision of job description and role specification should be made.
- Specific protocols for trauma patients should be tailored to their needs.
- Further studies should be done to assess the long term effects of such study on trauma patient's outcome at the emergency department, added to triage intervention by emergency nurses.

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Corresponding author

Soad M. Hegazy
Medical–Surgical Nursing Department¹, Pediatric Nursing Department², Faculty of Nursing, Ain shams University
soadmahmoud43@yahoo.com

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Adsorptive features of polyacrylamide- bentonite and zeolite composites for Cs⁺ and Co²⁺

M. I. El- Dessouky¹, E. H. El- Masry^{*1}, H. S. Hassan¹ and M. F. El- Shahat²

¹Hot Labs, Atomic Energy Authority Inshas, Kaliobia,

²Faculty of Science, Chemistry Dept., Ain Shams University, Cairo, Egypt

*emanelmasry74@yahoo.co.uk

Abstract: Polyacrylamide- Bentonite and polyacrylamide- Zeolite composites were synthesized and evaluated, as ion exchangers, for the removal of cesium and cobalt ions from aqueous solutions in batch operations. Batch experiments were carried out as a function of pH, initial ion concentration and temperature. Simple kinetic and thermodynamic models have been applied to the rate and isotherm sorption data and the relevant kinetic and thermodynamic parameters were determined from the graphical presentation of these models.

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Key Words: Ion exchange/ Cesium and Cobalt ions / polyacrylamide/ Zeolite/ Bentonite.

1. Introduction

There are a number of liquid processes and waste streams at nuclear facilities that require treatment for process chemistry control reasons and/or the removal of radioactive contaminants. Cesium, cobalt and strontium are the most abundant radionuclides in nuclear fission products that are routinely or accidentally released. They have relatively long half-life of about 30 years and are considered as hazardous elements for the environment. Different techniques such as chemical precipitation, ion exchange, and evaporation are used for the treatment of aqueous waste solutions containing these ions. Ion exchange technique has become one of the most commonly used treatment methods for hazardous and radioactive aqueous streams due to its simplicity, selectivity and efficiency. A wide range of materials having different chemical and physical properties, which can be naturally occurring or synthetic, is available for this technique. Inorganic ion exchange materials have emerged as an increasingly important replacement or complement for conventional organic ion exchange resins, particularly in liquid radioactive waste treatment due to their radiation stability and greater selectivity for certain radiological important species, such as cesium, cobalt and strontium. In the last decade, the natural clay minerals, such as montmorillonite (MMT), kaolinite⁽¹⁾, and palygorskite (attapulgite)⁽²⁾, are widely used in catalysis^(3,4), as adsorbents⁽⁵⁻¹⁴⁾, in nanocomposites⁽¹⁵⁻²⁰⁾, in sensors⁽²¹⁾, electrode^(22,24), as antibacterial materials⁽²⁵⁾ and in nuclear waste treatment and storage⁽²⁶⁻²⁹⁾. Nowadays, surface modification of clay minerals has become increasingly important for improving the practical applications of clays and clay minerals⁽³⁰⁾, Surface modification by polymers is found to be one of the

most effective methods, as the surface properties can be widely changed by a variety of functional polymers. The present work deals with the preparation and characterization of Polyacrylamide-Bentonite and Polyacrylamide- Zeolite composites and a series of experiments to assess the utility of these prepared composites for the removal of Cs⁺ and Co²⁺ ions from aqueous solutions under batch conditions. The relevant data, with respect to kinetic and equilibrium of the sorption and exchange of Cs⁺ and Co²⁺ ions, have been obtained using simple kinetic and thermodynamic models.

2. Experimental

2.1. Chemicals and reagents

All the reagents used in this work were of AR grade chemicals and were used without further purification. Cesium and Cobalt were supplied as cesium chloride and, Cobalt chloride, from Sigma-Aldrich Company. Stock solutions of the test reagents were prepared by dissolving CsCl and CoCl₂.6H₂O in distilled water.

2.2. Preparation

Includes the preparation of polyacrylamide, polyacrylamide- Bentonite and polyacrylamide-Zeolite composites. The preparation of polyacrylamide was carried out using Gamma radiation- initiated polymerization of acrylamide monomer in aqueous solution at a monomer concentration of 10% and a radiation dose of 10 KGy/hr. the prepared polyacrylamide gel was used to prepare the Bentonite and Zeolite composites at the same radiation dose.

2.3. Characterization

The crystallinity and functional groups of the

prepared material were investigated using X-Ray Diffraction (XRD), Fourier transformed infrared spectroscopy (FT-IR) and thermal analysis.

2.4. Batch sorption studies

Batch experiments were performed under kinetic and equilibrium conditions. To determine the pH range at which the maximum uptake of Cs⁺ and Co²⁺ ions would take place on the prepared composites. A series of 50 mL test tubes each containing 0.1 g of composite was filled with 10 mL of a desired concentration (10⁻⁴M). The initial pH was adjusted to values ranging from 2.0 to 8.0 using dilute solution of hydrochloric acid or Ammonia solution. The tubes were shaken for three hours to attain equilibrium. Preliminary investigations showed that the sorption process of each studied ion was completed after two hours. The suspension obtained was centrifuged to separate the solid from the liquid phase. The clear liquid phases obtained were used for the elemental analysis using Atomic Absorption Spectrophotometer (Buck scientific model VGP 210).

2.4.1. Kinetic studies

Kinetic studies were performed at three different temperatures (298, 313 and 333° K) for Cs⁺ and Co²⁺ ions at an initial ion concentration of 0.01 M. For these investigations, 0.1 g of polyacrylamide-bentonite and polyacrylamide- Zeolite composite was contacted with 10 mL solution containing known concentration of Cs⁺ and Co²⁺ ions and the solution in the vial was kept stirred in a thermostat shaker adjusted at the desired temperature. A fixed volume (2 mL) of the aliquot was withdrawn as a function of time while the solution was being continuously stirred. Thus, the ratio of the volume of solution to the weight of the composite in the vial does not change from the original ratio. The withdrawn solution was centrifuged to separate the composite and a fixed volume (1 mL) of the clear solution was pipetted out for the determination of the amount of unadsorbed metal ion still present in solution. The percent uptake was calculated using:

$$\% \text{ uptake} = \frac{(C_o - C_t)}{C_o} \times 100 \quad (1)$$

Where C_o and C_t are the initial and equilibrium concentrations (mmol/L) of metal ion in solution.

2.4.2. Sorption equilibrium studies

In the experiments of sorption isotherm measurements, 10 mL of the metal ion solution of varying concentrations (10⁻⁴ to 5x10⁻² M) were agitated with 0.1 g of the polyacrylamide- bentonite or Zeolite composite at different temperatures (298,

313, and 333° K) and at initial

PH of 6.0. After the established contact time was reached, aliquots of supernatants were withdrawn and the amount of the metal ion retained in the composite phase (mg/g) was estimated. All batch experiments were carried out in duplicate and the mean values are presented. The metal concentration retained in the solid phase was calculated using the following equation:

$$q_e = \frac{(C_o - C_e)V}{M} \quad (2)$$

Where, C_o and C_e are the initial and equilibrium concentrations of metal ion in solution (mg/l),

V is the solution volume (l), and

M is the weight of the solid (g).

3. Results and Discussion

3.1. Characterization of the prepared materials

The FT-IR spectra of the composites were compared in Fig (1). Broad and sharp appearance at 3200–3600 cm⁻¹ in the FT-IR spectra were of the bonded and free O-H stretches. The band at 1000-1700 cm⁻¹ and band at 400-700 cm⁻¹ corresponding to silicates. Bands at 1700, 3200cm⁻¹ are for C=O from amide group. Band at 2900 cm⁻¹ related to C-H group. Bands at 800 and 2500 cm⁻¹ are corresponding to Al-OH and Si-OH, respectively⁽³¹⁾. It was found that, from the XRD pattern of polyacrylamide- zeolite and polyacrylamide-bentonite composites shown in fig (2), both of the composites are amorphous. Strong interaction between zeolite, bentonite and monomer in polymeric chains during synthesis takes place and leads to absence of diffraction peaks⁽³²⁾. TGA and DTA curves show that the composites are thermal stable at temperature greater than 300°C.

3.2. Effect of pH

The effect of pH on the sorption removal of Cs⁺ and Co²⁺ ions from aqueous chloride solutions using prepared polyacrylamide- bentonite and polyacrylamide- Zeolite composite material was investigated over the pH range from 2.0 to 8.0. It was observed that each metal ion sorption process is dependent on the initial pH of the solution and the amount of metal ion sorbed increased with increase in the pH value. At lower values, the metal ion uptake was inhibited in this acidic medium and this can be attributed to the presence of H⁺ ions competing with the Cs⁺ and Co²⁺ ions for the sorption sites. The uptake continuously increases with the increase in pH value and the highest uptake was observed at pH range from 6.0 to 8.0. Also the increasing of the pH value decreases the degree of protonation of the amid and amino groups cross linking between polymeric chains which leads to an increase in the probability of interaction between composites and metal ions⁽³²⁾.

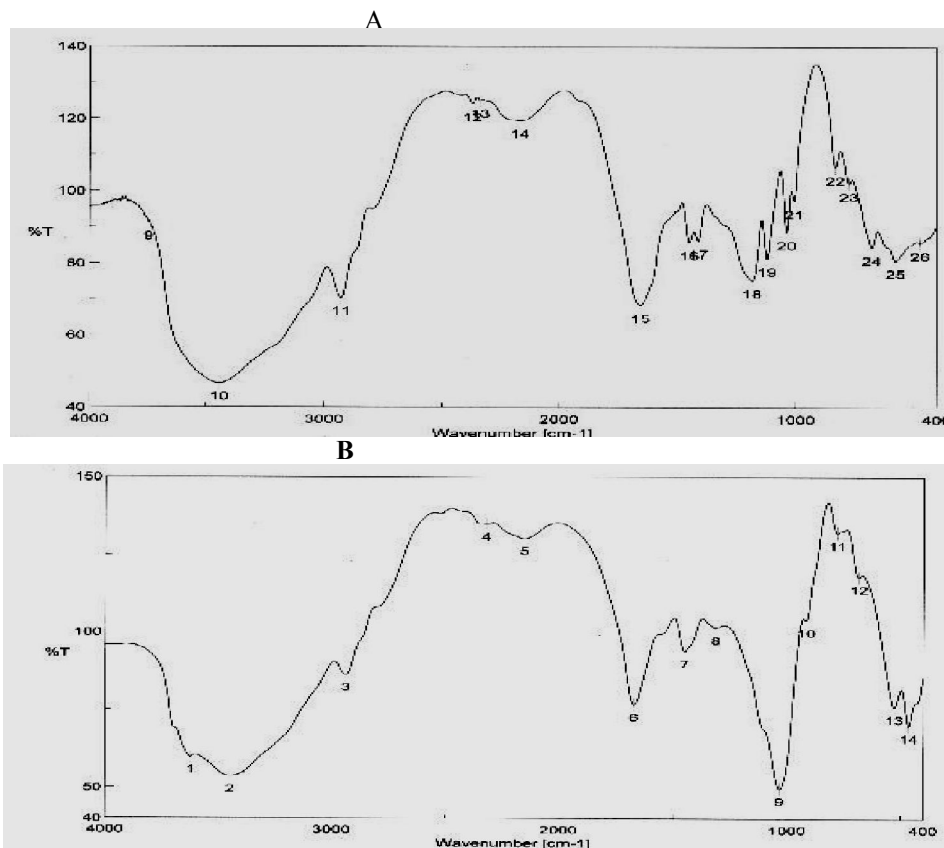


Fig (1): FTIR pattern of a) polyacrylamide- zeolite b) polyacrylamide- bentonite composite

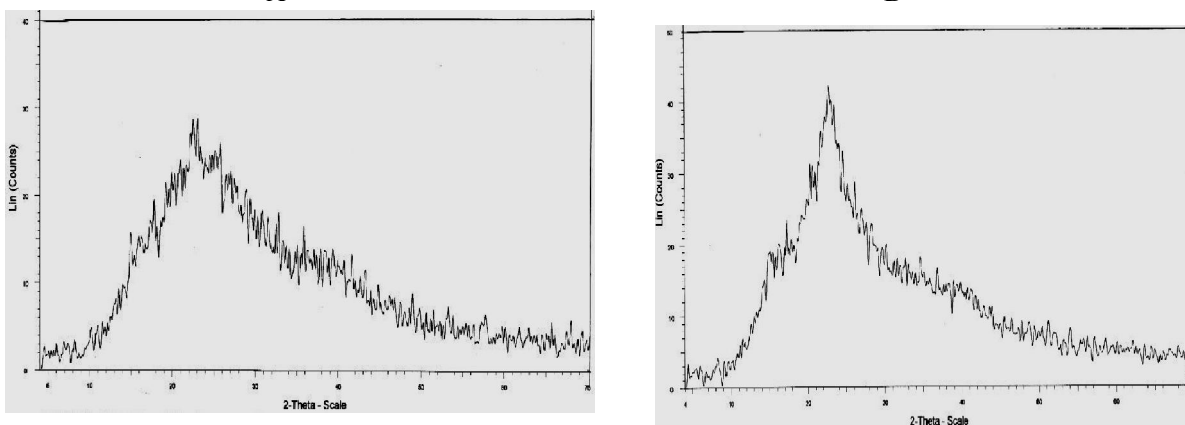


Fig (2): XRD pattern of a) polyacrylamide- zeolite b) polyacrylamide- bentonite composite

3.3. Effect of contact time

The variation of the amounts of Cs⁺ and Co²⁺ ions sorbed at different time intervals, for the fixed initial ion concentration of 0.01 M and at different sorption temperatures of 298, 313 and 333° K are represented in Figs (3, 4). From these figures it can be concluded that the amount of the sorbed Cs⁺ and Co²⁺ ions increases with the increase in temperature indicating an endothermic nature of the sorption processes, while the time required reaching

equilibrium remained practically unaffected .

3.4. Sorption kinetic

To describe the changes in the sorption of metal ions with time, three simple kinetic models were tested. The rate constant of each metal ion removal from the solution by polyacrylamide- bentonite and polyacrylamide- Zeolite composite was determined using pseudo first-order and pseudo second-order rate models.

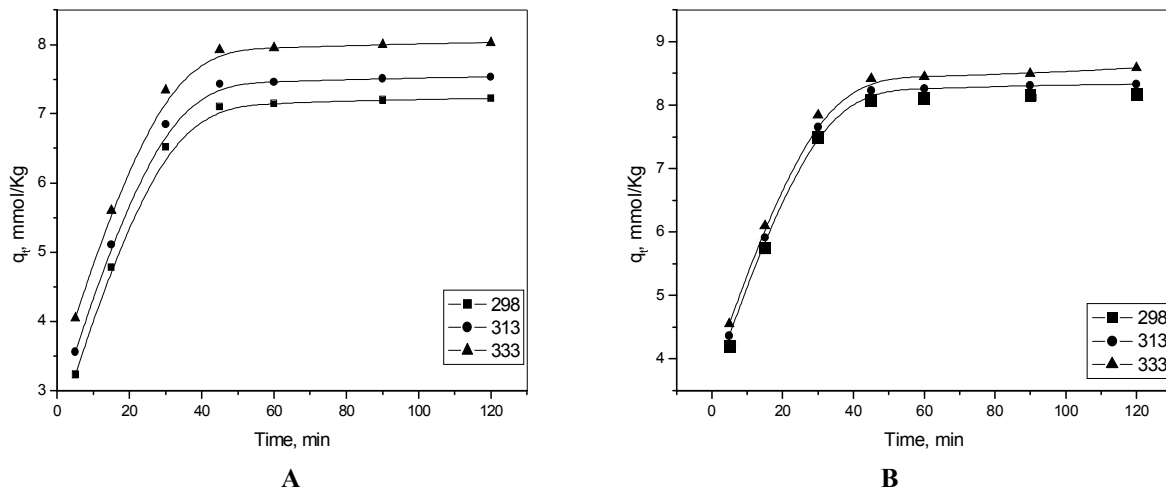


Fig. (3): Effect of contact time on sorption of a) Cs⁺ and b) Co²⁺ ions onto PAM- Zeolite composite at different temperatures.

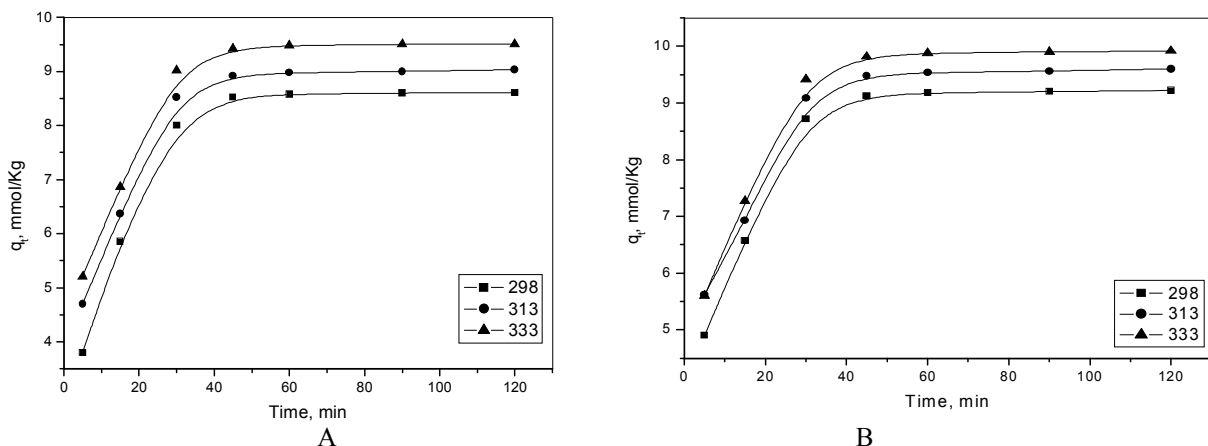


Fig (4): Effect of contact time on sorption of (a) Cs⁺ and (b) Co²⁺ ions onto PAM- Bentonite composite

The pseudo first order model

The sorption kinetics of metal ions from liquid phase to solid is considered as a reversible reaction with an equilibrium state being established between two phases. A simple pseudo first-order model⁽³³⁾ was therefore used to correlate the rate of reaction and expressed as follows:

$$\text{Log}(q_e - q_t) = \text{log}q_e - \frac{k_1}{2.303} t \quad (3)$$

where q_e and q_t are the concentrations of ion in the adsorbent at equilibrium and at time t , respectively (mmol/g) and k_1 is the pseudo first-order rate constant (min^{-1}). The sorption of metal ions was tested using the pseudo first order model, although the linear correlation coefficients of the plots are so good, the q_e (calculated) values are not in agreement with q_e (experimental) for all studied sorption processes. So, it could suggest that the sorption of Cs⁺ and Co²⁺ metal ions onto polyacrylamide- Zeolite and polyacrylamide- bentonite composites is not a first-order reaction. (Figures were omitted because

the model is not applicable).

The pseudo second order model

A pseudo second-order rate model⁽³⁴⁾ is also used to describe the kinetics of the sorption of ions onto adsorbent materials.

$$\frac{t}{q} = \frac{1}{K_2 q_e^2} + \frac{1}{q_e} t \quad (4)$$

Where k_2 is the rate constant of pseudo second-order equation. The q_e and K_2 values of the pseudo second-order kinetic model can be determined from the slope and the intercept of the plots of t/q versus t , respectively. The compatibility of experimental data to the second-order kinetics model was evaluated. The kinetic plots of t/q_t versus t for both Cs⁺ and Co²⁺ ions sorption at different temperatures are presented in Figs. (5, 6). The relation is linear, and the correlation coefficient (R^2), suggests a strong correlation between the parameters and also explains that the sorption process of each ion

follows pseudo second-order kinetics. The product k_2q_{e2} is the initial sorption rate represented as $h = k_2q_{e2}$. From Table (1), it can be shown that the values of the initial sorption rate (h) and rate constant (K_2) were increased with the increase in temperature. The correlation coefficient R^2 has an extremely high value

(>0.99), and its calculated equilibrium sorption capacity (q_e) is consistent with the experimental data. These results explain that the pseudo second order sorption mechanism is predominant and that the overall rate constant of each sorption process appears to be controlled by the chemical sorption process⁽³⁵⁾.

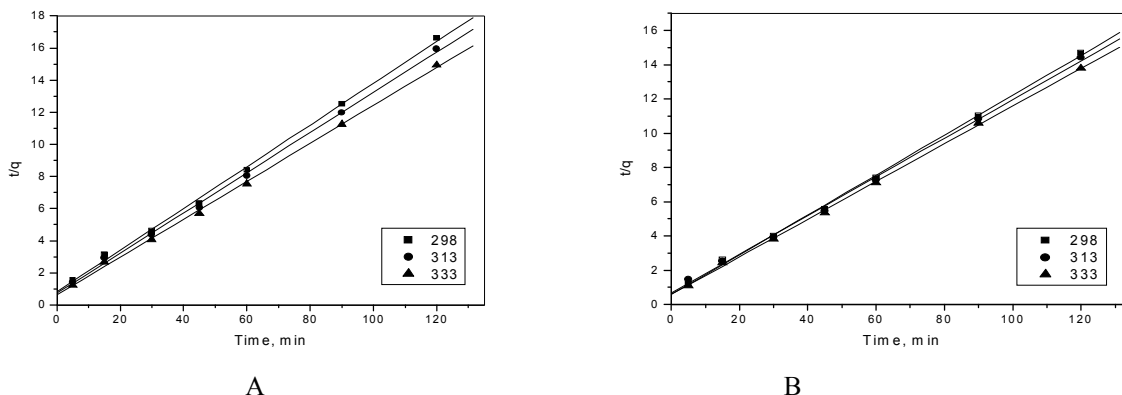


Fig.(5): Peseudo second order plots for a) Cs⁺ and b) Co²⁺ ions onto PAM- Zeolite composite at different temperatures.

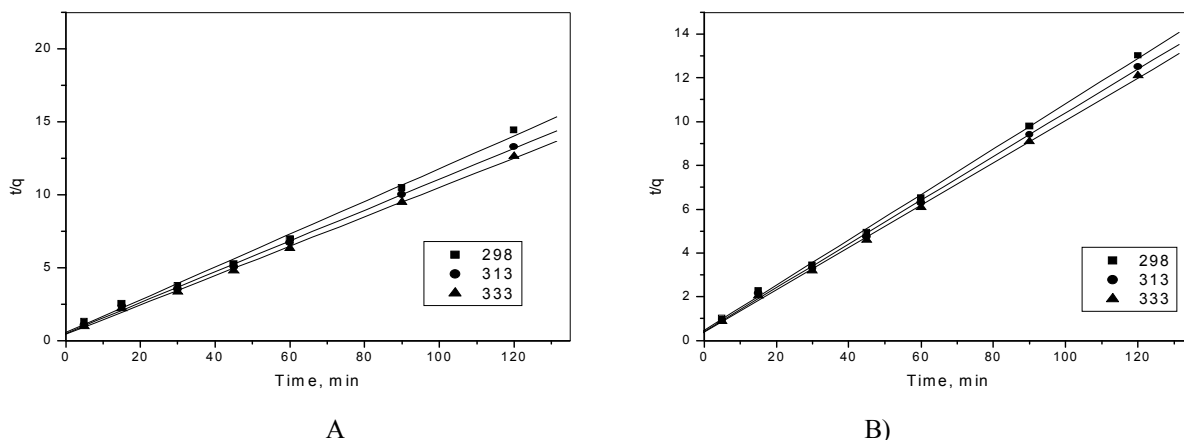


Fig.(6): Peseudo second order plots for a) Cs⁺ and b) Co²⁺ ions onto PAM- Bentonite composite at different temperatures.

Table (1): The calculated parameters of the pseudo second-order kinetic model for Cs⁺ and Co²⁺ ions sorbed onto PAM- Bentonite and PAM- Zeolite composites at diferent sorption temperatures

Metal ion	Temp., K	PAM- Zeolite			R^2	PAM- Bentonite			
		K_2 , g/mmol.min	q_e , calc. mmol/kg	q_e exp mmol/kg.		K_2 , g/mmol.min	q_e , calc. mmol/kg	q_e exp mmol/kg.	R^2
Cs ⁺	298	0.02	8.9	8.6	0.996	0.021	7.7	7.2	0.996
	313	0.02	9.5	9.0	0.998	0.022	8.0	7.51	0.998
	333	0.03	9.9	9.5	0.998	0.023	8.3	8.0	0.998
Co ⁺⁺	298	0.022	10.0	9.2	0.998	0.025	8.3	8.15	0.998
	313	0.023	10.1	9.56	0.998	0.02	9.1	8.31	0.997
	333	0.025	10.3	9.9	0.998	0.02	10.0	8.50	0.999

3.5. Sorption isotherms

Sorption equilibrium is usually described by an isotherm equation whose parameters express the

surface properties and affinity of the sorbent, at a fixed temperature and pH. An adsorption isotherm describes the relationship between the amount of

adsorbate on the adsorbent and the concentration of dissolved adsorbate in the liquid at equilibrium. In this concern, the sorption isotherms for removal of Cs^+ and Co^{2+} ions from aqueous solutions onto polymer composites at three different temperatures were determined as shown in Figures (7, 8). The isotherms are regular, positive. The convex isotherms at high concentrations indicated that sorption was reduced⁽³⁶⁾. The initial rapid sorption gives way to a slow approach to equilibrium at higher ion concentrations. These results reflect the efficiency of polymer for the removal of Cs^+ and Co^{2+} ions from aqueous solution in a wide range of concentrations. The uptake of ions increased with the increase in temperature thereby indicating the process to be endothermic.

3.6. Isotherm models

Freundlich Isotherm model

Freundlich equation is derived to model the multilayer sorption and for the sorption onto heterogeneous surfaces. The logarithmic form of Freundlich equation may be written as:

$$\log q_e = \log K_f + (1/n) \log C_e \quad (5)$$

where, q_e is the amount of metal ion sorbed per unit weight of sorbent (mmol/kg), C_e is the equilibrium concentration of the metal ion in the equilibrium solution (mmol/L), K is constant indicative of the

relative sorption capacity (mmol/kg) and $1/n$ is the constant indicative of the intensity of the sorption process. The pictorial illustration of $\log q_e$ vs. $\log C_e$ is shown in Figs. (9,10) which suggests that the sorption of Cs^+ and Co^{2+} ions obeys Freundlich isotherm over the entire range of sorption concentration studied. The numerical values of the constants $1/n$ and K_f are computed from the slope and the intercepts, by means of a linear least square fitting method, and also given in Table (2). It can be seen from these data that the Freundlich intensity constant (n) are greater than unity for all studied ions. This has physicochemical significance with reference to the qualitative characteristics of the isotherms, as well as to the interactions between metal ions species and the composite. In our case, $n > 1$ for all ion species, the composite shows an increase tendency for sorption with increasing solid phase concentration. This should be attributed to the fact that with progressive surface coverage of adsorbent, the attractive forces between the metal ion species such as van der Waals forces, increases more rapidly than the repulsive forces, exemplified by short-range electronic or long-range Coulombic dipole repulsion, and consequently, the metal ions manifest a stronger tendency to bind to the active sites of the compo site⁽³⁷⁾.

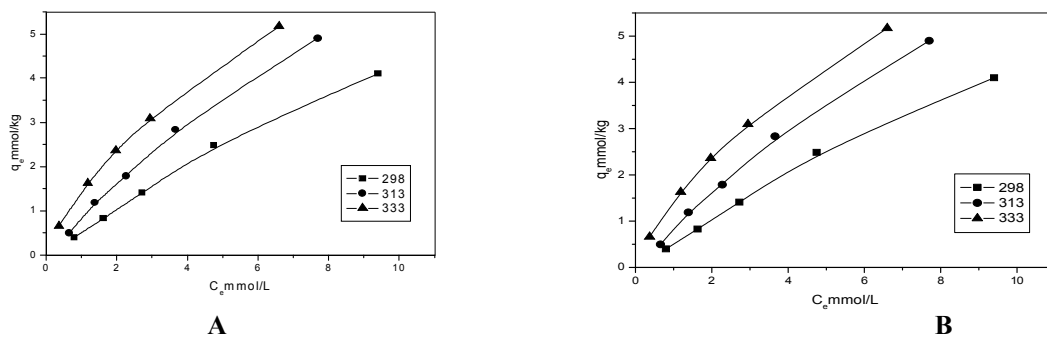


Fig (7): Sorption isotherms for a) Cs^+ and b) Co^{++} onto PAM- Zeolite composite at different temperatures.

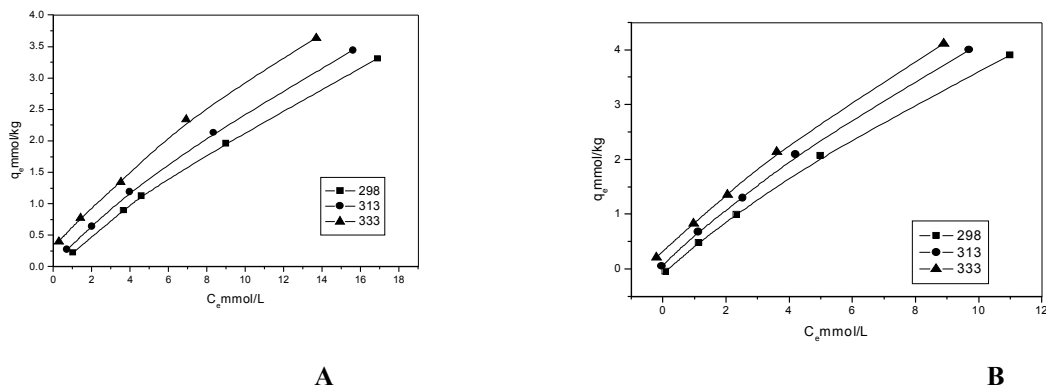


Fig (8): Sorption isotherms for a) Cs^+ and b) Co^{++} onto PAM- Bentonite composite at different temperatures.

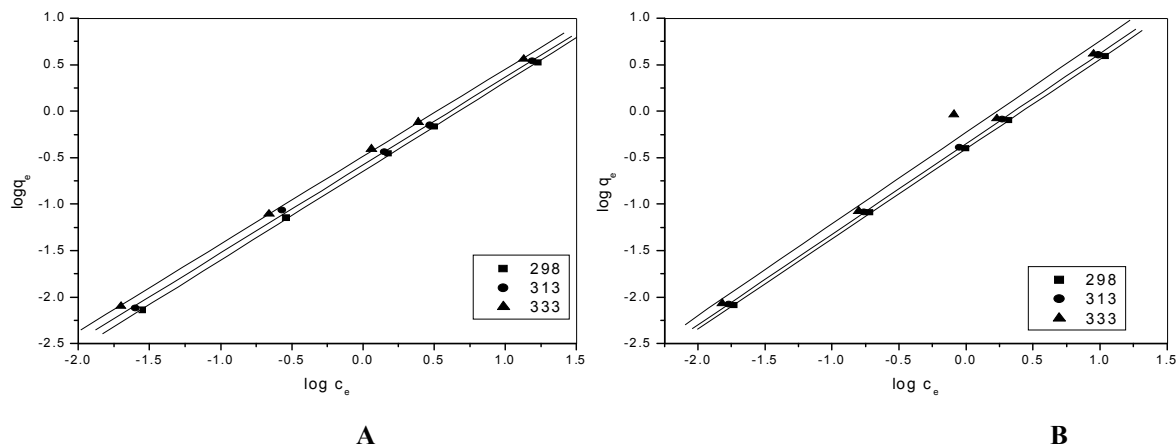


Fig.(9): Freundlich isotherm plots for the sorption of (a) Cs^+ and (b) Co^{2+} ions onto PAM- Zeolite composite at different temperatures

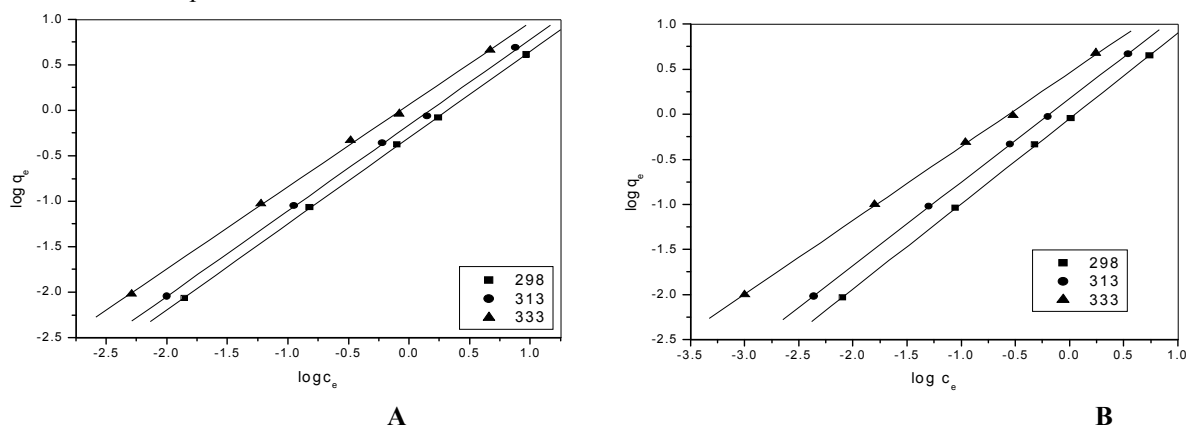


Fig. (10): Freundlich isotherm plots for the sorption of (a) Cs^+ and (b) Co^{2+} ions onto PAM- Bentonite composite at different temperatures

Langmuir Isotherm model

Langmuir sorption isotherm models the monolayer coverage of sorption surfaces and assumes that sorption occurs on a structurally homogeneous adsorbent and all the sorption sites are energetically identical. The linearized form of the Langmuir equation is given by the following formula:

$$\left(\frac{C_e}{q_e}\right) = \left(\frac{1}{Q_0 b}\right) + \left(\frac{1}{Q_0}\right)C_e \quad (6)$$

Where, q_e is the amount of metal ion sorbed per unit weight of adsorbent (mmol/g), C_e is the equilibrium concentration of the metal ion in the equilibrium solution (mmol/L), Q_0 is the monolayer adsorption capacity (mmol/g) and b is the constant related to the free energy of adsorption ($b \propto e^{-\Delta G/RT}$). Applying Langmuir isotherm by plotting C_e/q_e vs. C_e as shown in Figs (11, 12) for Cs and Co respectively straight lines are obtained for the three ions, confirming that this expression is indeed a reasonable representation of chemisorption isotherm. The Langmuir constants Q_0 and b for sorption of ions onto polymer, as showed in table (3), increased with temperature

showing that the sorption capacity and intensity of sorption are enhanced at higher temperatures. This increase in sorption capacity with temperature suggested that the active surfaces available for sorption have increased with temperature. One of the essential characteristics of the Langmuir model could be expressed by dimensionless constant called equilibrium parameters R_L ⁽³⁸⁾

$$R_L = 1 / (1 + b C_0) \quad (7)$$

where, C_0 is the highest initial metal ion concentration (mmol/L). The value of R_L indicates the type of isotherm to be irreversible ($R_L=0$), favorable ($0 < R_L < 1$), linear ($R_L=1$), or unfavorable ($R_L > 1$). All the R_L values were found to be less than 1 and greater than 0 indicating the favorable sorption isotherms of all metal ions. High values of regression coefficients between the sorbate and sorbent systems for both Langmuir and Freundlich models (around 0.99) indicated the applicability of this resin system for Cs^+ and Co^{2+} removal in both monolayer sorption and heterogeneous surface conditions.

Table (2): Freundlich parameters for sorption of Cs⁺ and Co²⁺ onto PAM- Bentonite and PAM- Zeolite composites

Metal ion	Temp.,k	PAM- Bentonite			PAM- Zeolite		
		1/n	K,mmol/kg	R ²	1/n	K,mmol/kg	R ²
Cs	298	0.90	1.15	0.998	0.94	3.1	0.992
	313	0.94	1.58	0.998	0.95	3.8	0.992
	333	0.95	2.00	0.997	0.96	4.4	0.995
Co	298	0.82	1.12	0.991	0.97	1.66	0.996
	313	0.93	1.50	0.993	0.97	2.24	0.999
	333	0.95	2.57	0.998	0.98	2.9	0.998

Table (3): Langmuir parameters for sorption of Cs⁺ and Co²⁺ ions onto PAM- Bentonite and PAM- Zeolite composites.

Metal ion	Temp.,k	PAM- Bentonite				PAM- Zeolite			
		Q ₀ , mmol/kg	b, l/mmol	R ²	R _L	Q ₀ , mmol/kg	b, l/mmol	R ²	R _L
Cs	298	23.7	0.25	0.988	0.074	56.8	0.08	0.981	0.20
	313	27.1	0.44	0.988	0.043	76.9	0.16	0.982	0.11
	333	33.5	1.70	0.999	0.012	135.1	0.20	0.991	0.09
Co	298	44.8	0.50	0.991	0.040	104.2	0.09	0.979	0.18
	313	49.0	1.20	0.997	0.020	155.0	0.11	0.999	0.15

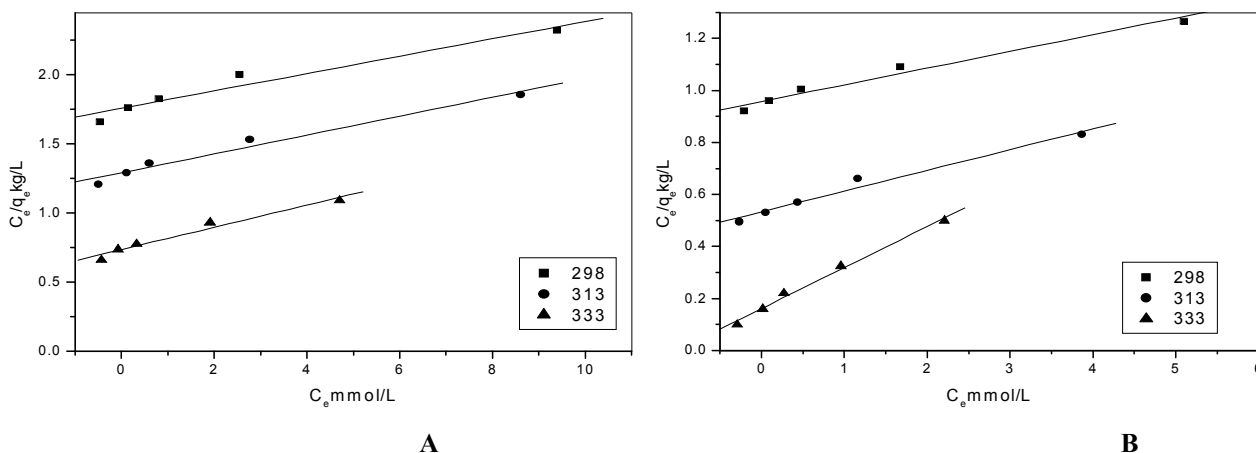


Fig (11): Langmuir isotherms for a) Cs⁺ and b) Co⁺ onto PAM- Zeolite composite at different temperatures.

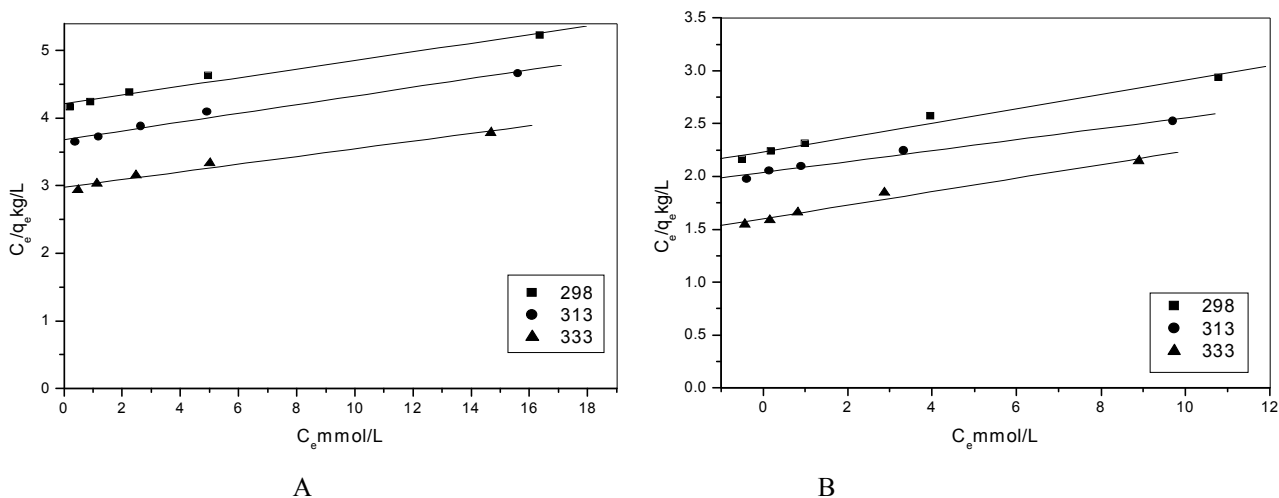


Fig (12): Langmuir isotherms for a) Cs⁺ and b) Co⁺ onto PAM- Bentonite composite at different temperatures.

3.7. Thermodynamic studies

To calculate the different thermodynamic parameters, Vant Hoff equation was used which is given as follow (39):

$$\Delta G = -2.303RT \log K_c$$

$$\text{therefore, } \log K_c = -\Delta G / 2.303R (1/T)$$

Therefore,

$$\log K_c = \frac{\Delta S}{2.303R} - \frac{\Delta H}{2.303R T} \quad (8)$$

ΔG : is the free energy, T : is the absolute temperature in Kelvin, R : is the general gas constant ($R=8.314 \text{ J/mol. K}$), ΔH : is the enthalpy change, ΔS : is the entropy change and K_c is the equilibrium

constant. The values of enthalpy change (ΔH) and entropy change (ΔS) calculated from the slope and intercept of the plot of $\log K_c$ versus $1/T$, shown in Fig (15), are also given in Tables (5, 6). The change in ΔH for all ions were found to be positive confirming the endothermic nature of the sorption processes. ΔS values were found to be positive due to the exchange of the metal ions with more mobile ions present on polyacrylamide- Zeolite and polyacrylamide-Bentonite composites towards Cs^+ , Co^{2+} and Sr^{2+} ions, which would cause increase in the entropy during the sorption process.

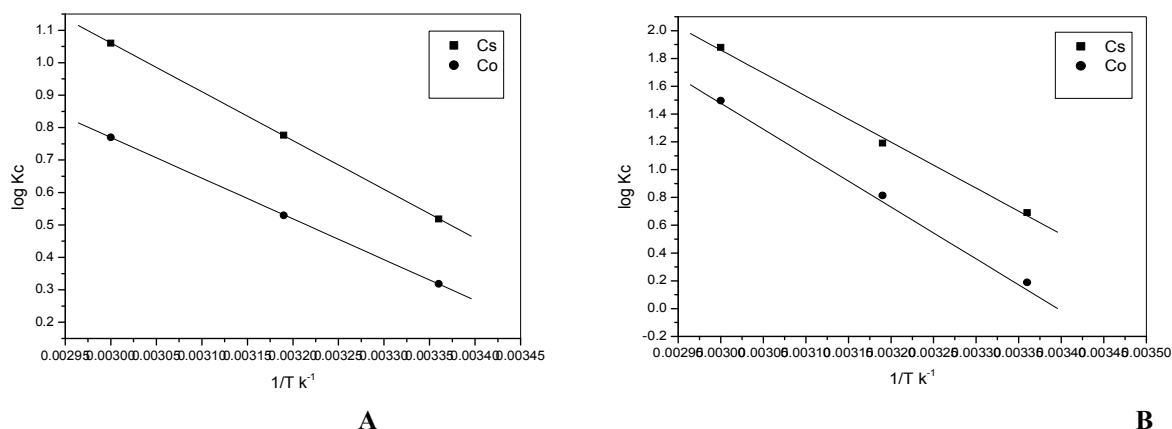


Fig (13): Vant Hoff plots for sorption of Cs^+ and Co^{2+} ions onto a) PAM- Zeolite, b) PAM- Bentonite at three different temperatures

Table (5): Values of the thermodynamic parameters for the sorption of Cs^+ and Co^{2+} ions onto PAM- Zeolite composite.

Temp, k	k_c		$\Delta G, \text{kJ/mol}$		$\Delta H, \text{kJ/mol}$		$\Delta S, \text{kJ/mol}$	
	Cs^+	Co^{2+}	Cs^+	Co^{2+}	Cs^+	Co^{2+}	Cs^+	Co^{2+}
298	2.71	1.50	-2.45	-1.03				
313	5.67	1.90	-4.49	-1.68	20.0	24.0	69.0	86.2
333	11.43	2.20	-6.76	-2.17				

Table (6): Values of the thermodynamic parameters for the sorption of Cs^+ and Co^{2+} ions onto PAM- Bentonite site

Temp, k	k_c		$\Delta G, \text{kJ/mol}$		$\Delta H, \text{kJ/mol}$		$\Delta S, \text{kJ/mol}$	
	Cs^+	Co^{2+}	Cs^+	Co^{2+}	Cs^+	Co^{2+}	Cs^+	Co^{2+}
298	4.90	1.17	-3.94	-0.40				
313	15.60	7.70	-7.13	-5.33	63.40	71.40	226	243
333	75.00	26.15	-12.00	-9.05				

4. Conclusion

In this study, the preparation and characterization of PAM-Zeolite and Bentonite composites and its comparative adsorptive features with bare Zeolite and Bentonite were investigated for Cs^+ and Co^{2+} removal. The FTIR and XRD analysis showed that PAM-Zeolite and Bentonite composites had a hybrid composition of PAM and Zeolite or Bentonite resembling colloidal dispersion of one solid phase in another. Sorption experiments were performed for the removal of cesium,

cobalt and strontium ions from their aqueous solutions using the two composites. The kinetic modelings suggest that, the pseudo second-order mechanism is predominant and that the overall rate constant of ions appears to be controlled by the chemisorption mechanism. Experimentally obtained isotherms were well compatible to Langmuir and Freundlich models, from which the derived parameters were, confirmed each other. Measurements of ΔG_0 are a negative value confirmed the adsorption reactions occurred

spontaneously at a given temperature.

Corresponding author

E. H. El- Masry

Hot Labs, Atomic Energy Authority Inshas, Kaliobia,
emanelmasry74@yahoo.co.uk

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An investigation on physical response of modified adhesives used in medical applications

Mohsen Gh Kharaji¹, Fakhroddin Alimoradi², Alireza Yekrangi³, Ameneh Langari*⁴

¹Shahrekord University of Medical Sciences, Shahrekord, Iran

²Civil Engineering Group, Chalooos Branch, Islamic Azad University, Chalooos, Iran

³Engineering Group, Ramsar Branch, Islamic Azad University, Ramsar, Iran

⁴North Khorasan University of Medical Sciences, Bojnurd, Iran

Corresponding author email: amenehlangari@yahoo.com

Abstract: Glues and adhesives are increasingly used in medical applications. They can be utilized in disposal medical devices, structural bonds, bone cement, prostheses and other applications. For improve the adhesion of these materials, the liquid Hycar modifier is added and mixed. However, despite liquid modifier might modify the adhesion properties of glues and adhesives, the physical properties of these materials might be deteriorated. In this paper the physical response i.e. thermal and mechanical behavior of the adhesive modified with liquid rubber has been investigated for medical applications. In addition, scanning electron microscopy (SEM) is utilized in this work for better understanding the behavior of adhesive.

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Keywords: Medical adhesive, HDT, Hycar, Physical properties.

1. Introduction

Recently, glues and adhesives are used in medical applications. Since the hardened, finished polymers are almost non-toxic, they can be used in many medical applications such as disposal medical devices, structural bonds, bone cement, prostheses, etc.. In figure (1) some medical applications of the cut-cure adhesives e.g. stopping bleeding, surgical operations are demonstrated. Each medical adhesive has its own benefits and limitations that should be matched with its appropriate application. The primary types of adhesives used in medical device applications includes Acrylics, Epoxies and Styrene polymers. Challenges for medical devices that require skin contact can often be reduced to a wrestling match between adhesion and irritation.

Adhesion, the attachment between glue and substrate may occur either by mechanical means, in which the glue works its way into small pores of the substrate, or by one of several chemical mechanisms (Ratna *et al.* 2000). The strength of glue depends on many factors. In some cases, an actual chemical bond occurs between adhesive and substrate (Takemura *et al.* 1985). In others, electrostatic forces, as in static electricity, hold the substances together. A third mechanism involves the molecular forces that develop between molecules. A fourth means involves the moisture-aided diffusion of the glue into the substrate, followed by hardening (Takemura *et al.* 1985; Ratna *et al.* 2000).

Thermal stability is very important for High-temperature applications of an adhesive material. In order to use these materials in high temperature

applications, the thermal properties of these materials should be evaluated (Takemura *et al.* 1985; Ratna *et al.* 2000; Ratna *et al.* 2001). In this work the Differential Scanning Calorimetry (DSC) is used to measure glass transition temperature (T_g) which is the temperature at which the adhesive sample become soft. Previous researchers studied the thermal behavior of materials (Jinen, 1988; Bhowmik *et al.* 1998; Xie *et al.* 2005; Ratna *et al.* 2001). The mechanical performance of the adhesive is another important factor for evaluating the performance of an adhesive. Several works shows that increasing liquid modifier to epoxy adhesive might reduce the mechanical properties of the adhesive (Rinde *et al.* 1980; Sanjana & Testa, 1985; Huang & Kinloch, 1992; Jang & Yang, 2000; Paul & Bucknall, 2000; Pearson *et al.* 2000; Chikhi *et al.* 2002; Gam, 2003; Atefi *et al.* 2012a; 2012b; Davoodi *et al.* 2012) Therefore one should not add a large amount of liquid rubber to epoxy adhesive system. In this regards investigators should investigate all aspects of physical behavior and artifacts of epoxy-based modified adhesive.

In this work an epoxy adhesive is studied and the modification of adhesion is preformed using Hycar modifier. This is mixed with epoxy before mixing with hardener. We focus on thermal behavior of the adhesion using DSC. The glass transition temperature of the formulations is measured in this study. Moreover we evaluated the mechanical behavior of the adhesion using Impact test and Flexural test. In addition, scanning electron microscopy (SEM) is utilized in this work for better understanding the

behavior of adhesive. The obtained data can be comprehensively useful for further manufacturer, investigators and industrial engineers who have investigated the physical performance of modified epoxy adhesives.

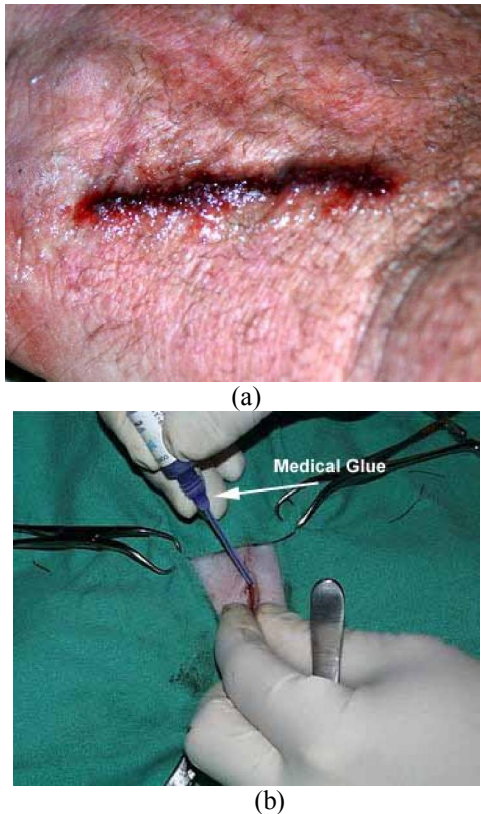


Figure 1. Use of adhesives in medical applications in (a) stopping bleeding and (b) surgical operation

2. Experimental

2.1 Materials

Two materials have been used: one is epoxy adhesive and the other is Hycar additive. Adhesive was made of two compounds: EPON828 epoxy resin and the Epikote DX6509 curing agent. These materials have been obtained from Shell Co. The adhesive is mixed in the ratio 80 part of EPON828 with 20 part of Epikote DX6509. The Hycar used is obtained from the Shimi-Mobtaker-Peivand (Islamic Republic of Iran) for modifying the adhesive material. This is a liquid state material that is added commonly to the adhesive for improving the adhesion properties of the epoxy adhesive

2.2 Sample preparation

First 0 to 24 percent of Hycar was appropriately weighted and nine samples are made (We present formulations that are used in Table 1). Then these

amounts are added and mixed with EPON828. Mixing is done to obtain uniform composition. Afterwards, appropriate amount of Epikote DX6509 has been added to the mixture and we mixed it again for several minutes. Then mixture was cast into an appropriate small can. Afterwards, this is heated one hour at eighty centigrade degree. Specimens were cut out from the prepared by cutter.

2.3 Thermal property measurement

In this work the glass transition temperature is applied as an indicative for thermal strength of adhesives. A Differential scanning calorimetry (DSC) is used based on a Setaram DSC machine. Samples with weight of 8 mg are weighted and pressed in aluminum cans. Then these samples are heated from ambient temperature till 130 centigrade degree. Finally, these are cooled back to room temperature. The glass transition temperature (T_g) of the samples were measured.

2.4 Flexural Test

Flexural samples with a gauge length of 50 mm were made cutting from molded sheets and both sides were polished by sand paper until all visible defects disappeared. Flexural tests were performed at a strain rate of 0.5 mm/min at room temperature using an Instron universal machine. The extensometer was utilized to obtain accurate data.

2.5 Impact Test

Izod impact test was accomplished according to ASTM D256 using an 1 J hammer energy. Samples were prepared and machined to the standard shape ($62 \times 12.7 \times 4.2 \text{ mm}^3$). Specimens were tested using at room temperature using an Impactor machine.

2.6 Microscopy

Fracture surfaces of the specimens were examined using scanning electron microscope (SEM). SEM samples were coated with a thin layer of gold before examination to protect the fracture surfaces from beam damage and also to prevent charge build up.

3. Results and discussion

It should be noted that different criterions are suggested by standard test methods for evaluating the thermal resistance of a glue or adhesive material. One important of these criteria is the Differential Scanning Calorimetry (DSC). DSC is used to measure glass transition temperature (T_g) which is the temperature at which the adhesive sample become soft. This property of a given plastic material is applied in many aspects of product design, engineering, and manufacture of products using

thermoplastic components. Thermal simulations of a system will show temperatures that will be encountered by a specific component of that system. Knowing what temperature that a specific component will have to endure during use will allow the determination of the best material for that application (www.Wikipedia.com). The results of T_g measurement has been presented in Table 2. This table reveals that the glass transition temperature of adhesive decreases as the amount of Hycar increases. This is very significant for contents higher than 6%.

An interesting finding is that one can conclude to not increase the Hycor content beyond the critical value due to the possibility of diminishing the efficiency of the adhesive for high temperature applications. It is well-established that using modifier can improve the adhesion properties of glues

(Bascom *et al.* 1975; Jinen, 1988). However, the modifier can reduce the thermal stability of adhesive.

Table 3 shows the flexural properties of epoxy as a function of Hycar content. As expected, the flexural strength radually decrease with increasing rubber content (Table 3). This is due to the fact that the strength and modulus of Hycar is much lower than that of the epoxy matrix. However note that the decreasing amount is not very considerable and therefore one conclude that the flexural strength remains constant over the wide range of Hycar.

The results of impact energy absorption (impact strength) versus Hycar content for notched test specimens are reported in Table 4. Note that modifier increases impact strength (Table 4). However for obtaining optimum physical properties, one should not use more than 9-12 % Hycar for modification of epoxy adhesive.

Table 1. Samples used

Sample Material	EP	EP-3	EP-6	EP-9	EP-12	EP-15	EP-18	EP-21	EP-24
EPON828	80	80	80	80	80	80	80	80	80
Epikote DX6509	20	20	20	20	20	20	20	20	20
Hycar	0	3	6	9	12	15	18	21	24

Table 2. T_g of formulations

Sample	EP	EP-3	EP-6	EP-9	EP-12	EP-15	EP-18	EP-21	EP-24
T _g	84.44	84.21	83.86	81.22	80.13	79.45	78.31	76.95	73.4

Table 3. Flexural strength

Sample	EP	EP-3	EP-6	EP-9	EP-12	EP-15	EP-18	EP-21	EP-24
Strength	105	104	105	103	105	102	103	102	101

Table 4. Impact strength

Sample	EP	EP-3	EP-6	EP-9	EP-12	EP-15	EP-18	EP-21	EP-24
Strength	2.4	2.8	3.3	3.8	4.1	3.6	3.1	3.2	2.9



Figure 2. SEM of neat epoxy without Hycar

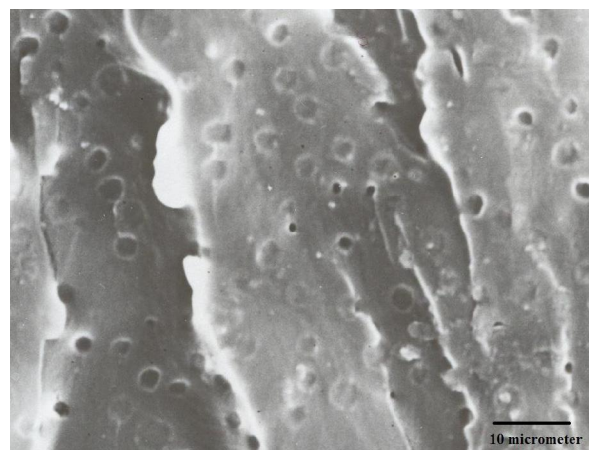


Figure 3. SEM of epoxy containing Hycar

Figures 2 and 3 shows the SEM fracture surface of specimens. Comparison between Figure 2 and Figure 3 reveals less roughness on the damaged surface of the Hycar modified epoxy specimen. Decrease in roughness corresponds to decrease in rigidity and increasing in energy absorption. It is in accordance with the Impact strength of Hycar-modified specimen in comparison with neat epoxy.

4. Conclusions:

Glues and adhesives are of important applications in medicine such as disposal medical devices, structural bonds, bone cement, prostheses, etc. In this paper the physical response i.e. thermal and mechanical behavior of the adhesive modified with liquid rubber has been investigated. In addition, scanning electron microscopy (SEM) is utilized in this work for better understanding the behavior of adhesive. Results show that adding liquid rubber decrease the T_g of the system. This does not change the flexural strength of the adhesive system. Using liquid rubber increase the Impact strength of the adhesive system. For obtaining an optimum physical properties, one should not use more than 9-12 % liquid rubber for modification of adhesive. The obtained data can be comprehensively useful for further investigators and medicine.

Corresponding Author:

Ameneh Langari
North Khorasan University of Medical Sciences
Bojnurd, Iran
E-mail: amenehlangari@yahoo.com

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Simulating The Cooling Of Medical Ct-Scanners: Part 1: FormulationMariam K. Hafshejani¹, Fakhroddin Dadjoo², Fakhroddin Alimoradi², Ali Falavand³, Armin Arad^{4*}¹Shahrekord University of Medical Sciences, Shahrekord, Iran²Engineering Group, Chalooos Branch, Islamic Azad University, Chalooos, Iran³Engineering Group, Ahvaz Branch, Islamic Azad University, Ahvaz, Iran⁴North Khorasan University of Medical Sciences, Bojnurd, IranCorresponding author email: aarad1384@yahoo.com

Abstract: CT-scanners are used as a non-invasive detecting method in medical applications as a powerful radiological device. Many patients are used CT-scanners for detecting disease. However CT-scanners required cooling due to the huge heat generated during the operation. In this work we modeled a convection-diffusion cooling process of CT-scan devices. A flow in a rectangular body with symmetry, constant heat flux and constant temperature boundary conditions has been considered. The governing equation has been discretized based on the finite volume method and has been solved using a fully implicit method. This paper is mostly concerned with the problem description and the results presents in part 2 of this paper.

[Hafshejani M K, Dadjoo F, Alimoradi F, Falavand A, Arad A. **Simulating the Cooling of Medical CT-Scanners: Part 1: Formulation.** *Life Sci J* 2012;9(1):1029-1034] (ISSN:1097-8135). <http://www.lifesciencesite.com>. 147

Keywords: CT-scanner, CFD simulation, Heat transfer

1. Introduction

Computed tomography (CT) creates two-dimensional images from body structures (S. Hassan and G. Hassan, 2011). Figure 1(a-c) shows the CT imaging system, the gantry and a typical produced image. As seen in figure 1(a), computed tomography imaging system primarily includes the gantry and patient table. The gantry (figure 1(b)) is a moveable frame that contains the x-ray imaging system. The load of heat can lead decreasing the CT-scanner life. When a CT-scanner x-ray tube reaches a maximum heat level, it is necessary to cool it using a cooling fluid.

CT scanners must possess the high amount of heat that a tube can store without any operational damage. The system must absorb high heat levels generated during the high speed rotation of the anode and the bombardment of electrons upon the anode surface (Reddinger, 1997; Ahmadi and Marghmaleki, 2011). The x-ray tubes heat capacity is expressed in heat units. Modern CT scanners bear a heat capacity of 3.5-5 million heat units (Reddinger, 1997). Many CT x-ray tubes utilize a combination of oil and air cooling systems to eliminate heat and maintain continuous operational capabilities (Reddinger, 1997).

In recent decades, CFD, is known as the powerful method that uses numerical methods to simulate problems involving fluid motion by solving the equations (<http://www.ehow.com>; Biao *et al.* 2012). Heat transfer analysis of CT-scanner using Computational Fluid Dynamics is an important process to understand the thermal history of any component or cooling fluid in the CT-scanner. Heat

transfer analysis plays a vital role in determining, monitoring, and controlling thermal loads across a CT-scanner. Heat transfer analysis is used in several industries such as engineering and medical sciences (McKenna *et al.*, 1999; Kuipers *et al.*, 1992; Owoyemi *et al.*, 2007; Behjat *et al.*, 2008; Piechowski, 1999). For example thermal analysis is used in areas where there is consistent interaction between fluid and component surfaces of CT-scanner. Some main applications of heat transfer analysis include cooling systems of CT-scanners, MRI devices and insulation etc. CFD thermal analysis allows an automated, multi-dimensional design optimization system to ensure accurate heat transfer simulation (www.cfdoutsourcingservices.com).

In this work, a Computational Fluid Dynamics simulation is done, to analyze the cooling system dynamic variation of temperature, during passing a cold fluid above a hot plate in a CT-scanner. The parametric characteristics of the problem, together with the domain and applied grid are the items discussed in this paper. The next part of this article is mostly dedicated to the results of this project on cooling CT-scanner surface.

2. Problem formulation

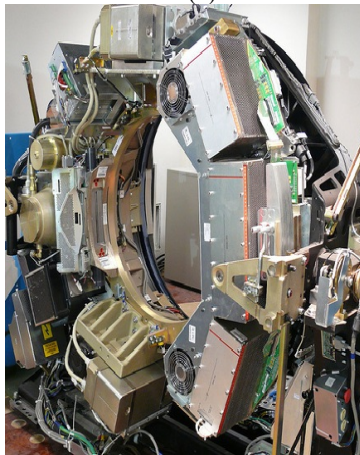
The governing equation is the conservation of energy, in an unsteady-state form as below:

$$\rho C_p \left(\frac{\partial T}{\partial t} + u \frac{\partial T}{\partial x} \right) = \frac{\partial}{\partial x} \left(k \frac{\partial T}{\partial x} \right) + \frac{\partial}{\partial y} \left(k \frac{\partial T}{\partial y} \right) \quad (1)$$

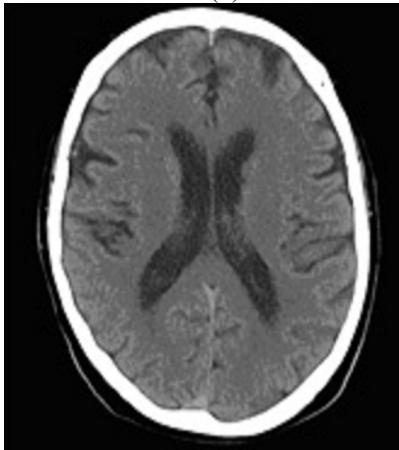
The parameters of the problem in the formula are all in SI units. FVM discretization is used to convert the PDEs to a set of ODEs. As it's a time dependent problem, FDM time discretization is also used. The written code is based on implicit formulas. Convergence of the solution is guaranteed, but more memory resource is required rather than explicit method. Table 1 includes specified parameters.



(a)



(b)



(c)

Figure 1. (a) the CT system, (b) the CT gantry and (c) a typical CT image.

Table 1. Specific parameters of the problem

Length	100
Width	50
Initial Temperature	20
Maximum time	500
number of mesh, x direction	30
number of mesh, y direction	15
time step	5
K1	{ $K_x = K_1 * (1 - \exp(-1000/T))$ }
K2	{ $K_y = K_2 * T^{(x+y)}$ }
Rho	500
Cp	10
T down	200
T left	120
H	200
T	300
Dp/dx	0.05
Heat generation rate	1

3. Geometry of the model

The geometry and boundary conditions applied for the problem of cooling CT-scanner anode surface is shown schematically in Figure 2 and 3. The values of L, W, T up, T left, T_∞ , h can be considered as input data.

Regarding the initial conditions of this problem, the following figure shows the state of the fluid at time 0. As shown, the fluid body of the fluid is at 20°C before we start to pass it over the hot plate.

4. Grid

A simple rectangular grid is used to simulate the problem. Figure 4. includes a schematic picture of the grid used in this study.

5. The in-house coding

The formulation and boundary conditions described above are all programmed in MATLAB 2006a. The following section of this paper includes the code we used. This code is based on the finite volume discretization and the schemes required for consistency, convergence and stability of the solution.

6. Discussion

CFD has many benefits to companies and to researchers. Some argue that using a computer to simulate cooling flow in CT-scanners using CFD is cheaper and easier than building the required machinery, tooling, and equipment to manufacture a test part of CT-scanner and then test it physically. CFD also produces accurate results and data for every

point of time and thus can be used to completely track even a single particle of a fluid from point A to point B.

One of the major issues of relying on CFD-produced data is verification and validation. Before a new CFD code or results can be trusted, it must first be verified that the codes are calculating the right equations, and then the results must be compared with physical, real-world tests in order to validate the accuracy of the calculations. Because of this, CFD will not be used as a sole source of data for the time being. Another major hurdle of using CFD is the limitation due to computing power. Supercomputers are getting more and more powerful every year, but

real complex CFD problems, such as problems with moving boundaries and many different flows, require computing power that, while it is possible today, is not fast enough to solve in due time.

Current research topics that use CFD is the thermal analysis of heat flux and temperature distribution through the heat transfer along a cold fluid, passing over a hot plate. This is the case that occurs during cooling CT-Scanner operation. The code we used is presented in this article. A comprehensive assessment on the results and their feedback on the criteria we used to solve this problem will be in the follow up paper.

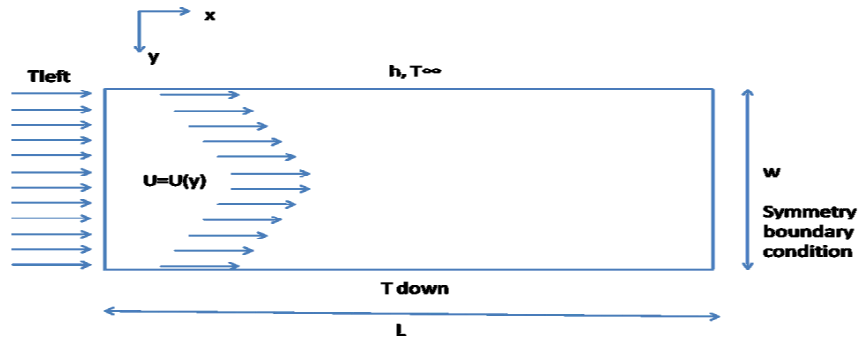


Figure 2. Boundary conditions

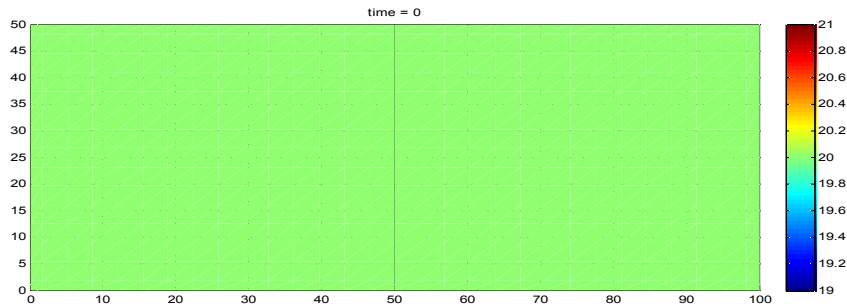


Figure 3. Domain at time=0

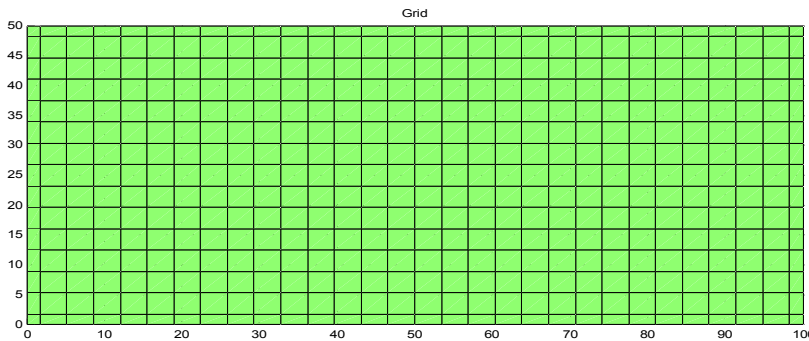


Figure 4. A schematic picture of the grid

7. Conclusions

While CT-scanners required cooling due to the huge heat generated during the operation, less work has been conducted on modeling a convection-diffusion cooling process of CT-scan devices. The cooling flow in a medical CT-scanner with constant heat flux and constant temperature boundary conditions has been considered in this work. Velocity profile comes from a one dimensional flow between parallel planes of CT-scanner. The governing equation has been discretized based on the finite volume method and has been solved using a fully implicit method. This paper is mostly concerned with the CT-scanner problem description and the results presents in part 2 of this paper. The computer code has been presented in appendix.

Corresponding Author:

Armin Arad
North Khorasan University of Medical Sciences
Bojnurd, Iran
E-mail: aarad1384@yahoo.com

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Appendix: MATLAB Code

```

clc
clear
n=1;
while n==1
    prompt = {' LENGTH ':'WIDTH:','initial
temperature','max time','number of mesh, x
direction','number of mesh, y direction','time step'};
    dlg_title = 'Input';
    num_lines = 1;
    def = {'100','50','20','500','30','15','5'};
    answer = inputdlg(prompt,dlg_title,num_lines,def);
    if size(answer)==0
        button = questdlg('Would you like to close this
simulation program?',...
        'simulation','yes','no','no');
        if strcmp(button,'yes')
            return;
        end;
    else
        n=0;
    end;
end;
L=str2num(cell2mat(answer(1)));
W=str2num(cell2mat(answer(2)));
T0=str2num(cell2mat(answer(3)));
t=str2num(cell2mat(answer(4)));
delta_x=L/(str2num(cell2mat(answer(5)))-1);
delta_y=W/(str2num(cell2mat(answer(6)))-1);
delta_t=str2num(cell2mat(answer(7)));
n=1;
while n==1
    prompt = {'K1 (Kx=K1*(1-exp(-1000/T))','K2
Ky=K2*T*(x+y)','Rho','Cp','T down','T left','h','T
inf','dp/dx','Heat generation'};
    dlg_title = 'Input';
    num_lines = 1;
    def =
{'600','1','500','10','200','120','200','300','0.05','1'};
    answer = inputdlg(prompt,dlg_title,num_lines,def);
    if size(answer)==0
        button = questdlg('Would you like to close this
simulation program?',...
        'simulation','yes','no','no');
        if strcmp(button,'yes')
            return;
        end;
    else
        n=0;
    end;
end;

```

```

end;
K1=str2num(cell2mat(answer(1)));
K2=str2num(cell2mat(answer(2)));
Rho=str2num(cell2mat(answer(3)));
Cp=str2num(cell2mat(answer(4)));
Tdown=str2num(cell2mat(answer(5)));
Tleft=str2num(cell2mat(answer(6)));
h=str2num(cell2mat(answer(7)));
Tinf=str2num(cell2mat(answer(8)));
dpdx=str2num(cell2mat(answer(9)));
Q=str2num(cell2mat(answer(10)));
x=[0,delta_x/2:delta_x:L-delta_x/2,L];
y=[0,delta_y/2:delta_y:W-delta_y/2,W];
[n,s1]=size(x);
[n,s2]=size(y);
[x,y]=meshgrid(x,y); %node locate
tt=zeros(s2,s1);
T0=T0+tt;
axes1 = axes(...
'CameraPosition',[2.54 41.94 916],...
'CameraUpVector',[1 0 0],...
'DataAspectRatio',[50 50 1]);
axis(axes1,[0 L 0 W]);
grid(axes1,'on');
hold(axes1,'all');
surf1 = surf(x,y,tt,'Parent',axes1);
pause(1)
delete(surf1);
delete(axes1);
axes1 = axes(...
'CameraPosition',[3*L/4 W/2 1000],...
'CameraUpVector',[1 0 0],...
'XGrid','off',...
'YGrid','off',...
'ZGrid','off',...
'DataAspectRatio',[50 50 1]);
axis(axes1,[0 L 0 W]);
grid(axes1,'on');
hold(axes1,'all');
surf1
surf(x,y,T0,'Parent',axes1,'FaceColor','interp',...
'EdgeColor','none','FaceLighting','phong');
colorbar1 = colorbar('EastOutside',...
'XLim',[-0.5 1.5]);
pause(1)
e=[];
for m=[1:s1-2]
for n=[2:s2-1]
% P, W, E, N, S
d=[s2*m+n,s2*(m-
1)+n,s2*(m+1)+n,s2*m+n+1,s2*m+n-1];
e=[e;d];
end
end
TT=T0;
T0=zeros(s1*s2,s1*s2);

for i=1:s1
for j=1:s2
T0(s2*(i-1)+j,s2*(i-1)+j)=TT(j,i);
end
end
KX=inline('K*(1-exp(-1000/T))','K','T'); % K1
KY=inline('K*T*(x+y)','K','T','x','y'); % K2
U=zeros(s2,s1);
for i=1:s2
for j=1:s1
Kx(i,j)=KX(K1,TT(i,j)); % Kx(i,j)=K1;
Ky(i,j)=KY(K2,TT(i,j),x(i,j),y(i,j)); %
Ky(i,j)=K2;
yy=y(i,j)-y(1,j);
U(i,j)=dpdx*yy/2*(W-yy);
end
end
for ti=delta_t:delta_t:tt
A=zeros(s1*s2,s1*s2); B=zeros(s1*s2,1);
j=0;
for m=1:s1
for n=1:s2
i=(m-1)*s2+n;
a=zeros(1,5); b=zeros(1); % a=[P W E N S]
if x(n,m)==0
a(1,1)=1; b=Tleft; p=0;
else if x(n,m)==L
a(1,1)=1; a(1,2)=-1; b=0; p=0;
else if y(n,m)==0
a(1,1)=1; b=Tdown; p=0;
else if y(n,m)==W
Dy=y(n,m)-y(n-1,m);
Kyn=(Ky(n-1,m)+Ky(n,m))/2;
a(1,1)=-Kyn/Dy-h;
a(1,5)=Kyn/Dy; b=-h*Tinf; p=0;
else
j=j+1;
Dxw=x(e(j,1))-x(e(j,2));
Dxe=x(e(j,3))-x(e(j,1));
Dyn=y(e(j,1))-y(e(j,4));
Dys=y(e(j,5))-y(e(j,1));
Dx=(Dxw+Dxe)/2; Dy=(Dyn+Dys)/2;
if m>2
Kxe=6/8*Kx(n,m)+3/8*Kx(n,m+1)-
1/8*Kx(n,m-1);
Kxw=6/8*Kx(n,m-1)+3/8*Kx(n,m)-
1/8*Kx(n,m-2);
else
Kxe=(Kx(n,m)+Kx(n,m+1))/2;
Kxw=(Kx(n,m)+Kx(n,m-1))/2;
end
Kxn=(Kx(n-1,m)+Kx(n,m))/2;
Kxs=(Kx(n+1,m)+Kx(n,m))/2;
if m>2
Kye=6/8*Ky(n,m)+3/8*Ky(n,m+1)-
1/8*Ky(n,m-1); %

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        Kyw=6/8*Kx(n,m-1)+3/8*Kx(n,m)-
1/8*Kx(n,m-2); %
        else
        Kye=(Ky(n,m)+Ky(n,m+1))/2;
        Kyw=(Ky(n,m)+Ky(n,m-1))/2;
        end
        Kyn=(Ky(n-1,m)+Ky(n,m))/2;
        Kys=(Ky(n+1,m)+Ky(n,m))/2;
        Ue=(U(n,m)+U(n,m+1))/2;
        Uw=(U(n,m)+U(n,m-1))/2;
        Un=(U(n-1,m)+U(n,m))/2;
        Us=(U(n+1,m)+U(n,m))/2;
        a(1,3)=a(1,3)+Kxe*Dy/Dxe;
        a(1,1)=a(1,1)-Kxe*Dy/Dxe;
        a(1,1)=a(1,1)-Kxw*Dy/Dxw;
        a(1,2)=a(1,2)+Kxw*Dy/Dxw;
        a(1,4)=a(1,4)+Kyn*Dx/Dyn;
        a(1,1)=a(1,1)-Kyn*Dx/Dyn;
        a(1,1)=a(1,1)-Kys*Dx/Dys;
        a(1,5)=a(1,5)+Kys*Dx/Dys;
        a(1,3)=a(1,3)+Rho*Cp*U(n,m)/2/Dx;
        a(1,2)=a(1,2)-Rho*Cp*U(n,m)/2/Dx;
        b=Q*Dx*Dy; p=1;
    end
end
end
end
if p==0
A(i,i)=a(1,1); B(i)=b;
if m==s1
    A(i,i-s2)=a(1,2);
end
if n==s2 & m>1 & m<s1
    A(i,i-1)=a(1,5);
end
else
A(i,i)=(Rho*Cp*Dx*Dy/delta_t)-a(1,1);
A(i,e(j,2))=-a(1,2); A(i,e(j,3))=-a(1,3);
A(i,e(j,4))=-a(1,4); A(i,e(j,5))=-a(1,5);
B(i)=b+(Rho*Cp*Dx*Dy/delta_t)*T0(i,i);
end
end
end
T=zeros(s2,s1);
X=inv(A)*B;
for i=1:s1
    for j=1:s2
        T(j,i)=X(s2*(i-1)+j);
    end
end
T0=T;
TT=T0;
T0=zeros(s1*s2,s1*s2);
for i=1:s1
    for j=1:s2
        T0(s2*(i-1)+j,s2*(i-1)+j)=TT(j,i);
    end
end
delete(surf1);
delete(axes1);
tft=num2str(t);
axes1 = axes(...
    'CameraPosition',[3*L/4 W/2 1000],...
    'CameraUpVector',[1 0 0],...
    'XGrid','off',...
    'YGrid','off',...
    'ZGrid','off',...
    'DataAspectRatio',[50 50 1]);
axis(axes1,[0 L 0 W]);
grid(axes1,'on');
hold(axes1,'all');
surf1
=
surf(x,y,T,'Parent',axes1,'FaceColor','interp',...
    'EdgeColor','none','FaceLighting','phong');
colorbar1 = colorbar('peer',...
axes1,'EastOutside',...
'Box','on',...
'XLim',[-0.5 1.5],...
'YLim',[20,200]);
pause(1)
T
ti
for i=1:s2
    for j=1:s1
        Kx(i,j)=KX(K1,T(i,j)); % Kx(i,j)=K1;
        Ky(i,j)=KY(K2,T(i,j),x(i,j),y(i,j)); %
Ky(i,j)=K2;
        yy=y(i,j)-y(1,j);
        U(i,j)=dpdx*yy/2*(W-yy);
    end
end
end
end

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