Afro-Egyptian Journal of Infectious and Endemic Diseases

المجلة الافريقية المصرية للامراض المعدية والمتوطنة

ISSN (Online): 2090-7184

ISSN (Print): 2090-7613

An Official Publication of Endemic and Tropical Medicine Department ,Faculty of Medicine ,Zagazig University ,Zagazig 44519 ,Egypt

Published by: Communication and Information Technology Center(CITC),Zagazig University , Zagazig ,Egypt
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11-US National Library of Medicine (NLM) Catalog [NLM ID: 101570965]:http://www.ncbi.nlm.nih.gov/nlmcatalog/101614559

Contents

Superproof about Sexual Reproduction and Life Cycle in the Parasitic Generation of *Strongyloides stercoralis* in Human Host

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Key words:
strongyloidiasis, morbidity, mortality, infection, fulminant death

Background and study aim: *Strongyloides stercoralis* was believed to have two kinds of life cycles. One of them was an internal parthenogenetic life cycle that constituted the parasitic generation. Some authors also stated that this parasitic generation of the internal life cycle used to reproduce only by protandrogony (i.e., hermaphroditism). The second type of life cycle was the external sexual cycle, interacting among free-living worms that represented the free-living generation in soil. The key objectives of this study were to: verify the type of reproduction in the parasitic generation of *S. stercoralis*, and demonstrate the complete integrated life cycles of both parasitic and free-living generations of *S. stercoralis*.

Patients and Methods: The major sites of stool sample collection were selected to be the elementary schools (from students) at Dilla District, because *S. stercoralis* was ascertained to be endemic in this region. The parasitic worms of *Strongyloides stercoralis* obtained from fresh stools were used to set growth culture of free-living generation of this very species and to find the free-living male and female in copulation from the growth culture. A set of parasitic male & female in copulation isolated from a fresh stool sample of a patient under medical care in a hospital was also included.

Results: The male and female worms of *S. stercoralis* had been isolated from both parasitic and free-living generations while they were in the actual copulation.

Conclusion: The method of reproduction in the parasitic generation of *S. stercoralis* in human host was practically proved to be certainly sexual. This verified conclusion was the first practical achievement in the entire globe by solving the persistent problems (i.e., erroneous concepts of parthenogenesis and protandrogony as the types of reproduction in the parasitic generation of *S. stercoralis*) that had been misleading and puzzling the minds of the concerned scientists of the world until the establishment of this very achievement. Additionally, this achievement had enabled the investigator to construct the complete integrated life cycles of both parasitic and free-living generations of *S. stercoralis*.

INTRODUCTION

Strongyloides (S). stercoralis is believed to have two kinds of life cycle: an internal parthenogenetic cycle that constitutes the parasitic generation, and the external sexual cycle, representing the free-living generation in soil **[1-3]**. It had been stated that the type of reproduction in the parasitic generation of *S. stercoralis* in human host was only by parthenogenesis of parasitic females in the absence of parasitic males **[4-8]**. Due to this concept of parthenogenesis the parasitic male had been omitted in the figures that demonstrated the life cycle of the parasitic generation of *S. stercoralis* in all modern and relevant textbooks, journals, and on the internet. In the parasitic generation, when the filariform larvae are in contact with skin, they penetrate the small cutaneous blood vessels and are carried through the right heart to the lungs **[9]**. Then, the mature parasitic females settle in the tissues of epithelial mucosa to lay eggs that hatch soon and are discharged in stools each day **[10,11]**. When all or some larvae metamorphose into infective filariform larvae autoinfection may be onset by invading the mucosa of the ileum or colon, travel to lungs and then return to the intestine to mature in the mucosa **[12-15]**.

Disseminated strongyloidiasis had been reported in both of two recipients of kidney allografts from a single cadaver donor **[16]**. It was also reported that in a 53-year-old man who had lung cancer, fulminantly fatal strongyloidiasis had developed following postchemotherapy of immunosuppression, resulting in the death of the patient within 48 hours **[16]**. The development of a florid strongyloidiasis was observed in a 45-year-old man, following anticancer chemotherapy when eggs of *S. stercoralis* were seen in the stools **[17]**. One scientific study has reported that almost all deaths due to helminth in the United States result from *S. stercoralis* hyperinfection mortality rates because the occurrence of hyperinfection can be as high as 87% **[18]**. Concerning some morphological features of this parasite, the part of the worm's body that is known as the tail is the posterior part of body beginning from cloaca in the parasitic males or beginning from anus in the parasitic females. Cloaca is the opening through which spicules are everted at times of copulation and fertilization; and it is also the outlet of the digestive tract. The 3 stages of human strongyloidiasis are: Intestinal Strongyloidiasis, Gastropulmonary Strongyloidiasis, and Disseminated Strongyloidiasis **[19-22]**. Some of the clinical presentations (manifestations) of strongyloidiasis can be highlighted as: Cutaneous with larva currens (racing larvae), pruritic linear or serpiginous, creeping urticarial eruption, dermatologic lesions, and petechiae; Pulmonary with persistent wheezing, cough, and deteriorating respiratory status; and Intestinal with vomiting, abdominal pain, watery diarrhea and constipation.

Aim of the study

The aim of this study has two key objectives that are: to prove the fact that the type of reproduction in the parasitic generation of *S. stercoralis* is certainly sexual, and establish the complete integrated life cycles of both parasitic and free-living generations of *S. stercoralis*.

PATIENTS AND METHODS

The sites of collecting data were decided to be elementary schools at Dilla District, because S. stercoralis was ascertained to be endemic in this region. The purpose of collecting stools samples from different elementary schools was to set growth culture of free-living worms of *S. stercoralis* in sterilized topsoil from which the copulating free-living male (♂) and female (♀) of this species can be isolated. Then, the form of copulation of this set of free-living male & female was planned to be compared with that of parasitic male & female isolated from a fresh stool sample taken from an HIV/AIDS patient under medical care in a hospital.

Sample Size. A total of 300 students were randomly examined for *S. stercoralis*. Those children with relatively large number of rhabditiform larvae of this parasite were used as the sources of stools samples for inoculating the worms into the sterilized topsoil in petridihes.

Growth culture of free-living generation

Topsoil that contained organic substance was taken and put into four different petridishes. Each of the petridishes was closed with its own lid and labeled 1,2,3 and 4; and then, the following 4 steps were implemented. The petridishes with their contents of topsoil were autoclaved.

The topsoil autoclaved in each of the petridishes was inoculated with fresh stool sample infected with *S. stercoralis* and identified to harbor relatively heavy worm-load of this parasite, before they had been given treatment.

Excess water was added to the topsoil of all the four petridishes and were incubated at 28 ºC on the same day. With the aim to find the free-living male & female in copulation, using a dropper a drop of soil suspension from each of the four topsoil petri dishes incubated was placed on a clean slide & covered with a 22 square mm cover slip and examined under a compound light microscope everyday beginning from the 3rd day of incubation until the 5th day.

Treatment

The participant children who were found to be positive for *S. stercoralis* were given 400 mg albendazole tablet to take with a glass of water on empty stomach in the morning and to begin taking meal at noon. This prescription & clinical supervision was performed by a medical doctor (Dr.Corazon B. Jaca FMA).

RESULTS

On the 5th day from the date of setting the growth culture of free-living generation of S. stercoralis, the free-living male & female in copulation were found and isolated from the growth culture. The copulating pair was observed under a compound light microscope and microphotographed from the field of vision of the microscope using a digital camera. The

copulating pair of the parasitic generation which was isolated from a fresh stool sample of an HIV/AIDS patient was microphotographed from the field of vision of the microscope in the same way with the digital camera. Now, the free-living male & female in copulation, and the parasitic male & female in copulation isolated from a fresh stool sample are spectacularly displayed for comparison.

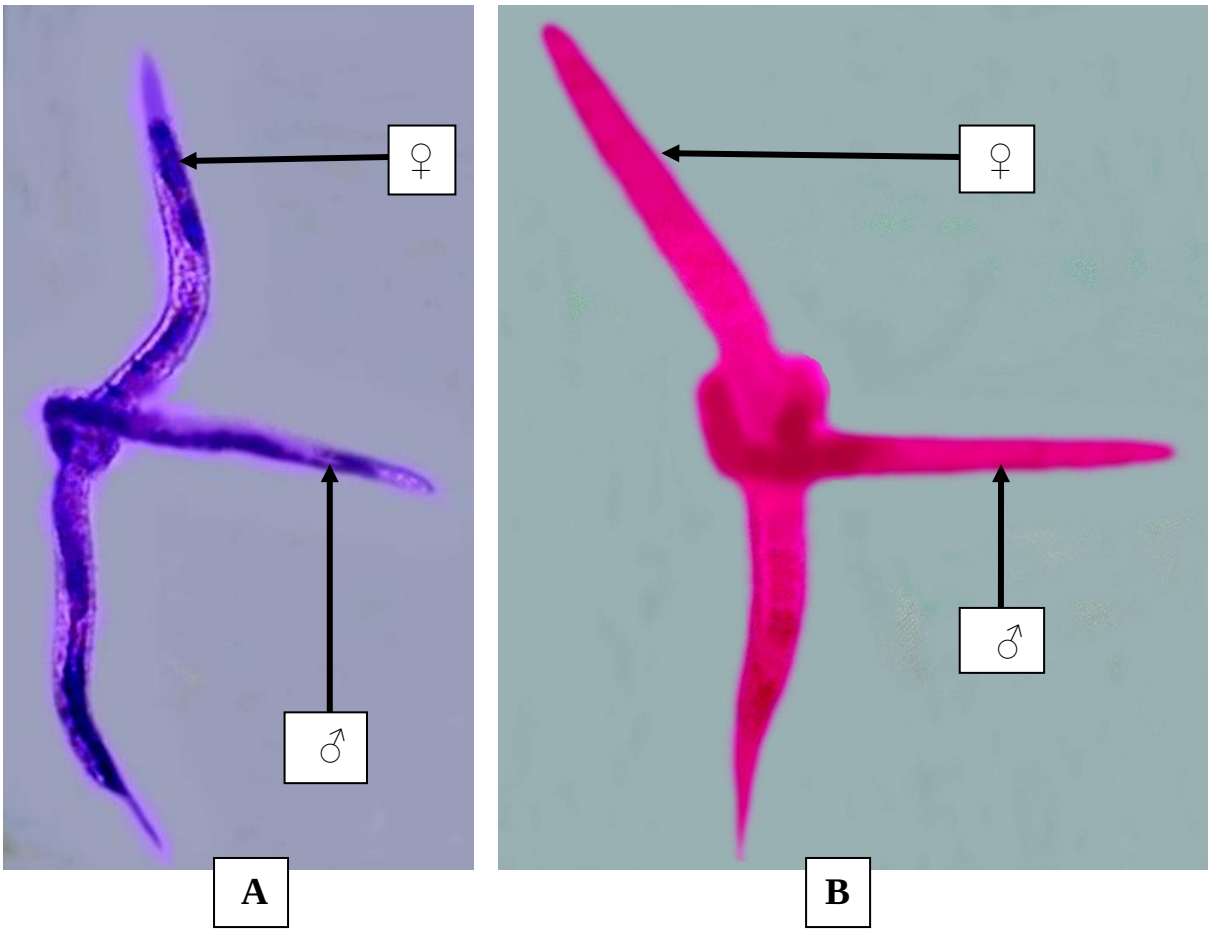

Figure (1): Copulation among the worms of *Strongyloides stercoralis*. *Strongyloides stercoralis*: **(A)**, parasitic male (♂) & parasitic female (♀) in copulation isolated from a fresh stool sample of an HIV/AIDS patient under medical care in a hospital & microphotographed with a digital camera; and **(B)**, free-living male (♂) & free-living female (♀) in copulation isolated and then microphotographed with a digital camera from the growth culture of this very species by inoculating in an sterilized set of topsoil placed in a petridish and incubated at 28 ºC.

- The curved/coiled posterior body part of the male that coils around a specific body region of the female bears the genitals of the male everted at times of breeding (i.e., spicules everted out of spicule pouches found in cloaca).
- The specific body region of either parasitic or free-living female around which the curved/coiled posterior body part of the male coils bears vulva. The opening of vulva leads to a short muscular vagina which in turn leads to a pair of uteri of the adult female worm of *S. stercoralis*.

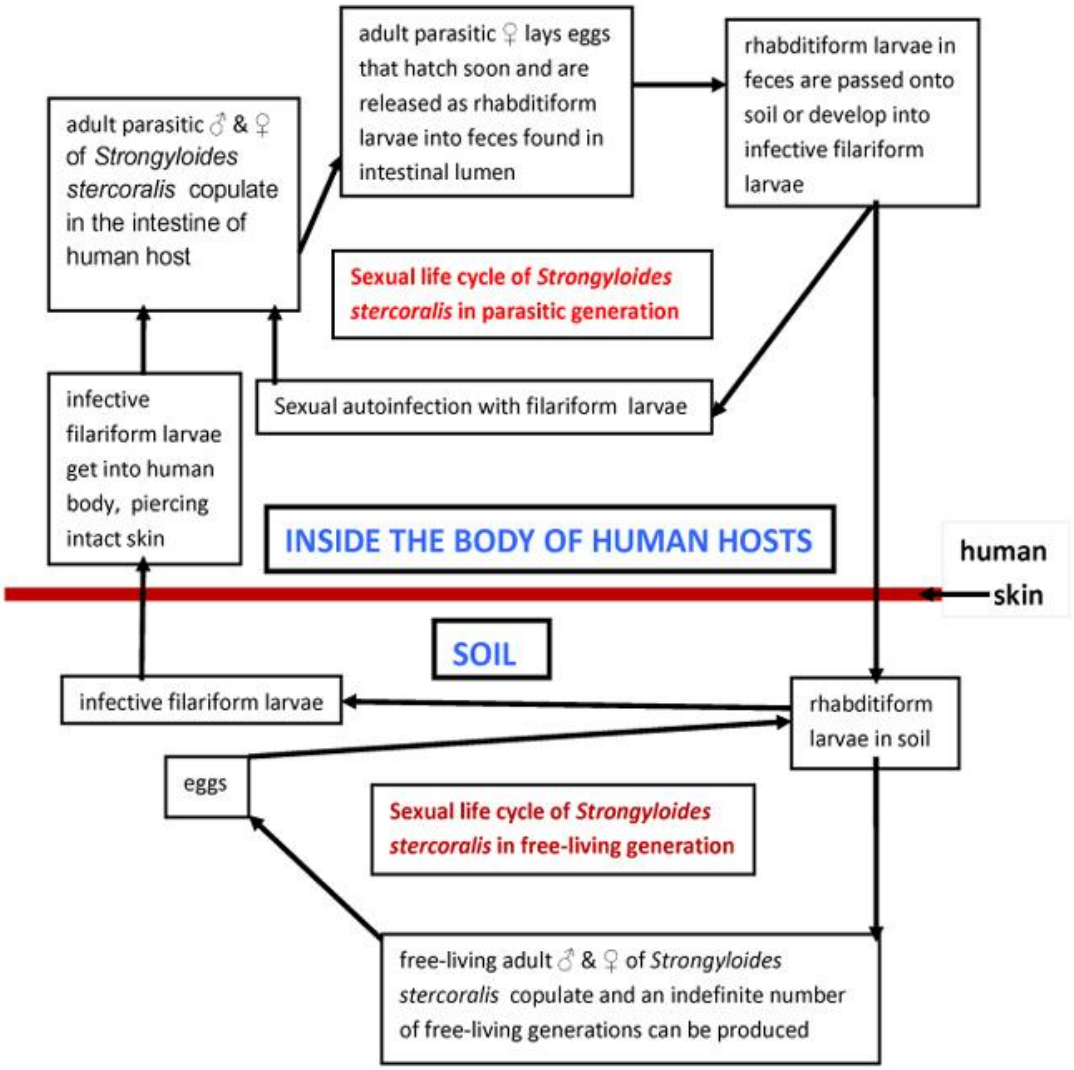

Figure (2): Complete integrated sexual life cycles of *Strongyloides stercoralis* in: parasitic generations in human host, and free-living generations in soil.

DISCUSSION

It has been believed that the type of reproduction of *Strongyloides stercoralis* in human host is parthenogenesis. In other words, parasitic males of *S. stercoralis* are never seen in human host until now. This is what has been written and reported: in modern textbooks of human/medical parasitology, in articles of famously reputable journals, on the internet, and from the minds of students of biology, and health sciences.

However, the presence of the parasitic males (just as there are parasitic females) of *S. stercoralis* in human host had been shown in a demonstrative practical study project executed by the researcher of this paper **[23]**.

Authors of modern textbooks of the subject stated that parthenogenesis was the only method of reproduction; still some others had concluded that protandrogony (i.e., hermaphroditism) was the actual method of reproduction for *S. stercoralis* in human host **[6]**. Neither the belief of parthenogenesis nor that of protandrogony is acceptable/true as each of them does not have any scientific evidence or persuasive scientific background. Actually the presence of the parasitic males of *S. stercoralis* in human host had been reported and published in 2014, being enough to block the epidemic spread of erroneous concepts of parthenogenesis and protandrogony. Anyhow, the report of this paper is crucial at global level to irreversibly delete the misleading generalizations of parthenogenesis and protandrogony without

delay: from modern textbooks of human/medical parasitology, from related articles of journals, from concerned sites of internet, and from the minds of students of biology, and health sciences.

It is because of this massive strength of eradicating/eliminating these erroneous concepts from the scientific discipline of the subject that the title of this paper begins with the word "Superproof". This is so because when the free-living male & female copulate the curved/coiled

posterior body part of the male coils around a specific body region of the female in order to ejaculate (introduce) the male gametes into the female reproductive tract through the opening of vulva. As it has been seen in the copulation of free-living male and female, the same positional form of copulation is spectacularly observed in the copulating parasitic male & female isolated from a fresh stool sample of a patient (Figure 1).

In conclusion, seeing the copulating of parasitic male and female of *S. stercoralis* with the observers' own eyes proved the fact that the method of reproduction of *S. stercoralis* was doubtlessly sexual. The usefulness of the integrated life cycles of the free-living & parasitic generations has been kept in mind, because efficient preventive measures against parasitosis and devising effective treatment depend on understanding the biology & life cycle of a parasite (which means understanding the nature of the disease).

Conflict of interest

I confirm that I don't have any competitive conflict of interest with anybody.

Financial support

The financial support to cover the cost of this study project was given by the Research & Dissemination Office of Dilla University.

Ethics

Ethical permission/clearance to perform the research work to contribute to the well-being of human subjects was obtained from Dilla University, the Office of Gedeo-Zone Administration, and the Directors of Elementary Schools of Dilla District.

Acknowledgements

I am very much thankful to Dilla Ethiopian Catholic Church for its assigning a medical doctor (Dr. Corazon B. Jaca FMA) for prescription and clinical supervision in the process of treating the infected participant student children. I am also grateful indeed to Dilla University for its providing me with the necessary fund to cover the cost of this study project and writing the letters of cooperation or ethical permission to the Directors of Elementary Schools of Dilla District.

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Peer reviewers: Hala A El-Nahas, Professor of Medical Parasitology Faculty of Medicine, Mansoura University, Mansoura, Egypt; **Ekhlas Hamed Abdel-Hafeez,** Assistant Professor, Department of Parasitology, Faculty of Medicine, Minia University, Minia, Egypt. **Maha Eissa,** Professor of Medical Parasitology Faculty of Medicine, Alexandria University, Alexandria, Egypt

Editor: Mohamed H Emara; Lecturer of Tropical Medicine, Faculty of Medicine, Zagazig University, Egypt.

The Fibrosis-Cirrhosis Index (FCI) for Staging of Liver Fibrosis in Chronic Hepatitis C

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Key words:
liver fibrosis, liver biopsy, The Fibrosis-Cirrhosis Index (FCI).

Background and study aim : Chronic hepatitis C (CHC) is a major global health problem with its consequences of liver fibrosis and cirrhosis. Liver fibrosis is the main predictor of the progression of chronic hepatitis C, and its assessment can help determine therapy. Several indices are available to predict cirrhosis. The fibrosis – cirrhosis index (FCI) was proposed to be efficient for none invasive staging of liver fibrosis besides being simple and inexpensive. This study is designed to assess the accuracy of the fibrosis-cirrhosis index (FCI) for non-invasive staging of liver fibrosis in patients with chronic hepatitis C compared to the liver biopsy findings.

Patients and methods: This study was conducted on 150 chronic hepatitis C patients who attended the Hepatology, Gastroenterology and Infectious Diseases Department at Benha University Hospital and El Mansoura Health-Insurance Hospital. Another group of 30 healthy subjects (with negative hepatitis viral markers) represented the control group.

Results: FCI was a relatively sensitive, specific and accurate marker of fibrosis. At a cutoff value of < 0.12, FCI had a NPV of 81.7% for the exclusion of significant fibrosis, while at cutoff value > 0.19 it had a PPV of 82.5% for the diagnosis of advanced fibrosis.

Conclusion: FCI is a simple index that integrates ALP, bilirubin, albumin and platelet count for staging fibrosis from absent up to cirrhosis.

INTRODUCTION

Chronic hepatitis C (CHC) is a major global health problem with its consequences of liver fibrosis and cirrhosis up to hepatocellular carcinoma **[1].**

In Egypt, Being a developing country, CHC represents a real medical and financial problem as it affects about 14.7 % of the Egyptian population making it one of the countries with highest prevalence worldwide **[2].**

Liver fibrosis is the main predictor of the progression of CHC, and its assessment can help determine therapy **[3].**

Liver biopsy is still considered the gold standard for staging fibrosis, however it is invasive, expensive and not suitable for all patients who may get severe side effects that may lead to death **[4].** There is a need for a simple, safe, noninvasive and inexpensive test to assess fibrosis and cirrhosis **[5].**

Several indices are available to predict cirrhosis but no method or score is available on exclusive basis to diagnose earlier fibrosis stages **[6].**

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. However, mild fibrosis (F0) to end stage cirrhosis cannot be predicted accurately using a single system **[7].**

The fibrosis–cirrhosis index (FCI) was proposed and comprised serum alkaline phosphatase (ALP), bilirubin, albumin and platelet count, where FCI =[(ALP × Bilirubin)/(Albumin × Platelet count)]. It was supposed to be efficient for none invasive staging of liver fibrosis besides being simple and inexpensive **[8]**.

PATIENTS AND METHODS

Study design: Cross-sectional study.

This study:
Was carried out on 150 patients with evidence of CHC (positive HCV-Ab and HCV-RNA- PCR with elevated ALT level for more than 6 months). They were 94 males (62.7%) and 56 females (37.3%), and their ages ranged between 18 and 60 years.

All cases were selected from the department of Hepatology, Gastroenterology and Infectious Diseases, Benha University Hospitals, and Mansoura Health Insurance Hospital, within the period between October 2013 to October 2014.

The criteria for exclusion were; any contraindication to percutaneous liver biopsy, hepatitis with causes other than HCV, age under 18 years, severe systemic illness and pregnancy.

Patients were sex and age matched with 30 healthy subjects who were volunteer blood donors visiting Benha University Hospitals-blood bank within the same period of the study and who were having normal liver profile with absence of HCV-Ab, HBsAg and HBc Ab (total) in their sera.

Patients were subjected to the following:
Full history taking, thorough clinical examination.
Routine laboratory investigations, that included: Complete blood picture. Liver profile tests: prothrombin time and concentration, serum albumin, AST, ALT, Alkaline phosphatase, γ-GT and serum bilirubin (total and conjugated). Hepatitis viral markers; that included: Detection of HCV-Ab by ELISA technique using Biochem Kit., Detection of HCV–RNA–PCR. Detection of HBsAg by ELISA technique using Sorin Biomedic Kit (for exclusion of +ve cases).

Abdominal Ultrasonography.
Scoring of king's score: Age (years) x AST (IU/L) x INR/platelet count (109/L)[9], Age-platelet index (API)= Age (year)/PLT (× 10^9/L)[10], AST to platelet ratio index (APRI): [(AST of the sample/ reference AST)x100]/ platelets count (10^9/L) **[11]**, FIB-4 score = [age (years)] × AST (U/L)]/ [number of platelets (10^9/L)] × ALT (U/L) ½] **[12]**, FCI= (ALP x bilirubin)/ (albumin x platelet count) [8].

Liver biopsy and histopathological examination for necro Inflammatory grading and fibrosis staging applying the METAVIR scoring system **[13,14]**.

Samples collection, preparation and handling:
A sample of 8-10 ml of venous blood was with-drawn under aseptic condition. One ml of blood was added to an anticoagulant- (EDTA) containing tube for complete blood count. The rest of the blood sample was left to clot in a sterile, clean and dry tube. After clotting, samples were thoroughly separated from all cellular material by centrifugation. Multiple freeze-thaw procedures were avoided. Any precipitates in specimens were removed by centrifugation before testing, and specimens with obvious hemolysis were excluded.

Abdominal Ultrasonography:
Liver was assessed for: size (span), echogenicity, surface, thickening of portal tracts, portal vein diameter, hepatic veins, inferior vena cava and presence or absence of focal lesions.

Spleen was assessed for: size, echogenicity, splenic vein diameter and presence or absence of collaterals. Other data concerning the gall bladder, both kidneys, pancreas, para aortic region as well as detection of ascites all were fulfilled.

Biopsy of the liver:
N.B: Patients with ascites were excluded from this study.

Histopathological examination:
The stained sections were examined for: Evidence of etiology: HCV, Schistosomiasis. Assessment of necro inflammatory grading and fibrosis staging applying the METAVIR scoring system of **[15].** Where Activity was graded according to the intensity of necro inflammatory lesions: A0=no histological activity, A1=mild activity, A2= moderate activity, A3=severe activity. The stage of fibrosis (F) was assessed on a five Point scale: F0= no fibrosis, F1=Portal fibrosis without septa, F2=Few septa, F3=numerous septa without cirrhosis, F4=cirrhosis.

Statistical Analysis
Data were tabulated, coded then analyzed using the computer program SPSS (Statistical package for social science) version 17.0to obtain

Descriptive statistics were calculated in the form of: A- Mean ± Standard deviation (SD) for quantitative parametric data. **B-** Median and range (Minimum–maximum) for quantitative non-parametric data. **C-** Frequency (Number-percent) for qualitative data.

Analytical statistics:
In the statistical comparison between the different groups, the significance of difference was tested using one of the following tests:-

A-Student's *t*-test:-Used to compare between mean of two groups of numerical (parametric) data. **B-** Mann Whitney U test: Used to compare between two groups of numerical (non-parametric) data. **C-** Kruskal Wallis test**:** Used to compare between more than two groups of numerical (non-parametric) data followed by Mann-Whitney for multiple comparisons.

The sensitivity and specificity of FCI, FIB4, King's score, APRI and API to differentiate between F0-F1 and F2-F4 and also between F0-F2 and F3-F4 fibrosis grades were examined at different cutoff points using ROC curve analysis to determine the best cutoff point as well as the diagnostic power of each test. A *P* - value < 0.05 was considered statistically significant in all analyses.

RESULTS

The study was conducted on 150 patients (cases group) {94 males (62.7%), 56 females (37.3%)}. The (control group) 30 subjects {22 males (73.3 %), 8 females (26.7%)} (Figure 1).

There is a statistically significant difference between cases and control groups (p 0.05) as regards platelets, ALT, AST, ALP, APRI, FCI, FIB4, king's score, and API. Cases group had significantly lower platelets count, higher ALT, AST, ALP levels and higher APRI, FCI, FIB4, king's score and API values (Table 1).

Albumin, INR, Bilirubin, AST, ALP, APRI, FCI, King's score, API & FIB4 where significantly lower, while platelets were significantly higher in patients with METAVIR (F≤1) compared to those with METAVIR (F ≥ 2) (P<0.001) (Table 3).

Albumin, INR, Bilirubin, AST, ALP, APRI, FCI, King's score, API & FIB4 were significantly lower, while platelets were significantly higher in patients with METAVIR (F≤2) compared to those with METAVIR (F≥3) (P<0.001). (Table 4).

As shown in table (5) and figure (20):

- ROC curves of biomarkers for discriminating CHC patients with no or minimal liver fibrosis (F≤1) by METAVIR score from those with significant liver fibrosis (F≥2).
- Using cutoff values (0.705 - 0.125 - 1.445 - 13.06 - 2.5) the areas under the curve (AURC) were (0.79 – 0.90 – 0.89 – 0.86 and 0.77) for APRI , FCI , FIB4 , King's score and API respectively . sensitivity and specificity of FCI and FIB4 (87.6%, 83.1% - 86.5 % , 81.7%) were higher than King's score, APRI and API (81.6 % ,79.7 % - 74.2 ,70.0 % and 77.8 %, 56.7 respectively) in discriminating patients with (F≤2) from those with (F≥2). PPV of FCI and FIB4 (88.6% and 87.5 %) was >King's score (85.5 %) >APRI (78.6%) >API (72.9 %). NPV of FCI and FIB4 (81.7% and 80.3) was > King's score (74.6%) >APRI (72.3%) > API (69.3 %). The accuracy of FCI (85.6 %) was > FIB4 (84.6 %) > King's score (80.8 %) >APRI (72.3 %) >API (69.3 %). Out of assessed fibrosis biomarkers, FCI was the most accurate one for discrimination of significant fibrosis (F≥2).At a cut off value 0.12 it was 87.6% sensitive &86.5% specific with AUROC= 0.90.

As shown in table (6) and figure (21):

- ROC Curves of biomarkers for discriminating CHC patients with (F≤2) liver fibrosis from those with advanced liver fibrosis (F≥3).

Using cutoff values (0.195 - 2.115 - 15.06 - 0.975 and 3.5) the areas under the curve (AURC) were (0.91 - 0.87 - 0.89 - 0.85 and 0.79) for FCI, FIB4, King's score, APRI and API respectively. Increase sensitivity and specificity of FCI and FIB4 (88.1%, 87.6% and 83.05%, 80%) compared with King's score, APRI and API (86.2%,72.7%-74.6%, 76.7% and 78.3%, 72.7%) respectively. PPV of FCI (82.5%) was >FIB4 (73.1%) >APRI (67.7%) >King's score (67.6%) >API (65.3%). NPV of FCI (91.8%) >King's score (88.9%) > FIB4 (87.8 %) > API (83.3 %) > APRI (82.1 %). The accuracy of FCI (87.8%), FIB4 (81.8%), King's score (78.1%), APRI (75.8 %), API (74.7%). Out of assessed fibrosis biomarkers, FCI was the most accurate one for discrimination of advanced fibrosis (F≥3).At a cut off value 0.19 it was 88.1% sensitive & 87.6% specific with AUROC= 0.91.

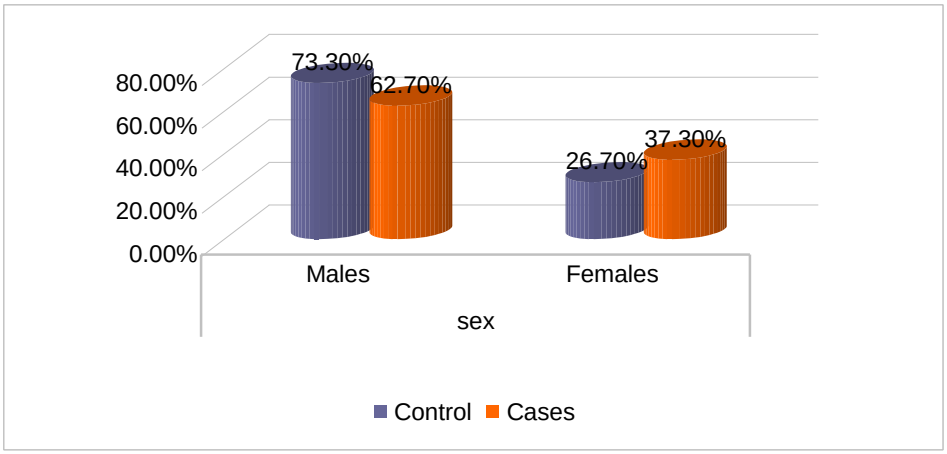

Figure (1): Gender of the studied groups within the study

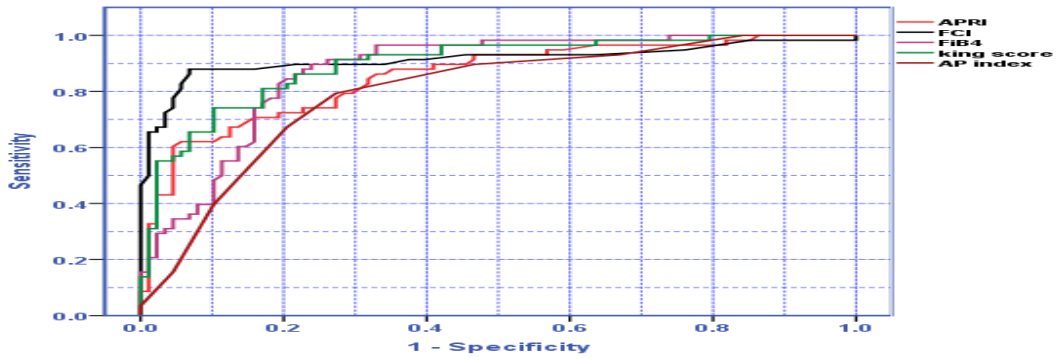

Figure (2): ROC1: Differentiation between (F≤1) and (F≥2) using different fibrosis biomarkers.

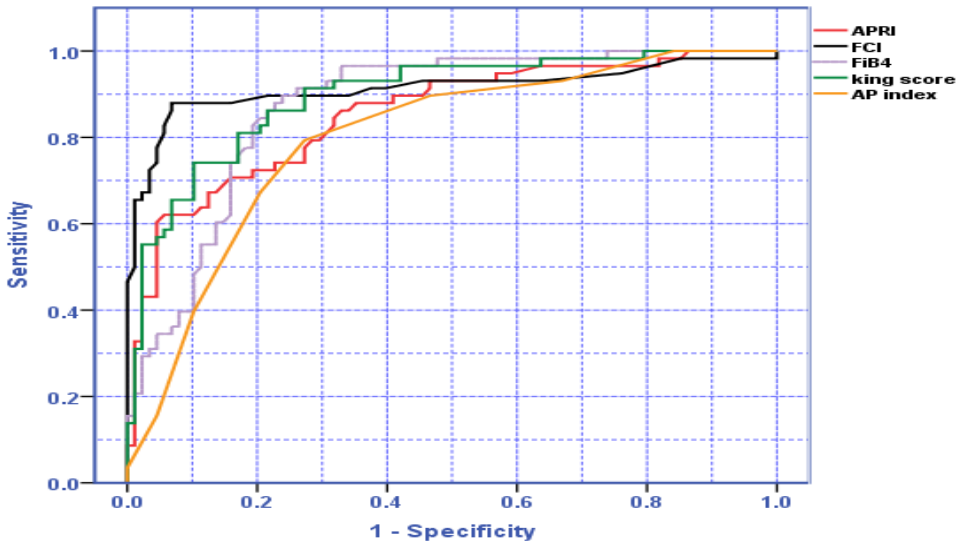

Figure (3): **ROC2**: Differentiation between **(F≤2)** and **(F≥3)** using different fibrosis biomarkers.

Table (1): Laboratory findings of the studied cases and control groups.

	Cases	Control	Test used	P
HB(gm/dl)	13 (11 - 13.8)	13.5 (11.3- 15.5)	*Mann-Whitney*	0.9
WBCsx10^3(/mm^3)	6.2 (4.2 - 11.9)	6 (3.6 11.9)	*Mann-Whitney*	0.9
Plateletsx10^3(/mm3)	158.87 ± 44.66	340.83 ± 47.64	*Unpaired t-test*	<0.001
Albumin(g/l)	4.2 (3.3- 4.8)	4.2 (3.5- 4.9)	*Mann-Whitney*	0.7
INR	1.07 (0.8 - 1.7)	1 (0.6 - 1.3)	*Mann-Whitney*	0.007
Bilirubin(mg/dl)	0.9 (0.5 – 2)	0.85 (0.6 - 1.1)	*Mann-Whitney*	0.14
Alk.ph(IU/L)	131 ± 56	98 ± 23	*Unpaired t-test*	<0.001
AST(U/L)	57 (7 - 198)	24 (6 - 40)	*Mann-Whitney*	<0.001
ALT(U/L)	40 (12 - 73)	21 (6- 40)	*Mann-Whitney*	<0.001
AST/ALT	1.5 (0.18 - 5)	1.15 (0.18 – 6)	*Mann-Whitney*	0.4
APRI	0.86 (0.06- 4.4)	0.15 (0.04 -0.26)	*Mann-Whitney*	<0.001
FCI	0.17 (0.00 - 1.13)	0.06 (0.0 - 0.1)	*Mann-Whitney*	<0.001
FiB4	1.86 (0.07 - 22.77)	0.44 (0.06- 1.11)	*Mann-Whitney*	<0.001
King's score	15.45 (0 - 100.9)	1.6 (0.21 – 2.8)	*Mann-Whitney*	<0.001
API	3 (0 - 8)	0 (0- 1)	*Mann-Whitney*	<0.001

Table (2): Age, Laboratory data and Fibrosis biomarker in patients with different METAVIR fibrosis stages (F0-F4)

		METAVIR FIBROSIS STAGE					P
		F0	F1	F2	F3	F4	
Age (y)	Median	25	35 [a]	43[a]	45.5[ab]	46[ab]	<0.001
	Range	18 - 35	20 - 56	19- 57	27- 59	34 - 56	
Hb (mg/dl)	Median	13.5	13.2	12.9	13	13.45	0.2
	Range	11-13.4	11.5-13.8	11.4-13.8	11.5-14.6	11.8-15.6	
WBCsx10^3 (/mm^3)	Median	6.1	6.4	6.2	5.8	5.95	0.88
	Range	3.6-11	3.6-11.9	3.6 - 11	3.6 - 11.9	4 - 11	
Plateletsx10^3 (/mm^3)	Median	197.5	194.5	161[a]	134[abc]	101[abcd]	<0.001
	Range	167- 289	99-250	122 - 278	100 - 175	69 - 164	
S.albumin (g/dl)	Median	4.5	4.3 [a]	4.2 [a]	3.8[abc]	3.6[abcd]	<0.001
	Range	4.3-4.6	4.1-4.5	4.0-4.8	3.5-4.2	3.3-3.9	
INR	Median	0.9	1	1.04 [a]	1.08 [a]	1.3[abcd]	<0.001
	Range	0.8-1.1	0.95-1.2	1.0 -1.34	1-1.3	1.2-1.7	
S.bilirubin (mg/dl)	Median	0.7	0.75	0.8	1.15[abc]	1.7[abcd]	<0.001
	Range	0.6 -1	0.5-1.1	0.6-1.3	0.7-1.5	1.2 - 2	
Alk.ph (U/L)	Median	76	114 [a]	143 [a]	159[a]	165[ab]	<0.001
	Range	38 - 180	37 - 287	28 - 310	52 - 270	58-200	
AST (U/L)	Median	46	40	50	56	89 [abcd]	<0.001
	Range	7-145	10-148	12-102	10 - 198	60 -135	
ALT (U/L)	Median	37	41	40	43	44	0.07
	Range	12 - 55	19 - 57	19 - 66	12 - 64	23 - 73	
AST/ALT	Median	1.39	1.09	1.40	1.58	2.07[abc]	0.001
	Range	0.18 - 3.21	0.19 - 4.11	0.24 - 4.02	0.18 - 5.25	1.06 - 4.17	
viral load (IU/ml) X10^3	Median	588.5	728	662.5	402	325	0.3
	Range	17.9 - 2920	17.9 - 2920	22.4 - 2290	21.2 - 2100	18 - 4880	
APRI	Median	0.47	0.53	0.69	0.95	2.06[abcd]	<0.001
	Range	0.06-1.67	0.1-2.57	0.18-1.69	0.16-4.4	1.1 - 3.07	
FCI	Median	0.06	0.11 [a]	0.18[ab]	0.3[abc]	0.6[abcd]	<0.001
	Range	0.03- 0.1	0.03 - 0.34	0.03- 0.47	0.07- 0.6	0.0-1.13	
FIB4	Median	0.5	1.06 [a]	1.9 [a]	2.4[ab]	6.1[abcd]	<0.001
	Range	0.07 - 1.6	0.23 - 4.8	0.5 - 9	0.5 - 3.7	2.6 - 22.7	
King's score	Median	4.2	7.8	13.8 [a]	20.7 [ac]	55.9[abcd]	<0.001
	Range	0 - 19.6	1.6 - 65.5	4.2 - 34.3	3.6 - 54.3	28.6 -100.9	
API	Median	2.0	3.0	3.0 [a]	5.0[ac]	6.0[abcd]	<0.001
	Range	0 - 4	0- 7	0 - 7	1 - 7	3 - 8	

P: Probability Test used: Kruskal-Wallis test followed by Mann-Whitney for multiple comparisons
a: significance relative to F0 group c: significance relative to F2 group
b: significance relative to F1 group d: significance relative to F3 group

Table (3): Age, Laboratory data and fibrosis biomarkers in patients with (F≤1) METAVIR stages compared to those with (F ≥2) stages.

	F≤1		F≥2		P
	Median	**Range**	**Median**	**Range**	
Age (y)	29	18 - 56	45.00	19-59	<0.001
Hb (gm/dl)	13.5	11-13.8	13	11.4 -13.8	0.3
WBCs x10^3 (/mm^3)	6.2	3.60-11.9	6.2	4.0-11.90	0.9
Plateletsx10^3(/mm^3)	195.5	99-289	133	69-278	<0.001
S.albumin (gm/dl)	4.4	4.1-4.6	3.90	3.30-4.80	<0.001
INR	1	0.8-1.5	1.14	1-1.7	<0.001
S.bilirubin (mg/dl)	0.7	0.5-1.1	1.20	0.6-2	<0.001
Alk.ph (IU/l)	88	37-287	158	28-310	<0.001
AST (U/L)	43	7 - 148	68	10 - 198	<0.001
ALT (U/L)	38	12 - 57	42	12 -73	0.02
AST/ALT	1.2	0.18 - 4.11	1.67	0.18-5.25	0.005
HCV-RNA viral load (IU/ml)x10^3	728	17.9 -2920	402	18-4880	0.2
APRI	0.49	0.06-2.57	1.2	0.16-4.4	0.02
FCI	0.07	0.03- 0.34	0.31	0-1.13	<0.001
FIB4	0.75	0.07 - 4.8	2.8	0.5 - 22.7	<0.001
King's score	7.18	0 - 65.5	23.11	3.6 -100.9	<0.001
API	2	0 - 7	5	0 - 8	<0.001

Table (4): Age, Laboratory data and fibrosis biomarkers in patients with (F≤2) METAVIR stages compared to those with (F ≥3) stages.

	METAVIR (F≤ 2) stages		METAVIR (F ≥3) stages		P
	Median	**Range**	**Median**	**Range**	
Age (y)	32.5	18 - 57	46	27 - 59	<0.001
Hb (gm/dl)	13.00	11-138	13	11.5-15.6	0.8
WBCsX10^3 (/mm)	6.2	4.05-11.9	5.8	4 -11.9	0.7
PlateletsX10^3(/mm)	185.5	99-289	116	69 - 175	<0.001
S.albumin (gm/dl)	4.4	4 - 4.8	3.7	3.3 - 4.2	<0.001
INR	1	0.8 - 1.5	1.2	1 - 1.7	<0.001
S.bilirubin (mg/dl)	0.8	0.50 - 1.3	1.4	0.7 - 2	<0.001
Alk.ph (IU/l)	105.5	28 - 310	161	52 - 270	<0.001
AST (U/l)	46	7 -148	79	10 - 198	<0.001
ALT (U/l)	39.3	12.7 - 65.9	43.6	12.3 -73.2	0.02
AST/ALT	1.25	0.18 - 4.11	1.79	0.18 - 5.25	0.001
HCV-RNA viral load (IU/ml) x10^3	728	17.9 - 2920	325	18 - 4880	0.06
APRI	0.59	0.06 - 2.57	1.5	0.16 - 4.4	<0.001
FCI	0.1	0.03 - 0.47	0.45	0 - 1.13	<0.001
FIB4	1.08	0.07-9	3.21	0.57-22.77	<0.001
king score	9.66	0.0 - 65.53	36.49	3.63 -100.9	<0.001
API	2	0 - 7	5	1 - 8	<0.001

DISCUSSION

Viral hepatitis C is a major global health burden affecting 180 million people worldwide. The severity of the disease associated with HCV infection varies between asymptomatic, acute and chronic hepatitis, cirrhosis and HCC **[16].** Liver fibrosis is the main predictor of the progression of CHC, and its assessment can help to determine therapy **[3].** Liver biopsy is considered the gold standard for staging fibrosis, however it is invasive, expensive, has both intra- and inter-observer variability as well as sampling errors and it is not suitable for all patients who may get severe side effects that might lead to death **[18]& [19].** Blood-based biomarkers offer a number of advantages over liver biopsy, including safety, cost-savings and wide spread accessibility **[20].** Several scoring systems**,** like AST to ALT ratio (AAR), AST-Platelet ratio index (APRI), Fibro test (FT), Fibrosis Index (FI) and FIB-4**,** with different thresholds to predict presence or absence of fibrosis or cirrhosis in patients with CHC had been proposed. However, mild fibrosis (F0) to end stage cirrhosis cannot be accurately distinguished using a single system **[7].** The fibrosis–cirrhosis index (FCI) was proposed comprising serum alkaline phosphatase (ALP), bilirubin, albumin and platelet count, where FCI=[(ALP × Bilirubin)/(Albumin × Platelet count)]. It was supposed to be efficient for none invasive staging of liver fibrosis besides being simple and inexpensive **[8]**.

For all these considerations, the main aim of the present study was to assess the accuracy of the fibrosis-cirrhosis index (FCI) for non-invasive staging of liver fibrosis in patients with CHC, compared to the liver biopsy findings.

In the current study serum bilirubin showed a statistically highly significant (P<0.001) rise with advancement of stages hepatic fibrosis from F0 to F4 respectively as shown in table (2). This comes in agreement with other studies who reported that serum bilirubin level showed a statistically significant increase with advancement of hepatic fibrosis from F1 to F4 **[8,17]**. On the other hand, another study reported that Serum total bilirubin did not differ significantly within different fibrosis stages **[21]**.

In the present study the level of serum albumin showed a statistically highly significant decline with advancement of stages of hepatic fibrosis from F0 to F4 as shown in table (2). This comes in agreement with other studies that reported that serum albumin showed a statistically significant decline with advancement of hepatic fibrosis **[8]**. In the contrary side, this result does not come in agreement with another study which reported a non-significant change in serum albumin level with different stages of fibrosis **[17].**

In the current study the platelet count showed a statistically highly significant decline with advancement of hepatic fibrosis as shown in table (2). This comes in agreement with other studies that reported a highly significant decline in platelet count with the progression of fibrosis **[22].** On the other hand another study reported that differences in platelet count were not significantly affected with the progression of liver fibrosis. This disagreement may be explained by the character of patients included in their study as they had genotype 1 and the small number of their cases with advanced hepatic fibrosis stages compared to the present study **[23]**.

The present study showed that APRI had a statistically significant higher values with advancement of hepatic fibrosis, as shown in table (2). Similar results obtained by study **[8]**.

In the present study, the age-platelet index (API) showed a statistically significant higher values with advancement of hepatic fibrosis, as shown in table (5). These results came in agreement with study which created this index. Reported that there was a significant positive correlation between API and necro-inflammatory activity and stage of fibrosis **[24]**.

In the present study, FIB4 showed a statistically higher significant values with advancement of hepatic fibrosis, as shown in table (2). Similar results were obtained by study which reported that FIB4 was significantly increased with progression of fibrosis stages **[8]**.

In the present study, King's sore showed a statistically significant higher values with advancement of hepatic fibrosis as shown in table (5).

In the present study FCI showed a statistically significant higher values with advancement of hepatic fibrosis (P<0.001), as shown in table (2).

In the present study, the independent variables for prediction of liver fibrosis were FCI, FIB4, King's score, API and APRI. Using ROC curves to assess and compare the diagnostic accuracy of blood markers as FCI, FIB4, King's score, API and APRI in patients with different stages of

liver fibrosis. As shown in table (5) and figure (20).

Acknowledgment

The authors would thank Dr. Amr Ibrahim Eisa and Dr. Ayman Elkholi who helped in conducting this study.

Funding: None.
Conflicts of interest: None.
Ethical Approval:Approved.

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Peer reviewer: Rashed Hasan ,Professor of Tropical Medicine and Hepatogastroenterology, Faculty of Medicine, Zagazig University, Egypt. **Editor: Tarik Zaher**, Professor of Tropical Medicine and Hepatogastroenterology, Faculty of Medicine, Zagazig University, Egypt

Impact of Training Education Program on Improving of Nurses Performance Regarding Infection Control in Endoscopy Unit

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Key words:
Educational Training Program, Gastrointestinal Endoscopy, Infection control, Endoscopy Reprocessing

Background and study aim: Endoscopes are complex and reusable devices. Understanding infection control in the context of endoscopy is important in reducing the transmission of infection. This study aims to evaluate the effect of an educational program on improving nurse's knowledge and practice regarding infection control in endoscopy unit at Zagzig University Hospital.

Subjects and methods: A quasi experimental research design was used. The study was conducted in the Endoscopy Unit at Zagazig University Hospitals. The study samples were all available endoscopy nursing staff . First tool was Questionnaire sheet, to assess nurses' knowledge. The second tool was observational checklist to assess nurses' practice.

Results: this study revealed that two third of nurses were in the age group of more than 40 years with mean age 42.2 ± 8.4 years and the majority of the sample had more than 10 years experience. There was improvement in total level of nurses'knowledge and practice regarding infection control with highly statistically significant difference between pre- post and pre-follow up program phase as regarding to infection control.

Conclusion: The implementation of health educational program had improved nurse's knowledge and practice regarding endoscopy infection.

Recommendations: Continuous educational and infection control training programs are recommended in endoscopy units. So incorporation of such interventions apply in all endoscope unite all over Egypt.

INTRODUCTION

The endoscopy itself is not dangerous, but the current cleaning process used between procedures leaves patients susceptible to infection and troubles many healthcare practitioners **[1].** The Emergency Care Research Institute**,** listed that, inadequate reprocessing of endoscopes as one of its "Top 10 Technology Health Hazards," asserted that reprocessing guidelines should be continuously reviewed and technicians should be better trained **[2]**. Bommarito reported that about 15% of endoscopes in Untied States (US) hospitals failed to achieve an accepted standard of cleanliness after liquid reprocessing (the prevailing disinfection process used between patient procedures in his study, duodenoscopes were the dirtiest at a 30% contamination rate, and colonoscopes were the cleanest at a 3% contamination rate **[3]**.Peery et al. **s**tated that more than 18.6 million gastrointestinal endoscopies were performed at least every year in the US alone **[4].** While The Gastrointestinal Endoscopy Center at Zagazig University Hospital offers a highly specialized medical service for many clients suffering from different GIT problems. The monthly reports of that center indicated that, during the first four months of 2013, more than 500 GIT endoscopy were performed each month**[5]**. Approximately 3 million health care workers (HCWs) experience percutaneous exposure to blood borne viruses (BBVs) each year. This results an estimated 16,000 hepatitis C, 66,000 hepatitis B, and 200 to 5000 human immunodeficiency virus (HIV) infections annually.

More than 90% of these infections are occurring in low-income countries and most of are preventable. Several studies report the risks of occupational BBV infection for HCWs in high-income countries where a range of preventive interventions have been implemented. In contrast, the situation for HCWs in low-income countries is not well documented, and their health and safety remains a neglected issue **[6]**.

Nurses have a professional and legal responsibility in preventing cross-infection from reaching to the patient **[7]**. Teaching and training are essential for the nursing staff members to improve the quality of health care and to acquire new knowledge and skills. Educational programs are considered as means for providing nurses with theoretical and technical information needed to acquire new skills and to continually improve nursing practice. Also help nurses to accept responsibilities for their professional development. The knowledge and practices of nurses in relation to infection control were deficient. The implementation of a specially developed program has led to statistically significant improvements in nurses' knowledge and practices **[8]**.The well-trained nurse is the backbone of a well-organized department. Today's technical and scientific advances in nursing and increasing consumer demand for high quality health care urged the nurse to keep current in a field that is exploding with new information and increases the need for developing nursing staff education **[9]**.

Significance of the study

Infection control has recently received a considerable amount of attention. Each year, there are more than 2 million healthcare-associated infections causing 90,000 deaths in the United States. Gastrointestinal (GI) endoscopes are used in more than 10 million procedures annually **[10]**, and contaminated endoscopes have been linked to more outbreaks of healthcare-associated infections than any other medical device **[11]**.All staff in any setting where gastrointestinal endoscopy is performed must adhere to infection control principles that will maintain a safe environment, free from the possibility of spreading disease to patients and co-workers. This is true regardless of the setting (hospital, clinic, ambulatory care center, and office), relative to any and all types of gastrointestinal (GI) procedures performed. Each individual who reprocesses instruments should complete the initial infection control orientation and reprocessing competency. Competency review and infection control updates should be validated and documented annually **[12]**.

Aim of study:

This study aimed to evaluate the effect of an educational program on improving nurse's knowledge and practice regarding infection control in endoscopy unit at Zagazig University Hospital.

SUBJECTSAND METHODS

Research design:
A quasi experimental design was utilized in this study.

Research Setting:
The study was conducted in endoscopy units in Zagazig University Hospitals.

Subjects:
The subjects of the study compromised of all available endoscopy nursing staff.

Tools for Data Collection:
Two tools were used for data collection from endoscopy nurse which include:

1- Questionnaire sheet to assess the endoscopy nurses knowledge regarding infection control (Pre, during, and Post) procedure.

2- Observational check list for endoscopy nurses to assess their practice level regarding infection control (Pre, during, and Post) procedure.

Tool I -Interview questionnaire sheet :
It was developed and written in Arabic language by researcher after reviewing relevant literature and agreed upon by a panel of medical surgical experts to assess nurse's knowledge in form of multiple choice questions. It was divided into two major parts:

Part I: It was developed to assess socio-demographic characteristics of nurses such as age, education, occupation, experience in endoscopy unit, training course regarding infection… etc
It is composed of (12) closed ended questions (question 1-12)**.**
Part II: It was developed to assess the endoscopy nurse's knowledge related to infection, chain of infection, Universal Precautions, and principles of aseptic technique, hand wash, waste management, nurses' knowledge about cleaning, level of

disinfection, sterilization, and endoscopy reprocessing. It is composed of 193 questions.

Scoring System:
The rating scale was graded as follows: correct answer (1) and uncorrected one & don't know (zero). Rating scale of all questions was collected. Total score was 215 degrees. Total score represented 100%. It was evaluated as follows:

- Satisfied > 75%
- Unsatisfied ≤ 75%.

Tool II: Observational checklist :
It was developed by the researcher after reviewing a literature**[13-17]** to assess nurses' practices of infection control precautions in endoscopy unit.It is consisted of two checklist as following:

I- General observation checklist: for endoscopy nurses. It is consisted of 28 steps;total degrees equal 51 degrees

II- Reprocessing checklist: for endoscopy nurses. It is consisted of 125 steps;total practice steps 161 equal 176 degrees

Scoring System:
The rating scale was graded as follows: done correctly (1) one degree and not done or if the nurse did it incorrectly (0) zero degree. Total score of all practices were176 degrees. Total score represented 100%. Rating scale of all practices was collected and distributed as follows:

- Done correctly >75%
- Not done≤ 75%.

Pilot study:
A pilot study was conducted on 4nurses in order to test the clarity and applicability of the study tools. Required modifications were done in the form of adding or omission of some questions. The time needed to fill in the questionnaire was about (30-45 minutes). Nurses involved in the pilot study were excluded from the main study subjects.

Infection control educational program:
Infection control educational program was developed by the researcher based on the result of the nurses' practice, the related current literature and available structure guidelines. The content of the teaching guidelines included sixteen sessions that divided in two types of sessions: educational and training sessions. An educational program about infection control in endoscopy unit.

General objectives
By the end of this educational program, the nurses were able to upgrade nurses' knowledge, and practice related infection in endoscopy unit

Program Descriptions:
The program is designed to be practical in nature addressing the nurses' knowledge and practices necessary for performing infection control measures in the endoscopy unit**.** The program construction goes through the following phases:

1- Pre-planning phase: Included;

1. The framework for the program.
2. Setting the program, general and specific objectives.
3. Allocation of the program resources and facilities (Setting and printed materials).
4. Construction of evaluation device to measure the program effectiveness (pre-/ post-test and follow up questionnaire sheet, and observational checklist)

2- Planning phase: Included;

1. Determining the program strategies (time – table of sessions, teaching methods, media used, learner's activities and evaluation methods).
2. Selecting the teaching place (class room, nursing station, endoscopy unit).
3. Determining the program finances (supplied by the researcher such as all printing materials and media.
4. Determining the learning objectives of the program.
5. Determining the learner characteristics.
6. Determining the learning content of the program.
7. Identifying the teaching methods and determine teaching media.
8. Determining the evaluation tools.

3- Implementation phase: Included;
The implementation of this program was covered over small sessions, including theoretical and practical content developed and selected to meet the participants' needs and correspond to their interaction and level of understanding as stated by the pilot study. Teaching was done through classical lectures and group discussion which were strengthened by demonstrating the role played by using suitable teaching aids prepared specially for the program such as lecture, handout, posters, colored pictures and simulators.

The program was implemented through the theoretical and practical sessions. Informed participant with time tables as follow:

1. Maintaining of administration acceptance.
2. Timing of the program implementation, which used to be in the morning and the follow up used to be conducted in endoscopy (9 AM o'clock until 1 PM o'clock).
3. Informing the nurses with time table chart.
4. Conducting the program.

The educational program for nurses working in endoscopy unit included a guidance book which covered theoretical and Practical parts through the following items:

Theoretical part included:

Theoretical part: it covered by two sessions about definition of endoscope, uses, importance, indication, patient preparation, and complication, and two session covered definition of infection, nosocomial infection, chain of infection, disease transmitted through endoscope, tow session about standers universal precautions, and aseptic techniques.

Practical part included:

Practical part: 10 sessions for practical, it composed of the application of patient preparation before procedure, care during procedure, and care after procedure, endoscopy reprocessing as (pre-cleaning, cleaning, rinsing, disinfection, drying, and storage. Demonstration and re-demonstration several time until correct done of the pre- during- and post endoscopy use.

4- Evaluation phase:

Evaluation was done immediately post program implementation and after six months of the follow up phase. The follow up was done to assess the nurses knowledge and practice of infection control and prevention in endoscopy unit through comparing the results of the pre, post and follow up phases to assess the continuous effect of educational program and the sheets were answered within 30-45 minutes, then collected.

III- Administrative design:

An official permission was obtained from Medical director and nursing director of the study setting. After clear explanation about the study title, aim of the study and setting where the study would be conduct and its benefits.

Ethical consideration:

The purpose of the study was explained to the nurses and oral consent was obtained from them to participate in this study. They were given an opportunity to withdraw from the study without given a reason and they were assured that anonymity and confidentiality of information was protected. Ethics, values, culture, and beliefs were respected.

VI -Statistical Analysis

All collected data were organized, categorized, tabulated, entered, and analyzed by using SPSS (Statistical Package for Social Sciences); a software program version 15, which was applied to frequency tables and statistical significance. The statistical significance and associations were assessed using, the arithmetic mean, the standard deviation (SD), Wilcoxon Signed Ranks test (Z test), Pearson chi-square test (X^2) and Pearson Correlation (r) to detect the relation between the variables. Graphs were done for data visualization and using Microsoft Excel.

RESULTS

Table 1: It was found that two third 66.7% of nurses were in the age group of more than 40 years with mean age 42.2 ± 8.4 years. In relation to the training 63.3% of them were not receive any training. Meanwhile ninety percent of the sample had completed their Secondary nursing school education while 10% had Bachelor in Nursing. As regards to occupation most of studied nurses 80% were staff nurse. As well as majority 93.3% of the sample had more than 10 years of experience with mean 23.8 ± 8.6 years.

Table (1) shows also 60% of nurses had medical examination pre working, while only 10% of them had medical examination during working. Figure 1: shows that regarding to Hepatitis B Vaccination; 76.7% of study sample were received the vaccine.

Table (2) and Figure (2) show that there was highly statistically improvement in of total nurses knowledge including :infection in general, nosocomial infection, principle of disinfection, infection control precaution, and endoscopy reprocessing when comparing with pre and post, pre and follow up implementation of educational program, at P≤0.01**. Also represented that total nurses knowledge in per program was 3.3% only

satisfactory increased to 80% in post phase and 70% in follow up phase of educational program.

Table (3) shows that there were highly statistically improvement of Practice Level regarding standard precaution as hand washing- wear protective cloth (Wear overshoes, wear mask and goggles, wear sterile gown, wear sterile gown, and wear sterile gloves), when comparing with pre and post, pre and follow up of implementation of educational program, at (p≤ 0.01**).

Figure (3) shows that there were highly statistically improvement of practice level regarding adherence to infection control principles restrictedly during procedure as satisfactory level increase from 10% in pre phase to 83.3% inpost, pre and follow up of implementation of educational program.

Table (4) Shows that regarding to Nurses' Practices of Infection Control Precautions post endoscopy procedures there were highly statistically improvement of practice level regarding of infection control in all table items namely pre-cleaning, -leakage testing, manual cleaning, high level disinfected, manual disinfecting, endoscopy storage, and nurses total post procedure when comparing with pre and post, pre and follow up of implementation of educational program, at (P≤0.01**).

Table (5) and figure (4): Illustrated that there were a highly significant statistical improvement of total nurses practice level regarding infection control in endoscopy unit (P≤0.01**). As satisfactory level was 0% in pre-program increased to 80% post, and 76.7% follow up of implementation of educational program.

Table(1): Socio Demographic Characteristic of Studied Nurses n= (30)

Socio Demographic Characteristic	No (30)	%
Age groups (in years)		
< 40y	10	33.3%
> 40y	20	66.7
Range	27: 57	
Median	41.5	
$\bar{X}$ ± **SD**	42.2 ± 8.4 years	
Level of education		
Bachelor in Nursing	3	10%
Secondary nursing school	27	90%
Occupation		
Chief of unit	3	10%
Nursing supervisor	3	10%
Staff nurse	24	80%
Years of experience		
< 10y	2	6.7%
> 10y	28	93.3%
Range	6 :40	
Median	24	
$\bar{X}$ ± **SD**	23.8 ± 8.6	
Training		
Attend	10	33.3%
Not Attend	20	63.3%
Medical examination pre working:		
Yes	18	60%
No	12	40%
Medical examination during working:		
Yes	3	10%
No	27	90%

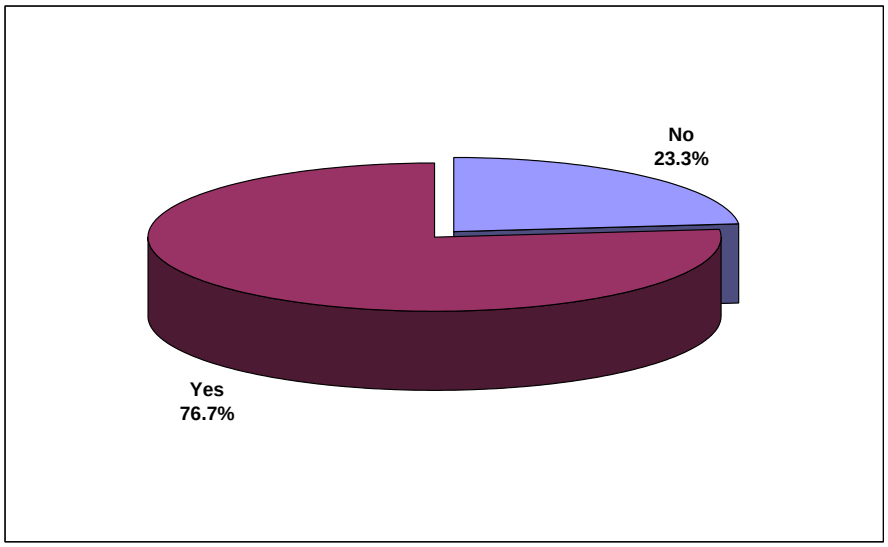

Figure (1) : Hepatitis B Vaccination

Table (2): Total Nurse's Knowledge Regarding Infection Control in Endoscopy Unit Throughout The Study Phases (n=30):

Total knowledge	Nurses' Knowledge						Wilcoxon signed rank test			
	Pre		Post		Follow up		Pre/Post		Pre/FU	
	N	%	n	%	N	%	Z	P	Z	P
Satisfactory	1	3.3	24	80.0	21	70.0	4.79	.000**	4.26	.000**
Unsatisfactory	29	96.7	6	20.0	9	30.0				

*Significant at P≤ 0.05 level
**highly significant at P≤ 0.01 level

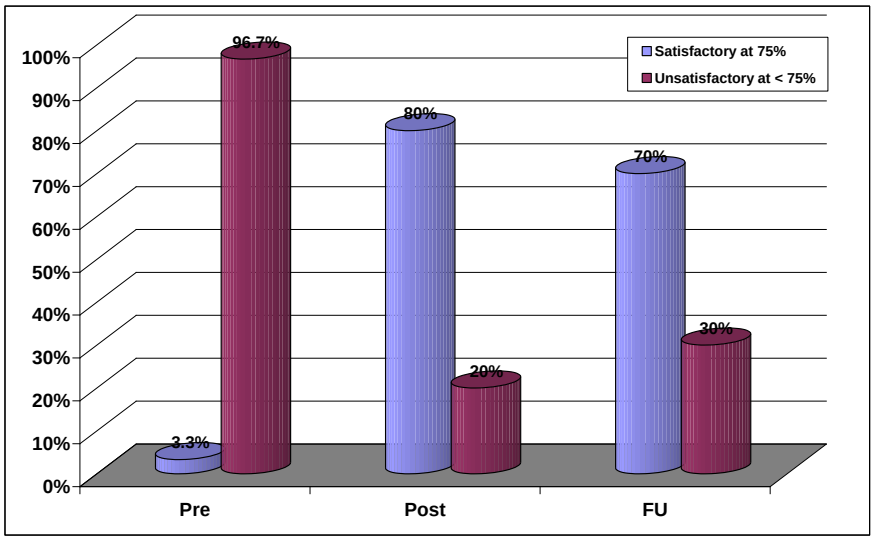

Figure (2): Total nurse's knowledge regarding infection control in endoscopy unit

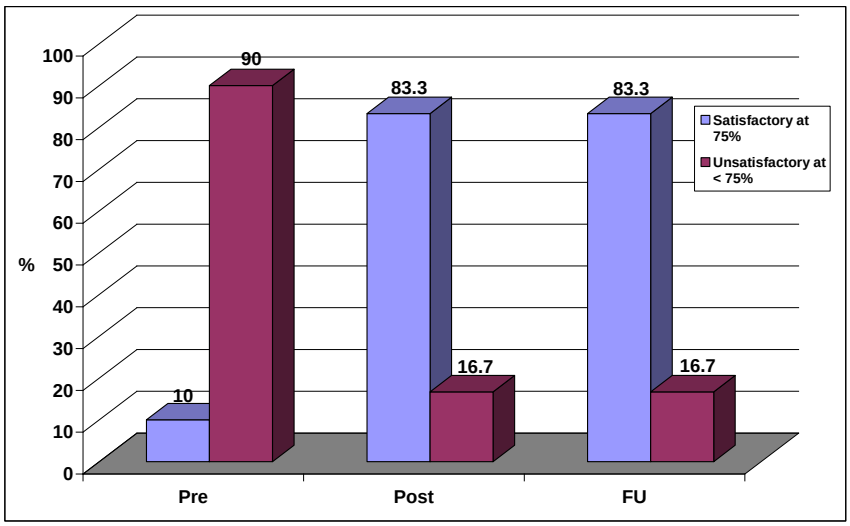

Figure (3): Adherence to infection control principles restrictedly during procedure

Table (3): Frequency Distribution regarding Nurses' Practices of Infection Control Precautions Prior to Endoscopy Procedures Throughout The Study Phases (n=30)

Procedure		Nurses' Practices						P- value	
		Pre		Post		Follow up			
		N	%	N	%	N	%	Pre/Post	Pre/FU
A- Hand washing	Satisfactory	1	3.3	28	93.3	26	86.7	0.000**	0.000**
	Unsatisfactory	29	96.7	2	6.7	4	13.3		
B- Wear protective cloth:									
1-Wear overshoes	Satisfactory	0	0.0	13	43.3	12	40.0	0.000**	0.001**
	Unsatisfactory	30	100.0	17	56.7	18	60.0		
2- Wear mask and goggles	Satisfactory	0	0.0	24	80.0	21	70.0	0.000**	0.000**
	Unsatisfactory	30	100.0	6	20.0	9	30.0		
3- Wear sterile gown	Satisfactory	3	10.0	11	36.7	11	36.7	0.033*	0.033*
	Unsatisfactory	27	90.0	19	63.3	19	63.3		
4- Wear sterile gloves	Satisfactory	6	20.0	30	100	30	100	0.000**	0.000**
	Unsatisfactory	24	80.0	0	0.0	0	0.0		

*Significant at $P \leq 0.05$ level
**highly significant at $P < 0.001$ level

Table (4): Frequency Distribution of Nurses' Practices regarding Infection Control Precautions Post Endoscopy Procedures

Procedure		Nurses' Practices						p- value	
		Pre		Post		Follow up			
		N	%	n	%	N	%	Pre/Post	Pre/FU
Pre-cleaning	Satisfactory	2	6.7	24	80.0	24	80.0	0 .000**	0.000**
	Unsatisfactory	28	93.3	6	20.0	6	20.0		
Leakage testing	Satisfactory	0	0.0	21	70.0	21	70.0	0.000**	0.000**
	Unsatisfactory	30	100	9	30.0	9	30.0		
Manual cleaning	Satisfactory	0	0.0	24	80.0	23	76.7	0.000**	0.000**
	Unsatisfactory	30	100	6	20.0	7	23.3		
High level disinfection	Satisfactory	4	13.3	22	73.3	22	73.3	0.000**	0.000**
	Unsatisfactory	26	86.7	8	26.7	8	26.7		
Manual disinfection and rinse	Satisfactory	0	0.0	15	50	14	46.7	0.000**	0.000**
	Unsatisfactory	30	100	15	50	16	53.3		
Automated disinfectionandrinse	Satisfactory	4	13.3	11	36.7	11	36.7	0 .035 **	0.035**
	Unsatisfactory	26	86.7	19	63.3	19	63.3		
Endoscopy handling	Satisfactory	25	83.3	29	96.7	28	70.0	0.102	0.257
	Unsatisfactory	5	13.7	1	3.3	2	30.0		
Endoscopy storage	Satisfactory	11	36.7	30	100.0	30	100	0.000**	0.000**
	Unsatisfactory	19	63.3	0	0.0	0	0.0		
Total post procedure	Satisfactory	0	0.0	16	53.3	12	40.0	0.000**	0.001**
	Unsatisfactory	30	100.0	14	46.7	18	60.0		

*Significant at P≤ 0.05 level
**highly significant at P < 0.001 level

Table (5): Total Nurses' Practices of Infection Control in Endoscopy Unit Throughout the Study Phases (n=30)

Total practices	Nurses' Practices						Wilcoxon signed rank test			
	Pre		Post		Follow up		Pre/Post		Pre/FU	
	N	%	N	%	N	%	Z	P	Z	P
Satisfactory	0.0	0.0	24	80.0	23	76.7	4.89	0.000**	4.97	0.000**
Unsatisfactory	30	100	6	20.0	7	23.3				

*Significant at P≤ 0.05 level **highly significant at P < 0.001 level

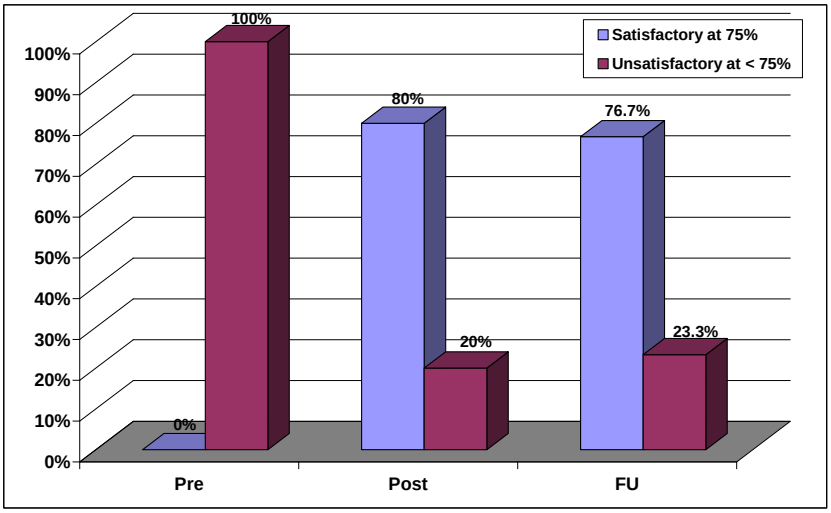

Figure (4): Total Nurses' Practices of Infection Control in Endoscopy Unit

DISCUSSION

In relation to nurses' characteristics, the finding of the present study revealed that more than two third of nurses were in the age group of more than 40 years with mean age 42.2 ± 8.4 years. This result was in disagreement with Abolwafa et al. who reported that the majority of the studied sample was in the age group from 20-30 years old **[18]**.Also Soliman found, in his study, that less than one third of the nurses were within age group of 25 – 35 years with a mean age of 32.61 ± 5.17 years **[17]**.

In relation to education level the majority of the sample had completed their secondary nursing school education while only ten percent of them had Bachelor in Nursing. This result was in agreement with El ghatey et al. who found that the majority of nurses had diploma of nursing while minority of them had bachelor degree in nursing **[6]**. This result was nearly the same as that reported by Abolwafa et al. who reported that most of nurses have Diploma of Secondary Nursing School **[18]**.

As regards to occupation the present study revealed that, most of studied nurses were staff nurse. As well as majority of the sample had more than 10 years of experience with mean 23.8 ± 8.6 years. This may be due to stop of supplement new nurses into work in endoscopy unit.All of them were female and about one third of the studied sample had previous attendance to training courses about infection.

These findings in contrast with Soliman who found that, slightly more than half of the nurses had more than 10 years of experience in hemodialysis unit (56.8%).The highest percentage of the studied nurses did not receive training program about infection control (75.5%)**[17]**. This result also was in disagreement with Abolwafa et al. who reported that the current job experience from 5<10 years. Additionally, about 10% of the studied sample had previous attendance training courses about infection **[18]**.

Also, Hosoglu et al. mentioned that more than two-thirds of the participants, in their study, had not been trained on the prevention of blood-borne diseases and the risks of occupational injuries[19]. The finding of the current study can be explained in the light of the belief that training within the unit is enough , also it may be due to lack of nurses' interest about the infection control training that done by the infection control team in the hospital. This justify is appreciated by Kandeel et al. who stated that the experience of developing and implementing an infection control (IC) program in Egypt has highlighted many constraints that are common in developing countries, including the lack of trained health care professionals and IC specialists who can implement IC programs**[20]**.

In relation to pre-employment screening and periodic examination during the work, three fifth of nurses had medical examination before working, while only ten percent of them had medical examination during working, thus there is increased risk of spread of infection, absenteeism and disability. This finding goes in the same line with Ghonaiem, and Mohammed who found that the majority of nurses had no pre employment screening and periodic examination during the work**[21,22]**.

Regarding to Hepatitis B Vaccination the current study revealed that, three fourths of study sample were received the vaccine. This is may be due to the increased awarenessof the hospital administration about the importance of the vaccine. Also Ghonaiem, and Mohammed revealed that the majority of nurse had not immunized against viral hepatitis **[21,22]**. This result is in agreement with EL-Badawy who found that, in the Liver Institute and in Shebin EL Kome Teaching Hospitals, Menofuya, Egypt, about 8% of nurses had positive hepatitis B surface antigen, 28% of them had positive anti- hepatitis B surfaces, and 24% had positive anti-hepatitis C**[23]**. This finding also consistent with Daniels who mentioned that the nurses should be vaccinated to prevent the potential risk of HBV **[24]**. Our results is consistent with those of Duval who assured that, HBV can be prevented by the vaccine **[25]**.

The current results shows that there was highly significant statistical difference regarding the improvement in the total nurses knowledge including (infection in general, nosocomial infection, principle of disinfection, infection control precaution, and endoscopy reprocessing) when comparing pre and post, pre and follow up implementation of educational program, (P≤0.01**). This study also revealed that the total nurses knowledge in per program was 3.3% only satisfactory increased to most of study subject in post phase and more than two third in follow up phase of educational program.

This findings are in agreement with those of Sliman who mentioned that, there were an improvement in nurses knowledge about infection control precaution throughout the program **[26]**. Also our results going parallel with those of Soliman who mentions that the majority of nurses were having good level of knowledge about infection control measures after implementation of program regarding infection control **[17]**.

These finding is in agreement with Abd Elaziz et al. who reported an improvement on nurse's knowledge pre and post implementation of the infection control program. There was a statistical significant difference ($p<0.01$) between the improvement of nurse's knowledge and the implementation of the infection control program**[27]**. In additional the present study finding was supported by the study carried out by Marchiam, and Keith who stated that, after implementation of IC program the nursing staff had good knowledge about infection control**[28]**. This was in line with study done by Mohamed, and Wafa who stated that the results of their study emphasized that scores of knowledge and practice among studied subjects were increased after implementation of the infection control program **[29]**.

The current results revealed that there were highly statistically significant improvement of practice level regarding standard precaution, as hand washing wear protective cloth (Wear overshoes, wear mask and goggles, Wear sterile gown, Wear sterile gown, and Wear sterile gloves) when comparing with pre and post, pre and follow up of implementation of educational program ($p\leq 0.01$**).

In the same line with these findings Sliman also reported that the majority of observed nurses had adequate practice scores in relation to hand washing, wearing sterile and non sterile gloves. While the least percentage of studied samples had adequate practice related to wear mask. These findings indicate a progressive increase in practice scores after program implementation **[26]**. This finding supported by study carried by Zatton who showed that (66.7%) of their study sample wash their hands with soap and water immediately after contact with blood or other body fluid **[30]**.

These results are in agreement with those of Abd El Lateef who reported a nurses practice score pre/post program implementation regarding wearing protective clothes **[31]**. Also this is supported by El Ghatey et al. who stated that, as regard to sterile glove technique, minority of nurses showed unsatisfactory level of practice with the same score in the pretests. This could be attributed to the lack of information regarding the importance of using sterile gloves in aseptic technique and lack of motivation **[6]**.

The current results revealed that none of nurses had satisfactory practice in leakage testing, manual cleaning, and - manual disinfecting pre-program and improved to (more than two third, most, and half) of nurses in post program. As regarded to importance of manual cleaning, Society of Gastroenterology Nurses and Associates mentioned that, meticulous manual cleaning of endoscopes and accessories is critical to the success of subsequent disinfection. Manual cleaning refers to the physical removal of organic material and/or soil. The presence of residual organic material and/or soil may protect microorganisms from penetration and destruction by germicides, therefore contributing to disinfection or sterilization failure. This must be beginning immediately after the patient procedure to prevent drying of secretions on both the external surface and inner channels of the endoscope **[32]**.

Similar to this Weber and Rutala reported that, outbreaks associated with flexible endoscopy have most often been associated with breaks in the cleaning and/or disinfection/sterilization stage of flexible endoscope reprocessing **[33]**. As well Cowen has described how the currently used reprocessing protocols provide a very narrow margin of safety and any slight deviation from the recommended steps may result in an increased risk of infection transmission by flexible endoscopes**[34]**.

In relation to pre-cleaning, and high level disinfection the present study showed that there was a minority of study subject pre- program had satisfactory practice increased to(most & near to three fourth) post program implementation. As well Merritt et al. stated that High-level disinfection refers to the use of a disinfectant (e.g., FDA-cleared chemical sterilant or high-level disinfectant) that inactivates all microorganisms (i.e. bacteria, viruses, fungi, mycobacteria) but not high levels of bacterial spores **[35]**. Burdick, and Hambrick added that the disinfection process requires immersion of the endoscope in the high-level disinfectant and

ensuring all channels are perfused for the approved contact time **[36]**.

The current study also showed that regarding automated disinfected and rinse the study represented that minority of nurses in pre-program increased to one third in post program, while endoscopy handling were most of nurses increased to majority post program, and endoscopy storage more than one third increased to 100% post program. Regarding their total practice, none of the nurses had satisfactory level which improved to half and two fifth in post and follow up respectively.

This finding supported by the study carried by Ali and Taha who reported that, in relation to applying reprocessing steps correctly which include rinsing, storage, documentation and decreasing microbial contamination the results of our study indicated effectiveness of the provided program toward this issues**[37]**.

The current study illustrated that there were a highly significant statistical improvement of total nurses practice level regarding infection control in endoscopy unit, ($p \leq 0.01$**). As satisfactory level was 0% in pre - program increased to most of study subject in post program, and more than three fourth in follow up of implementation of educational program. This result was congruent with El ghatey et al. who reported that there were highly statistical significant differences between nurses practice pre and posts program implementation**[6]**. This result consistent with Ali and Taha who stated that the improvement in nurses' practices after the intervention was also noticeable since their practices before the guidelines were even worse compared with knowledge. In fact none of them had adequate practice at the pre-program phase. Like knowledge, the adequate practice continued throughout the follow-up, and the attendance of the program were the only independent predictors that positively influenced the practice score **[37]**.

CONCLUSION:

There is improvement in total level of nurses' knowledge, and practice regarding infection control when comparing with pre- post, post/ follow up implementation of educational program.

RECOMMENDATIONS:

Continuous educational infection control training programs are recommended in endoscopy units. So incorporation of such interventions apply in all endoscope units all over Egypt.

ACKNOWLEDGEMENT:

I would like to express my deepest thanks and gratitude to professor Dr Mohamed El-khashab Professor of Tropical Medicine, Faculty of medicine, Zagazig University, professor Dr Nadia Mohamed Taha Assistance professor and the Head of Medical Surgical Department, Faculty of Nursing, Zagazig University, Dr Maha Desoke Saleh Lecturer of Medical Surgical, Faculty of Nursing ,Zagzig University, for unlimited help, constructive criticism, continuous encouragement, continuous assistance and supervision throughout all phases of producing of this work

Funding: None.
Conflicts of interest: None.
Ethical Approval:Approved.

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Peer reviewer: Maysaa Saeed ,Professor of Tropical Medicine and Hepatogastroenterology, Faculty of Medicine, Zagazig University, Egypt. **Editor: Mohamad Emara**, Assistant Professor of Tropical Medicine and Hepatogastroenterology, Faculty of Medicine, Zagazig University, Egypt

Evaluation of Granulocyte Elastase Enzyme in Diagnosis of Spontaneous Bacterial Peritonitis

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Key words:
Granulocyte elastase, spontaneous bacterial peritonitis, Cirrhosis

Background and study aim: The most common infections in decompensated liver cirrhotic ascites patients are cases of spontaneous bacterial peritonitis (SBP), which account for 40%–70% of cases. SBP is a bacterial infection that occurs in absence of an evident intra-abdominal or surgically treatable source of infection.

Patients and methods: This study was conducted on 80 patients with liver cirrhosis and ascites; 40 patients of them without SBP (group A) and 40 patients of them with SBP (group B) who were admitted to the Hepatology, Gastroenterology and Infectious Diseases Department, Benha University Hospital in the period between April 2014 and October 2014. Full history taking, clinical examination and laboratory investigation were done. Ascitic fluid analysis was done including detection of granulocyte elastase level.

Results: Granulocyte elastase was markedly elevated in group B; mean ascitic fluid GE ELISA (4.1±2.8) comparing with group A (0.8±0.7) and it revealed a high statistically significant association between SBP and GE (P value <0.05). SBP was more common in child C. Fever, hypotension and abdominal pain were more common in SBP group.

Conclusion: Granulocyte elastase is increased in cases of SBP, cutoff value of ascitic fluid (GE) for diagnosis of SBP at 0.88 ng/mL had 100% sensitivity, 75% specificity, 80% positive predictive value, 100% negative predictive value and 87.5% accuracy.

INTRODUCTION

Liver cirrhosis is the clinical end-stage of different entities of chronic liver disease when patients suffer from substantial mortality and morbidity, both of which are positively correlated with disease severity **[1]**. Cirrhosis represents the final common histological pathway for a wide variety of chronic liver diseases. Cirrhosis is defined histologically as a diffuse hepatic process characterized by fibrosis and the conversion of normal liver architecture into structurally abnormal nodules. Some patients with cirrhosis are completely asymptomatic and have a reasonably normal life expectancy. Other individuals have a multitude of the most severe symptoms of end-stage liver disease and have a limited chance for survival. Common signs and symptoms may stem from decreased hepatic synthetic function, decreased detoxification capabilities of the liver (e.g, hepatic encephalopathy), or portal hypertension (e.g, variceal bleeding) **[2]**.

Ascites is defined as an accumulation of excessive fluid within the peritoneal cavity and may be a complication of both hepatic and non-hepatic diseases. The 4 most common causes of ascites are cirrhosis, neoplasm, congestive heart failure, and tuberculous peritonitis **[3]**.

Spontaneous bacterial peritonitis (SBP) is defined as the infection of ascitic fluid (AF) in the absence of a contiguous source of infection and/or an intra-abdominal inflammatory focus. An ascitic fluid polymorphonuclear (PMN) leucocyte count ≥ 250/mm^3 irrespective of the AF culture result is universally accepted nowadays as the

best surrogate marker for diagnosing SBP. Frequently the results of the manual or automated PMN count do not reach the hands of the responsible medical personnel in a timely manner **[4]**.

Alternative methods using automated PMN counting **[5]**, reagent strips **[6]**, or ascitic fluid lactoferrin **[7]** have been developed. Unfortunately, their diagnostic accuracies are limited. Therefore, an accurate and convenient method of rapid diagnosis of SBP remains an unmet clinical need **[8]**.

Granulocyte elastase (GE) is a powerful proteolytic enzyme that is released by PMNs when degranulated in infectious processes [9].However more studies are needed to evaluate the accuracy of this test in diagnosis of SBP.

PATIENTS AND METHODS

Study design:
Cross-sectional study**.**

Patients:
We enrolled in the study 80 patients with decompensated chronic liver disease and ascites 40 patients of them without SBP (group A) and 40 patients of them with SBP (group B) who were admitted to the Hepatology, Gastroenterology and Infectious Diseases Department, Benha University Hospital in the period between April 2014 and October 2014 after approval of ethical committee of Benha Faculty of Medicine. The study was performed after written informed consent from all patients.

Fulfilling all criteria detailed below.

Inclusion criteria:
Ascitic patients with clinical, laboratory and ultrasongraphic findings of liver cirrhosis were included when:
1. Age >18 years.
2. Symptoms and signs suggest SBP as fever and abdominal pain.

Exclusion criteria:
Patients were excluded when they had any of the following criteria:
1. Patients with antibiotic therapy within one month before.
2. Recent abdominal surgery (< 3 months).
3. Abdominal malignancy as HCC and Colorectal carcinoma.
4. Secondary peritonitis due to intra-abdominal infection for example: abscess, appendicitis, cholecystitis and pancreatitis.
5. Other comorbidities e.g chronic obstructive pulmonary disease, chronic renal failure and ischemic heart disease.

Clinical and Laboratory Assessment:
All patients were subjected to the following: Thorough history taking, through clinical examination, ultrasonographic evaluation and routine laboratory investigations including blood picture, liver and kidney function tests, viral markers.

Sampling:
1. Five ml blood was withdrawn by veni-puncture, one ml in EDTA tube for CBC and four ml delivered into plastic tube and allowed to clot. Non-hemolyzed sera was separated by centrifugation and used for determination of creatinine, urea and liver functions (ALT, AST, total bilirubin, albumin, PT and INR).
2. Ascitic fluid sample was taken by paracentesis performed under aseptic conditions from a puncture site in the left or right lower quadrant with the patient in the supine position. All samples for diagnostic testing were immediately collected at the bedside and processed by laboratory personnel without further delay.

Methodology:
1- Complete blood picture using (Sysmax 5, Chuo-ku, Kobe, Japan) **[10]**.
2- Renal function test: blood urea and serum creatinine were determined calorimetrically on Dialab auto analyzer **[11]**.
3- Liver function tests were determined calorimetrically on Dialab auto analyzer and include the following:
- Serum alanine transaminase (ALT).
- Serum aspartate transaminase (AST).
- Serum bilirubin (Total and direct).
- Serum albumin.
- Prothrombin time and INR were done using coagulometer **[12]**.

4- Serological tests for viral markers using:
A. HBs Ag was determined using non – competitive sandwich assay on (ELISA) based technique **[13]**.
B. HCV Antibodies were detected using a third generation enzyme linked immunosorbent assay (ELISA) technique **[14]**.

5- Ascitic fluid examination for total protein content, albumin, glucose, Lactate dehydrogynase (LDH) and total and differential WBCs counting.

6- Ascitic fluid granulocyte elastase was measured by an enzyme-linked immunosorbent assay specific for human granulocyte elastase by a laboratory blinded to the patients' clinical information and other laboratory results. The kit was supplied from *Sunred-bio*, Shanghai, China.

Statistical analysis:

Statistical presentation and analysis of the present study was conducted SPSS V.20. Data was expressed into two phases:

I Descriptive 1- Mean value (X) and Standard Deviation [SD]: for quantitative data. 2- Frequency and percentage for qualitative data.

II Analytic by t-student test and Chi-square test. P value >0.05 was considered statistically non significant P value ≤0.05 was considered statistically significant. P value ≤0.001 was considered statistically highly significant.

RESULTS

Sixty one of them (76.3%) were males and nineteen (23.7%) were females (Table 1).

By comparison between group A and group B regarding demographic data, there was no statistical significant difference regarding gender, age or residence (P value >0.05) (Table 2).

Seventy seven patients (96.3%) of studied patients were HCV Ab positive and three patients (3.7%) were HBsAg positive and no patient has co-infection (Table 3).

By comparison between group A and group B regarding clinical presentation; abdominal pain and vomiting were founded in 55% and 25% of studied patients in group A. While in group B they had founded in 75% and 45% without statically significant difference (P value >0.05) (Table 4).

Jaundice, disorientation and flapping tremor were present in 60%, 52.5% and 50% respectively in group A. While they were present in 65%, 75% and 42.5% respectively in group B without statistical significant difference between both groups (P value >0.05).There was statistical significant difference between both groups regarding systolic blood pressure and temperature (Table 5).

By comparison between group A and group B regarding initial laboratory data. There was no statistical significant difference between both groups (P value >0.05) (Table 6).

By comparison between group A and group B regarding ascitic fluid protein, glucose and LDG, there was no statistical significant difference (P value > 0.05) (Table7).

By comparison between group A and group B regarding ascitic fluid granulocyte elastase. There was high statistically significant difference between both groups regarding granulocyte elastase (P value < 0.05) (Table 8).

SBP was more common in Child Turcotte Pugh Score class C (87, 5%), while 65% of patients with sterile ascites are Child Turcotte Pugh Score class C (Table 9).

There was a highly statistical significant difference regarding TLC and PMN count in ascitic fluid of SBP group compared to non SBP group (Table 10).

There was a highly statistical significant difference regarding PMN count in ascitic fluid, ascitic fluid (GE) test of SBP group compared to non SBP group (Table 11).

A cutoff value of ascitic fluid (GE) for diagnosis of SBP at 0.88ng/mL had 100% sensitivity, 75% specificity, 80% positive predictive value, 100% negative predictive value and had 87.5% accuracy (Figure 1).

Table (1): Demographic description of studied patients

Variable	Number (80)	%
Gender:		
Male	61	76.3%
Female	19	23.7%

Table (2): Comparison between group A (Non SBP) and group B (SBP) regarding demographic features

Variable	Group A Non SBP (N = 40)	Group B SBP (N = 40)	P. Value
Female Male	13 (32.5%) 27 (67.5%)	6 (15%) 34 (85%)	0.07
Rural Urban	27 (67.5%) 13 (32.5%)	34 (85%) 6 (15%)	0.07
Age	58.3±8.3	55.8±7.5	0.2

Table (3): Etiology of the chronic liver disease in studied patients. (Virological markers)

Variable	Number (80)	%
HCV Ab(+ve)	77	96.25%
HBS Ag(+ve)	3	3.75%

Table (4): Comparison between group A (Non SBP) and group B(SBP) regarding clinical presentations

Variable	Group A Non SBP (N = 40)	Group B SBP (N = 40)	P. Value
Main Complain:			
1-Abdominal pain	19 (47.5%)	23 (57.5%)	0.4
2-Marked abdominal enlargement	9 (22.5%)	4 (10%)	0.1
3-Fever	11 (27.5%)	13 (32.5%)	0.6
5-Vomiting	0	3 (7.5%)	0.08
6-Hematemesis	7 (17.5%)	4 (10%)	0.3
Symptoms			
1-Abdominal pain	22 (55%)	30 (75%)	0.06
2-Vomiting	10 (25%)	18 (45%)	0.06
3-Diarrhea	4 (10%)	8 (20%)	0.2
4-Hematemesis	6 (15%)	6 (15%)	1
5-Melena	6 (15%)	6 (15%)	0.5

Table (5): Comparison between group A (Non SBP) and group B (SBP) regarding clinical examinations

Variable	Group A Non SBP (N = 40)	Group B SBP (N = 40)	P. Value
Vital signs:			
1-systolic BP	101.9±14.8	96±9.1	0.03
2-diastolic BP	65.5±10.4	64.8±5.9	0.7
3-Temperature	37.5±0.8	37.9±0.8	0.05
4-Respiratory rate	17.6±2.3	17.3±2.9	0.6
General examination:			
1-Jaundice	24 (60%)	26 (65%)	0.6
2-lower limb edema	40 (100%)	40 (100%)	NA
3-disorientation	21(52.5)	30(75%)	0.4
4-Flabbing tremor	20(50%)	17(42.5%)	0.5
5-Hepatic encephalopathy	19(47.5%)	14(35%)	0.2

Table (6): Comparison between group A (Non SBP) and group B (SBP) regarding initial laboratory data

Parameters	Group A Non SBP (N = 40)	Group B SBP (N = 40)	P. Value
Hemoglobin (g/dl)	9.5±0.9	9.6±0.9	0.8
WBC×1000/mm^3	8.7±4.1	8.7±3.4	0.9
Platelet×1000/mm^3	83.83±29.03	93.5±23.73	0.12
Prothrombin time (second)	16.2±1.5	16.6±2	0.4
INR	1.6±0.2	1.6±0.3	0.3
ALT (IU\L)	50.7±21.2	52.1±16.3	0.7
AST (IU\L)	58±21	53.6±17.8	0.3
Albumin (g/dl)	2.1±0.4	2.1±0.3	0.7
Bilirubin Total (mg/dl)	3.5±1.6	3.8±1.7	0.5
Bilirubin Direct (mg/dl)	2.3±1.2	2.4±1.2	0.6
Urea (mg/dl)	73.3±35.5	73±20	0.9
Creatinine (mg/dl)	1.6±0.6	1.8±0.6	0.07

Table (7): Comparison between group A (Non SBP) and group B(SBP) regarding ascitic fluid analysis

Parameters	Group A Non SBP (N=40)	Group B SBP (n=40)	P value
Ascitic fluid protein (g/L) Normal(0.3-4.0g/dL)	1.5±0.5	1.3±0.4	0.09
Ascitic fluid Glucose (mg/L)	156.8±63	146.9±49.3	0.4
Ascitic fluid LDH (IU/L) Normal<400 IU/L	185.3±64.9	201.6±55.9	0.2

Table (8): Comparison between group A (Non SBP) and group B(SBP) regarding ascitic fluid granulocyte elastase

Variable	Group A (mean± SD)	Group B (Mean ± SD	P value
Ascitic fluid granulocyte elastase (ng)	0.8±1.1	4.1±2.8	≤0.001

Table (9): Comparison between group A (Non SBP) and group B(SBP) regarding Child-Turcotte-Pugh classification

Child Turcotte Pugh Score	Group A Non-SBP patients (N=40)	Group B SBP patients (n=40)
Child(A)	0(0%)	0(0%)
Child(B)	14(35%)	5(12.5%)
Child(C)	26(65%)	35(87.5%)

Table (10): Comparison between group A (Non SBP) and group B(SBP) regarding ascitic fluid WBCS counts and differential

Variable	Group A Non-SBP patients (N=40)	Group B SBP patients (n=40)	P value
Ascitic fluid **TLC**	95.5±50.4	2300.3±2157.8	≤0.001
Ascitic fluid **PMN**	76.3±39.8	1671.2±1096.5	≤0.001

Table (11): Comparison between group A (Non SBP) and group B(SBP) regarding ascitic fluid WBCS counts and granulocyte elastase

Variable	Group A Non-SBP patients (N=40)		Group B SBP patients (n=40)		P value
Ascitic fluid **PMN > 250**	0	0	40	100.0	≤0.001
Ascitic fluid **granulocyte elastase > 0.88**	2	5	40	100.0	≤0.001

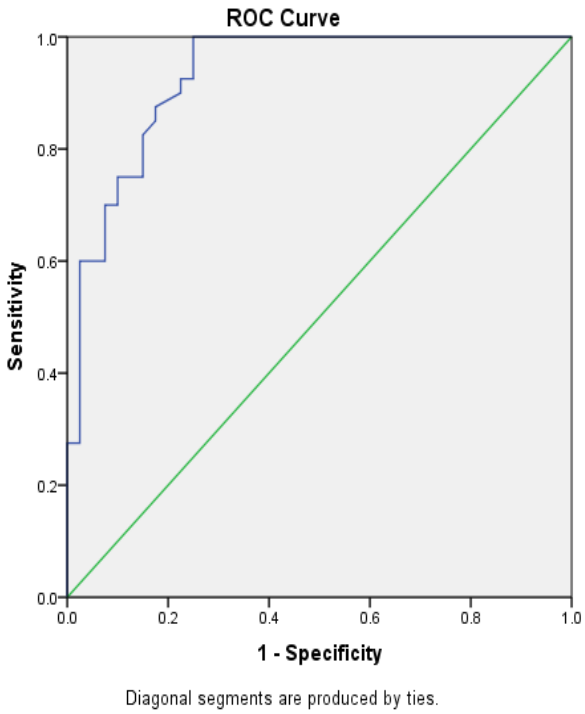

Figure (1): ROC curve for diagnosis of SBP by ascetic fluid granulocyte elastase by ELISA test

DISCUSSION

Ascites is the most common complication in patients with decompensated cirrhosis. Approximately 50% of patients with compensated cirrhosis will develop ascites over a 10-year period **[15]**.

The most common infections in decompensated cirrhotic patients are cases of spontaneous bacterial peritonitis (SBP), which accounts for 40%–70% of cases, followed by urinary tract infections, pneumonia and cellulitis **[16]**.

In this cross sectional comparative study which conducted on 80 patients with cirrhotic ascites, there was no statistical significant difference regarding gender and this in agree with study performed by Puri et al. **[17]** who reported that gender had no effect on incidence of SBP. Also the studies done by Chang et al.; Amany et al. and Nouh et al. **[18-20]** had found no statistical significant relation between SBP and gender. This result was not in agreement with the study done by Ageely et al. **[21]** included 115 cirrhotic ascitic patients, 46 of them had SBP who stated that SBP was frequent in males but was not influenced by the severity of liver disease or age. In this study mean age of patients in group A (58.3±8.3) and mean age of patients in group B (55.8±7.5) without statistically significant difference. Puri et al.; Chan et al.; Amany et al. and Nouh et al.**[17-20]** had founded that age seem to have no effect on the incidence of SBP.

In this study, seventy seven patients (96.3%) of the studied population were HCV Ab positive and three patients (3.7%) were HBsAg positive in agree with Amany et al.; Nouh et al. and Rizk et al. **[19,20,22]** demonstrated that HCV infection being the most frequent cause of chronic liver disease in Egypt. This was documented by Strickland **[23]** that Clinical studies showed 70% to 90% of patients with chronic hepatitis, cirrhosis, or hepatocellular carcinoma had HCV infections.

In this study, abdominal pain and vomiting were the main clinical presentations in group B compared to group A. These results were found to be close to that reported by Chang et al. and Kaymakoglu et al. **[18,24]** they stated that abdominal pain and fever are the most characteristic symptoms in patients with spontaneous ascitic fluid infection.

Abdominal pain was detected in 75% of SBP patients. This result was close to the study done by Wallersted et al. and Bibi et al. **[25,26]** demonstrated that abdominal pain was detected in 70%, 68.5% of SBP cases respectively and also was close to the study done by Badawy et al. **[27]** who eilicited abdominal pain in 80.2% and 84.2% of two groups of SBP cases. On the other

hand study performed by Rizk et al. and Ibrahim et al. **[22,28]** eilicited abdominal pain in 43% and 55.7% of SBP cases respectively.

In this study, hypotension and elevated temperature had statistically significant association with SBP group. These results were close to that reported by Zalam et al. **[29]** who stated that fever was detected in 75% of SBP cases with statistically significant association with SBP. On the other hand study performed by Bibi et al. **[26]** eilicited fever in 52.6% of SBP cases without statistical significant difference.

In this study, hepatic encephalopathy was detected in 35% of SBP and 47.5% of non SBP cases with statistically insignificant difference. These results were close to that reported by Wallersted et al. and Bibi et al. and Nobre et al. **[25,26,30]** who stated that hepatic encephalopathy was detected in 20%, 24.5% and 28.9% of SBP cases respectively. These results conflict the fact that the presence of hepatic encephalopathy in the course of liver cirrhosis is a marker of severe hepatic dysfunction that correlates with high prevalence of bacterial infections most commonly SBP **[31]**. This can be explained by selection bias of the two groups.

On the contrary, these results were against to that reported by Zalam et al.; Llovet et al. and Elsaaed et al. **[29,32,33]** who stated that hepatic encephalopathy was detected in 40%, 40.4% and 50% of SBP cases respectively with high statistical significance. This can be explained by development of portosystemic encephalopathy indicates decompensated liver disease and therefore, other features of decompensation, such as varices, ascites, and portal hypertension.

In this study we had found that mean hemoglobin was (9.5±0.9), mean total leucocytic count was (8.7±4.1)×1000/cm^3, mean platelet count was (83.8±29.03) ×1000/cm^3 and mean prothrombin time was (16.2±1.5) second in group A while mean hemoglobin was (9.6±0.9), mean total leucocytic count was (8.7±3.4) ×1000/cm^3, mean Platelet count was (93.5±23.7) ×1000/cm^3 and mean prothrombin time (16.6±2) second in group B without statistical significant difference.

This go on line with study done by Nouh et al.; Zalam et al. and Elsaaed et al. **[20,29,33]** demonstrated that no statistical significant differences were detected as regard TLC among both patient groups.

On the contrary, these results were against to the study done by Amany et al.; Rizk et al.; Rodriguez-Ramos et al.; Syed et al. and Lutz et al. **[19,22,34-36]** reported that there was statistically significant high serum total leucocytic count in SBP group. This could be explained by that bacterial translocation to mesenteric lymph node had important immunological function associated with local/systemic inflammatory response leading to peripheral leukocytosis **[37]**.

The present study had showed that decrease Hb %, platelet count and prolonged prothrombin time in comparison to non infected cases, this was in agreement with Gschwantler et al. and Kawasaki et al. **[38,39]** who stated that in patients with CLD, a sort of pancytopenia would be expected due to increased blood sequestration in the spleen and to low thrombopoietin levels.

In this study, there was no statistical significant difference between both groups regarding serum alanine transaminase, serum aspartate transaminase, serum albumin and bilirubin (total & direct). This in accordance with Nouh et al.; Rizk et al. and Zalam et al. **[20,22,29]** who reported no statistical significant differences were detected as regard liver function test among both groups.

On the contrary, Amany et al. and Elsaaed et al. **[19,33]** reported statistically significant lower serum albumin level in SBP group. And also El-Gendy et al. **[40]** stated statistically significant elevated serum bilirubin and prolonged prothrombin time in SBP group.

In this study regarding the kidney function test, there was no statistical significant difference and this agree with Amany et al.; Nouh et al. and Zalam et al. **[19,20,29]** who reported no statistical significant difference comparing both groups as regards kidney function tests.

These results were coinciding with the study done by Rizk et al.; Lutz et al.; Ruiz Del Arbol et al. and Gill et al. **[22,36,41,42]** found that patients with SBP frequently develop a rapidly progressive impairment in systemic hemodynamics, leading to severe renal and hepatic failure, aggravation of portal hypertension, encephalopathy, and death. This occurs despite rapid resolution of infection and is associated with an extremely poor prognosis. Also the study performed by Follo et al. **[43]** stated that one third of patients with SBP develop renal impairment and it is common in patients with severely impaired liver functions. It is not worthy that hepatorenal syndrome is the extreme expression of this circulatory dysfunction **[44]**.

In this study, SBP was more common in advanced Child-Pugh class C (87.5%) of patient in group B (SBP) compared with (65%) of patients in group A (Non SBP) meaning that the severity of the liver disease is probably an important risk factor for the development of SBP **[45]**. This was close to Quenzer **[46]** who reported that about 70% of the patients who develops SBP are in Child C class, with the remainder being class B.

By ascitic fluid analysis in studied groups, the mean ascitic fluid protein was (1.5±0.5 g/dl), mean ascitic fluid glucose was (156.8±63 mg/l) and mean ascitic fluid lactate dehydrogenase [LDH] was (185.3±64.9 IU/L) in group A. While in group B mean ascitic fluid protein was (1.3± 0.4 g/dl), mean ascitic fluid glucose was (146.9±49.3 mg/l) and mean ascitic fluid LDH was (201.6±55.9 IU/L).

By comparison between studied groups, there was no statistical significant difference regarding ascitic fluid analysis (P>0.05), and this in accordance with Zalam et al. and Elsaaed et al. **[29,33]** who reported that there was no statistical significant difference regarding ascitic fluid total protein, glucose and LDH levels between both groups. And also Bibi et al. **[26]** reported that there was no statistical significant difference regarding ascitic fluid total protein, glucose.

This result on disagreement with study performed by Amany et al. **[19]** stated statistical significant low ascitic fluid total protein, glucose and elevated LDH in SBP group. And also with study done by Abbass et al. **[47]** reported statistical significant low ascitic fluid albumin in SBP group while other parameters are statistically insignificant.

This result could be explained as both groups showed advanced liver disease so both have low proteins. Thus a follow up paracentesis is recommended as it may detect development of SBP being a high risk group. It is to be noted that, in contrast to other infected body fluids, ascitic fluid during SBP exhibited neither a rise in protein concentration nor a drop in absolute glucose concentration **[48]**. These results were contradictory to Sheer and Runyon **[49]** who reported that ascitic fluid total proteins were lower in patients with SBP than patients with sterile cirrhotic ascites and to Runyon **[50]** who stated that patients with low ascitic fluid total proteins (<1 g/dl) have to receive an antibiotic chemoprophylaxis as they are more prone to recurrence of SBP than those with high ascitic fluid total protein content (>l g/dl). Moreover, low ascitic fluid protein level <l g/dl considered to be the most important predisposing factor for developing the first episode of SBP **[51]** this was confirmed by Guarner et al. **[52]** who observed that about one fourth of patients with ascitic fluid protein levels less than 1g/dl developed SBP during a 3-year follow-up compared to only 4% of patients with higher levels.

By comparison between group A and group B regarding ascitic fluid granulocyte elastase. There was high statistically significant association between SBP and granulocyte elastase (P value < 0.05).

This result was in agreement with the study performed by **[9]** reported GE level was statistically significant higher in both ascitic fluid and plasma of SBP group than in non-SBP group at the time of diagnosis.

Also this result go on line with study done by Yamazaki et al. **[53]** stated that ascitic fluid GE levels were significantly higher in SBP group as detected by three different methods (latex immuonoassy , ELISA and reagent strip).

As regards this study, we found that cutoff of ascitic fluid (GE) for diagnosis of SBP at 0.88 ng/ml had sensitivity 100%, specificity 75% and these coincides with Yamazaki et al.s **[53]** who reported that cut-off diagnostic value for SBP for ascitic fluid GE latex immunoassy at 49.5 ng/ml had 85.7%sensitivity and 97.7%specificty.This difference may be due to small number of SBP patients in this study which include 58 cirrhotic ascitic patients, 12one of them only having SBP.

In conclusion, Diagnosis of SBP on clinical basis is difficult as there is extremely variable clinical presentaions. fever; hypotension and abdominal pain were more common in SBP group. Granulocyte elastase (GE) is increased in cases of spontaneous bacterial peritonitis (SBP) with cutoff value of ascitic fluid (GE) for diagnosis of SBP was at >0.88ng/mL had 100% sensitivity, 75% specificity, 80% positive predictive value, 100% negative predictive value with 87.5% accuracy.

Acknowledgment

The authors would thank Dr. Mohamed Abed El-Aziz Metwally, Dr. Mohammad El-Sayed El-Shewi and Dr. Jehan Hassan Sabry who helped in conducting this study. And staff of the Tropical Medicine Department, and to the laboratory technicians, for their valuable efforts.

Ethical approval: Approved.
Funding: None.
Conflict of interest: Authors declare no conflict of interest related to this article.

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Effect of Punica and Silymarin on Hepatotoxicity Induced by Pesticides

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Key words:
Pesticides – hepatotoxicity – punica – silymarin – antioxidant - enzymes

Background and Study Aim: Human beings are exposed to pesticides through consumption of contaminated food or exposure in the occupational environment. These compounds induce hepatotoxicity through generation of reactive oxygen species. There is much evidence indicating that natural substances from medicinal plants possess powerful antioxidant activities. The aim of the present study was to investigate the potential curative effects of punica and silymarin in rats exposed to fenitrothion.

Materials and methods: Animals were randomly allocated into one of the following groups (n= 10): (C) control group treated with oral distilled water, 3 ml/kg/day for 42 days, (F4) oral fenitrothion, 10 mg/kg/day for 28 days, (F6) oral fenitrothion, 10 mg/kg/day for 42 days, (Pun) fenitrothion, 10 mg/kg/day for 42 days and oral punica juice 3ml/kg/day for 14 days starting from day 29 of fenitrorthion administration and (Sil) fenitrothion, 10 mg/kg/day for 42 days and oral silymarin, 100 mg/kg/day for 14 days starting from day 29 of fenitrothion administration. Activities of hepatic enzymes including alanine amino-transferase (ALT), aspartate amino-transferase (AST), alkaline phosphatase (ALP) were evaluated. Serum albumin and total bilirubin concentrations were measured. Catalase (CAT) activity, reduced glutathione (GSH) and malondialdhyde (MDA) content in liver were determined. Total phenolic and flavonoids content were assessed in plant samples.

Results: Exposure to fenitrothion caused a significant increase in AST, ALT and ALP activities, total bilirubin concentration and a significant decrease in serum albumin. The hepatic antioxidant capacity was significantly lowered in fenitrothion- treated rats as compared to the control group ($p<0.05$). Treatment with punica or silymarin significantly ameliorated these changes.

Conclusion: This study indicated the promising therapeutic potential of punica and silymarin against hepatotoxicity induced by pesticides. These effects could be attributed to their antioxidant properties.

INTRODUCTION

In agriculture and in everyday life, to produce more and to get more profits, toxic chemicals, such as pesticides are used. But they are generating harmful consequences to animals, the environment and human health **[1].**

The liver plays a central role in the metabolism of substances and it can be considered as target organ of numerous chemicals used in the workplace. Many insecticides seem to be able to cause enzyme induction with the modification of hepatic metabolism of drugs and hormones, as well as significant changes in the liver. Pesticides may induce oxidative stress leading to generation of free radicals and alternated antioxidant or oxygen free radical scavenging enzyme system **[2].**

Minimizing oxidative stress will promote the physical condition and prevent some degenerative diseases in which free radicals are involved. There is a widespread agreement that synthetic antioxidants need to be replaced with natural antioxidants because some synthetic antioxidants have shown potential health risks. Therefore, it is

of great importance to find new sources of safe and inexpensive antioxidants of natural origin in order to use them in modulating oxidative stress associated with chronic diseases **[3].**

Punica granatum (punicaceae) commonly known as pomegranate is rich in antioxidant of poly-phenolic class which includes tannins, anthocynins and flavonoids. Pomegranate is the fruit of energy, vitality and medicinal value that have anthelmintic, immunostimulatory, hepatoprotective, anti-diarrhoeal and anti-cancer activities **[4].**

Silymarin, a polyphenolic flavonoid from the milk thistle plant (Silybum marianum), inhibits lipoprotein oxidation and acts as a free-radical scavenger. Its effectiveness against multiple disorders makes it a very promising drug of natural origin. It is beneficial too, because of its wide margin of safety, easy availability and low cost. Hence, this drug may have good potential towards the treatment of many diseases, both in human beings as well as animals **[5].**

The present study was designed to investigate the curative effect of punica as food supplement in a model of hepatotoxicity by the organo-phosphorous compound; fenitrothion in rats in comparison to silymarin.

MATERIALS AND METHODS

Materials:

Fenitrothion (Sumithion 50®, 50 mg/ml) was purchased from Kafr Elzayat Co. for Insecticide Ind., (Kafr Elzayat, Egypt). Fenitrothion suspension was freshly diluted in distilled water to 10 mg/ml and orally administered at a dose of 10 mg/kg **[6]**.

Punica granatum: Ripe Punica granatum fruits, family Punicaceae were obtained from local source in Zagazig, Sharkiah, Egypt. Pomegranates were washed and manually peeled without separating the seeds. Juice was prepared using a commercial blender (Braun, ZK 200, Germany), filtered and immediately diluted with distilled water (1:3) and stored at -20°C for no longer than 2 months. Aliquots were defrosted immediately and orally injected at a dose of 3 ml/kg **[7].**

The aqueous extract of silymarin was provided as a kind gift from MEBACO, (Arab Company for Pharmaceutical and Medicinal plants, Cairo, Egypt). Silymarin suspension was prepared by suspending 100 mg of Silymarin extract in 1 ml distilled water and orally injected at a dose of 100 mg/kg **[8].**

Voucher specimens (reference number VS-020713-02-Pun and VS-020713-03-Sil) were deposited in the herbarium of the department of Pharmacology, Faculty of Pharmacy, Zagazig University, Egypt.

Animals

Male albino rats weighing 160 ±10 g were obtained from National Research Center, Cairo, Egypt and were housed in plastic cages, allowed free access to a standard diet and tap water. The rats were housed at 23 ± 2° C 12 hr dark/light cycle. All experimental procedures were approved by the Ethical Committee for Animal Handling at Zagazig University (ECAHZU) (NO: P7-3-2013).

Animals were randomly allocated into one of the following groups (n= 10): C (control group treated with oral distilled water, 3 ml/kg/day for 42 days), F4 (oral fenitrothion, 10 mg/kg/day for 28 days), F6 (oral fenitrothion, 10 mg/kg/day for 42 days), Pun (fenitrothion, 10 mg/kg/day for 42 days and oral punica juice 3ml/kg/day for 14 days starting from day 29 of fenitrorthion administration) and Sil (fenitrothion, 10 mg/kg/day for 42days and oral silymarin, 100 mg/kg/day for 14 days starting from day 29 of fenitrothion administration).

At the end of the experiment, after overnight fasting, blood was collected from the retro-orbital plexus and centrifuged at 3500 rpm for 15 minutes with or without heparin and serum/ plasma was collected and stored at -20ºC. Animals were sacrificed by decapitation and liver were excised for preparation of tissue homogenates.

Methods

Total phenolic and total flavonoid contents in the plant materials were determined by colourimetric methods **[9-10].**

Catalase (CAT) activity, reduced glutathione (GSH) and malondialdhyde (MDA) content in liver were determined colorimetrically **[11-12- 13].**

The following parameters were assayed in serum using kits supplied by Biodiagnostic Co (Cairo, Egypt); ALT, AST and ALP activities, total bilirubin, albumin concentrations.

Statistical analysis:

Data are expressed as means ± SE. The statistical significance of the data was determined using one way analysis of variance (ANOVA) followed by Tukey's post hoc test using SPSS software

package version 10. The level of significance was taken as $P < 0.05$.

RESULTS

Determination of total flavonoid and total phenolic content of punica juice and silymarin extract.

The total flavonoids and total phenolics content of punica and silymarin were found to be (228.75 ± 6.91 and 217.35±5.65mg catechin/100g sample) and (37.16±0.49 and 36.41±0.47 mg gallic acid/ 100g sample) respectively as shown in (Table 1).

Determination of free radical scavenging activity of punica juice and silymarin extract.

The remaining percent of DPPH (2,2-diphenyl-1-picrylhydrazyl) and H_2O_2 which is indicative of the free radical scavenging activity of punica and silymarin were found to be (12.2% and 12.42%) and (14.45% and 15.08) respectively as shown in (Table 2).

Effects on different liver functions

Administration of fenitrothion for 28 and 42 days (F4 and F6 respectively), caused a significant increase in serum ALT, AST, ALP activities and serum total bilirubin compared with control group, while, treatment with punica or silymarin for 14 days (starting from day 29 along with fenitrothion) reversed these changes to near control values causing a significant decrease compared with F6 group (Table 3).

On the other hand, administration of fenitrothion for 28 and 42 days (F4 and F6 respectively), caused a significant decrease in albumin concentration compared with control group. Administration of punica or silymarin for 14 days (starting at day 29 days along with fenitrothion) caused a significant elevation in albumin concentration compared with F6 group (Table 3).

Effect on oxidative stress biomarkers

Administration of fenitrothion for 28 and 42 days (F4 and F6) caused a significant decrease in catalase (CAT) activity and glutathione (GSH) content in liver compared with control group. On the other hand, administration of fenitrothion for 28 and 42 days (F4 and F6) caused a significant increase in malodialdhyde (MDA) content in liver compared with control group (Table 4).

Treatment with punica or silymarin for 14 days (starting from day 29 along with fenitrothion) caused a significant increase in liver catalase activity and glutathione (GSH) content while liver malodialdhyde (MDA) content were significantly reduced (Table 4).

Table (1): Total flavonoid and phenolic content of punica juice and silymarin extract

	Total flavonoids (mg catechin/ 100g sample)	Total phenolics (mg gallic acid/ 100g sample)
Punica	228.75 ± 6.91	37.16 ± 0.49
Silymarin	217.35 ± 5.65	36.41 ± 0.47

Table (2): Free radical scavenging activity of punica juice and silymarin extract determined by the percent remaining of DPPH and H_2O_2

	% remaining of H_2O_2	% remaining of DPPH
Punica	12.2%	14.45%
Silymarin	12.42%	15.08%

Table (3): Effects of fenitrothion (10 mg/kg) alone or in combination with punica (3ml/kg) or Silymarin (100 mg/kg) on different liver functions. Data are presented as mean ± SE. (n = 10)

Parameter	Control	F4	F6	Pun	Sil
Serum ALT (U/L)	41.25±0.36	54.00±0.86*	55.17±0.42$^{*\$}$	45.0±0.29$^{*\#\$}$	42.17±0.60$^{\#\$}$
Serum AST (U/L)	62.67±0.88	146.00±4.37*	147.83±2.06$^{*\$}$	114.33±0.65$^{*\#\$}$	71.75 ± 2.57$^{\#\$}$
Serum ALP (U/L)	107.59±1.61	257.63±6.94*	236.69± 6.91*	118.84±7.54$^{\#\$}$	130.78±1.89$^{*\#\$}$
Serum total bilirubin (mg/dl)	0.32±0.01	0.89±0.01*	0.93±0.01$^{*\$}$	0.59±0.02$^{*\#\$}$	0.54±0.02$^{*\#\$}$
Serum albumin (gm/dl)	4.80±0.15	3.21±0.02*	3.28±0.04*	4.27± 0.01$^{*\#\$}$	4.29±0.06$^{*\#\$}$

* Significantly different from control group
$^{\$}$ Significantly different from F4 group
$^{\#}$ Significantly different from F6 group
$^{€}$ Significantly different from Sil group at p< 0.05 using ANOVA followed by Tukey's Post Hoc test.

Table (4): Effects of fenitrothion (10 mg/kg) alone or in combination with punica (3ml/kg) or silymarin (100 mg/kg) on liver oxidant state catalase glutathione and malondaldahyde. Data are presented as mean ± SE. (n = 10).

Level	Control	F4	F6	Pun	Sil
Catalase activity (µmole/ min/mg wet tissue)	0.60±0.01	0.31±0.004$^{*\#}$	0.25 ±0.002$^{*\$}$	0.62±0.0$^{*\#\$}$	0.61±0.01$^{*\#\$}$
GSH content (mg/gm wet tissue)	24.49±0.18	15.33± 0.14$^{*\#}$	12.09±0.07$^{*\$}$	25.26±0.21$^{*\$}$	25.06±0.29$^{*\#\$}$
MDA content (µmole/gm wet tissue)	56.16±1.41	95.44±1.83$^{*\#}$	95.94±3.33$^{*\$}$	66.33±0.77$^{*\#\$}$	64.66± 1.10$^{*\#\$}$

* Significantly different from control group,
$^{\$}$ Significantly different from fenitrothion 4 weeks (F4) group
$^{\#}$ Significantly different from fenitrothion 6 weeks (F6) group
$^{€}$ Significantly different from silymarin (Sil) group at p< 0.05 using ANOVA followed by LSD and Tukey' Post Hoc test.

DISCUSSION

Analysis of punica juice and silymarin extract showed the presence of flavonoids that may contribute to their antioxidant activity. These results are in accordance with previous studies showing the presence of flavonoids in punica **[14]** and silymarin **[15].**

Apart from being important dietary components, many therapeutic benefits of flavonoids are known in animal systems. Flavonoids have anti-oxidant, anti-proliferative, antitumor, anti-inflammatory, and pro-apoptotic activities **[16].**

The antioxidant activity of pomegranate aril juice, attributed to a great extent to total phenols and anthocyanins content **[17].** Similarly, silymarin possesses antioxidant, immunomodulatory, anticancer, antiinflammatory, antihepatoxic activities **[18].**

In the present study, results of DPPH and H_2O_2 reducing power indicated the high anti-oxidant activity **[19]** of punica juice and silymarin extract. These observations are in accordance with those reported for punica **[20]** and silymarin **[18]**.

Organophosphorus pesticides are widely used in the world and causing toxic effects on nontarget organisms especially mammalian. Due to the role of liver in detoxification of environmental xenobiotics, it is at great risk of injury and induces hepatotoxicity **[21].**

Hepatotoxicity by pesticides may occur in many ways, such as changes in the activities of liver enzymes, serum albumin and bilirubin concentrations which account for many indices of liver function **[22].**

The current study has shown that fenitrothion caused a significant increase in ALT, AST and ALP activities compared with control group. Pesticides may damage liver cells and liver transaminases may be used to monitor liver damage after exposure. These results coincide

with previous studies that showed a significant increase in liver enzymes in rats and humans exposed to organophosphorus insecticides (fenitrothion and chlorpyrifos) **[23]**.

Furthermore, it was found that serum bile acids were the most sensitive markers for detecting liver injury, suggesting that serum bile acids could be a valuable biomarker of hepatotoxicity caused by toxics. In the present study, fenitrothion treated rats showed significant increase in the level of bilirubin which is a normal metabolic product of haemoglobin in red blood cells **[24]**.

These results are in accordance with those obtained previously which indicated that the mechanism for pesticides hepatotoxicity is impairment of bile acid transport, causing cholestasis and subsequent hepatocellular apoptosis or necrosis **[22].**

The present study showed a decrease in albumin concentration following fenitrothion administration as previously stated **[25].** The albumin level suppression may be due to loss of protein either by reducing in protein synthesis or increased proteolytic activity or degradation **[26].**

There is a considerable importance of the investigation of free radical-mediated damage to biological systems due to pesticide exposure **[27-28],** the current study has shown that administration of fenitrothion resulted in an evident state of oxidative stress in the liver as evidenced by the decrease in catalase activity, glutathione content and increase in malodialdehyde content.

Previous studies indicate that fenitrothion intoxication can cause generation of free radicals and induce hepatic lipid peroxidation in rats [**29]**. Organophosphorus pesticide may induce oxidative stress which can be viewed as the disturbance in the oxidant-antioxidant balance in favor of the former **[30].**

Natural antioxidants from fruits and vegetables are reported to provide substantial protection that slows down the process of oxidative damage caused by reactive oxygen species (ROS) **[31].**

In the current study, coadministration of punica juice or silymarin extract with fenitrothion restored the altered liver functions as ALT and AST activities. These results are in accordance with those previously obtained for punica **[32]** and for silymarin **[33].** This alleviation in ALT and AST activities may be due to stabilizing membrane integrity that prevent the leakage of intracellular enzymes **[34-35].**

Similarly, treatment with punica juice or silymarin extract with fenitrothion, reversed the altered ALP activity and bilirubin concentration to near normal values. These results are in accordance with those obtained previously for punica **[36]** and for silymarin **[37].**

The amelioration in ALP activity and bilirubin concentration might be attributed to the ability of polyphenols and flavonoids present in punica **[32]** and silymarin **[33]** to treat the impairment of metabolism and excretion of bilirubin**.**

Interestingly, administration of punica juice and silymarin extract corrected the disturbed serum albumin content. These results match those obtained previously for punica and silymarin **[38].** The elevation of serum albumin might be attributed to the anabolic effect of flavonoids **[39]** or stimulation of RNA synthesis **[40]** and increased activity of mixed-function oxidation system **[41].**

Antioxidants play an important role in ameliorating the damaging effects of oxidative stress on cells **[42]**. In the current study, oral administration of punica juice or silymarin extract normalized the oxidative stress biomarkers; CAT, GSH and MDA in liver that were altered by fenitrothion. The antioxidant activity of punica and silymarin may be attributed to their content flavonoids which have been found to reduce xenobiotic-induced hepatotoxicity in animals and counteract the damaging effects of oxidative stress, cooperating with natural systems like glutathione and other endogenous protective enzymes **[43].**

CONCLUSION

Our findings demonstrated that punica and silymarin may be helpful in reducing the hepatotoxic adverse effects of fenitrothion by maintaining optimum cellular biochemical hemostasis.

Ethical approval: Approved.
Funding: None.
Conflict of interest: Authors declare no conflict of interest related to this article.

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Different Factors Correlated to Early Rebleeding in Cirrhotic Patients Treated by Variceal Band ligation versus Endoscopic Sclerotherapy

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Key words:
oesophageal varices, endoscopic sclerotherapy, endoscopic band ligation

Background and study aim : Endoscopic treatment has become the principal first-line intervention in patients with bleeding oesophegeal varices, both during the acute event and for long-term therapy to prevent recurrent bleeding. Several clinical considerations affect the prognosis in individual patients including the severity portal hypertension, the location of the bleeding varices, residual hepatic function, the presence of associated systemic disease, and others. Early rebleeding has been shown to be a strong predictor of mortality and recurrent variceal bleeding substantially increases the risk of complications which further contribute to mortality**.** This study aimed to evaluate early rebleeding after different methods of endoscopic intervention and investigate the different parameters of the patient that can be correlated to it.

Patients and methods: Hundred and four cirrhotic patients with first attack of variceal bleeding were included in this study. They were randomly allocated to two groups, group I: 52 patients who were managed by endoscopic variceal sclerotherapy and group II: 52 patients who were managed by endoscopic variceal band ligation to control their attack. The patients were followed up for six weeks and all their clinical, laboratory, endoscopic parameters were monitored. The rate of mortality and early rebleeding was measured and correlated to these different patients' parameters

Results: There was no significant difference between the two groups as regards rate of early rebleeding (15.4% in group I vs 9.6% in group II P= 0.374). The rate of early rebleeding was significantly correlated to Child's score (r=+0.136 P=0.014), PT (r=+0.35 P<0.001), INR(r=+0.419 P<0.001), grade of OV (r=+0.233 P=0.001), risky signs (r=+0.179 P=0.001), units of blood received (r=+0.387 P<0.001), amount of ethanolamine oleate (r=+0.329 P=0.017) and number of rubber bands used (r= +0.245 P=0.039). Mortality rates showed also no significant difference during the six weeks of follow up ,(19.2% in group I vs 21% in group II P= 0.647), as well as mortality rates in rebleeding cases (37.5% in group I vs 40% in group II P=0.925).

Conclusion: The factors that are strongly correlated to rate of early rebleeding after endoscopic management of OV are severely decompensated liver disease, larger OV size and presence of risky signs, use of more blood units during resuscitation, use of large amount of ethanolamine oleate during sclerotherapy and use of more rubber band during banding. Sclerotherapy and band ligation are comparable to each other in most outcomes especially early rebleeding and mortality.

INTRODUCTION

Portal hypertension commonly complicates liver cirrhosis and the development of oesophegeal varices is one of the major complications of portal hypertension **[1]**. The prevalence of oesophegeal varices at diagnosis ranges from 0-10% in patients with compensated cirrhosis, to 60% to 80% in patients with decompensated cirrhosis and the reported mortality from variceal bleeding ranges from 17% to 57% **[2]**. The progression from small to large varices occurs in 10% to 20% of cases annually **[3]**.

Endoscopic treatment has become the principal first-line intervention in patients with bleeding oesophegeal varices, both during the acute event and for long-term therapy to prevent recurrent bleeding **[4]**.

After control of the index bleed, there is a 40% chance of rebleeding with a similar mortality. The risk of rebleeding is greatest during the first few days after initial variceal hemorrhage **[5]**. Survival after variceal bleeding depends largely on the rapidity and efficacy of initial primary hemostasis and the presence and severity of underlying liver disease and hepatic functional reserve **[6]**.

Early rebleeding has been shown to be a strong predictor of mortality and recurrent variceal bleeding substantially increases the risk of complications which further contribute to mortality **[6]**. Rapid and sustained control of variceal bleeding remains the principal imperative of endoscopic intervention **[7]**. Several important clinical considerations influence the prognosis in individual patients. These include the natural history of the disease causing the portal hypertension, the location of the bleeding varices, residual hepatic function, the presence of associated systemic disease, continuing drug or alcohol abuse, patency of major splanchnic veins and the response to each specific treatment **[8]**.

Until now, there has been no general consensus on the risk factors and measures to prevent early rebleeding in cirrhotic patients in Egypt. Variceal Band ligation and Endoscopic Sclerotherapy can be effective methods to manage variceal bleeding and may be prevent it primarily and secondarily. However, early recurrent bleeding as a vital complication after variceal band ligation and endoscopic sclerotherapy has not been studied fully.

Aim of the work:

The aim of the present study was to evaluate the different factors that can affect the rate of early rebleeding of early rebleeding after different endoscopic treatments of variceal bleeding which help better management of variceal bleeding.

PATIENTS AND METHODS

This prospective randomized study was conducted in the Intensive Care Unit (ICU), In-patient and Endoscopy Units of Tropical Medicine Department, Faculty of Medicine Zagazig University, during the period from October 2012 to October 2014. It included 104 patients with first attack of hematemesis and melena diagnosed as bleeding esophageal varices by upper endoscopy. The Sample size was calculated using Epi info version 6.04.

They were divided into 2 groups (age, sex and severity of liver disease matched):

- **Group I:** included 52 patients who were treated by endoscopic sclerotherapy.
- **Group II:** included 52 patients were treated by endoscopic band ligation**.**

Inclusion criteria:

1- Presence of liver cirrhosis, the diagnosis of cirrhosis was based on clinical, biochemical and ultrasonographic findings with Child-Pugh grading.
2- First attack of upper GIT bleeding, which was proven by upper GIT endoscopy as bleeding esophageal varices.

Exclusion criteria:

1- Patients <18 and >60 years old
2- Patients who refuse participation in this study.
3- Hepatic patients with other causes of upper GIT bleeding than esophageal varices.
4- Patients with bleeding gastric varices.
5- Patients with recurrent attacks of bleeding oesophegeal varices.
6- Patients with intra or extrahepatic malignancy.
7- Patients who had uncontrolled bleeding for 24 h after endoscopic treatment.

All patients were subjected to the following:

1. Thorough medical history taking including:
2. Thorough clinical examination including:
3. The following laboratory investigations:
- Complete blood picture (haemoglobin level, red blood cell count, white blood cell count and platlet count)
- Biochemical liver tests on including: Total and direct serum bilirubin in mg\dl, Total serum protein and serum albumin in gm\dl, Serum Aspartate amino Transferase (AST) and serum Alanine amino Transferase (ALT) (IU\L), Prothrombin time in seconds and international randomization ratio (INR).
- Kidney function tests including blood urea and serum creatinine.
- Serum Bilharzial antigen: using ELISA/ soluble egg antigen (SEA)
- AST platelet ratio index (APRI): APRI = (AST/upper limit normal) x 100/platelet count. Score <0.5 excludes fibrosis.Score >2 suggests fibrosis.[9]

- Abdominal Ultrasonography : All the patients were examined using esaotemylab device. They were examined according to the standard maneuvers. Color Doppler ultrasound: All measurements were done by a single radiologist using color Doppler sonography with subjects in the supine or left lateral position. A Power Vision SSA-380A system (Esaotemy lab device) with (3 to 5 MHz) convex and sector pulsed probes. Sonographic examinations were carried out 8 hours after the last meal. In our study, we measured two parameters by Doppler ultrasound:
  - Portal vein velocity (cm/sec) PVV was measured directly using color Doppler ultrasound.
  - Hepatic artery resistive index (RI) = (peak systolic velocity - end diastolic velocity) / peak systolic velocity **[10]**.

4. Child-Pugh classification for all patients into: A,B, and C class according the severity of cirrhosis [11] :

Measure	1 point	2 points	3 points
Total bilirubin, (mg/dl)	<2	2-3	>3
Serum albumin, g/dl	>3.5	2.8-3.5	<2.8
Pt (seconds prolonged)	0-4	4-6	>6
Ascites	None	Mild	Moderate to Severe
Hepatic encephalopathy	None	Grade I-II (or suppressed with medication)	Grade III-IV (or refractory)

Points	Class
5-6	A
7-9	B
10-15	C

5. Upper gastrointestinal endoscopy :

Before endoscopy:

- Patients admitted to the Intensive Care Unit (ICU) for the first attack of variceal bleeding. Initial resuscitation following the classic Airway, Breathing, Circulation scheme was done followed by nasogastric lavage to remove particulate matter, fresh blood, and clots from the stomach to facilitate endoscopy.
- The patients were given the following medications along with volume replacement with plasma expanders: vitamin K 10mg/day IM, pantoprazole 40 mg/12 h IV, Somatostatin analogue (sandostatin) initial bolus 500 µg iv followed by 250 µg/hour for 24 hours and prophylactic antibiotic (cefotaxime sodium 1 gm IV /12h)

At time of endoscopy: Endoscopy was done by a single experienced endoscopist using end flexible videoendoscope (PENTAX VIDEO unit of endoscopy). The patients were positioned on their left lateral position, with head supported on a small firm pillow to remain in a comfortable neutral position and a bite guard in their mouth. Medazolam I.V. was used as sedation. Patients meeting the inclusion criteria were randomized alternatively to undergo Endoscopic Injection Sclerotherapy (EIS) or Endoscopic variceal Band Ligation (EBL).

- EIS was performed using a 25-gauge disposable injection needle for intravariceal and paravariceal injection. The sclerosant used was ethanolamineolyte.
- EVL was performed with PENTAX EG endoscope by the same experienced endoscopist using endoscopic ligating devices: an over tube or multi-band ligators.

Esophageal varices were graded into 4 grades as follows: [12]

- **Grade I:** small straight cords of varices continued to lower 1/3of the esophagus.
- **Grade II:** moderate sized clubbed varices with well-defined areas of normal mucosa between them, forming several distinct vertical cords and confined to lower third of esophagus.
- **Grade III:** gross varices extending into the proximal half of the esophagus, which is so large and tortuous, that normal mucosa may

not be visible in between unless the esophagus is fully distended with air.

- **Grade IV:** varices are like those of grade III but with dilated capillaries on top or in between varices, (varix over varix).

Portal hypertensive gastropathy was classified as follows: [13]

- **PHG grade I:** mild reddening and congestive mucosa, no mosaic like pattern**.**
- **PHG grade II:** Severe redness and a fine reticular pattern separating the areas of raised edematous mucosa (mosaic like pattern) or fine speckling.
- **PHG grade III:** Point bleeding + grade II.

After endoscopy:

The patients used non selective beta blocker carvidalol for prevention of recurrent variceal bleeding, starting with 12.5 mg orally single daily dose as recommended by Banares et al. **[14]**.

The patients were evaluated according to the presence or absence of the following symptoms: epigastric pain, heart burn, retrosternal chest pain, dysphagia, dyspepsia, and odynophagia upon discharge and during the follow up visits every two weeks.

Follow up:

The follow up of the patients was done every 2 weeks for 6weeks as regards the following:

1- The patients' general condition like development or improvement of ascites, lower limb edema, jaundice, and hepatic encephalopathy (HE).

2- Development of infections e.g diarrhea, chest infection, and abdominal pain and tenderness as indicators for SBP.

3- Laboratory tests; CBC, total and direct bilirubin, serum albumen and serum creatinine.

4- Upper GIT endoscopy with commenting on the variceal condition as previous, PHG, bleeding and development of sclerosant or post banding ulcer.

5- Rebleeding.

Statistical analysis:

Data were checked, entered and analyzed using SPSS version 19 EPI-INFO 6 and for data processing and statistic. Numerical data were expressed as mean and standard deviation and the comparison between numerical data is done with simple t test for normally distributed data and with Mann Whitney U test when data distribution is skewed. We used number and percentage to express categorial data and chi-square test to compare them. The correlation between numerical data was done by Spearman's correlation coefficient. The correlation between numerical and categorical data used Spearman's rank correlation.

RESULTS

Comparison between the two studied groups as regards age, gender distribution, incidence of diabetes, hypertension and bilharziasis revealed no significant differences as shown in table (1). Table (1) shows also that there were no significant differences as regards the cause of cirrhosis and the previous use of primary prophylaxis.

Table (2) shows that there were no significant differences between the studied groups as regards the liver and spleen size as detected by sonography. There were also no significant differences between the two studied groups as regards portal vein diameter and velocity as well as hepatic artery resistive index measured by coloured doppler, as shown in table (2). Table (2) also shows that there were no significant differences between the two groups as regards all laboratory parameters.

Comparison between the studied groups as regards the endoscopic examination revealed no significant differences between the two groups as regards grade of OV, number of cords, grade of PHG and incidence of duodenopathy at the beginning of the study as shown in table (3).

Table (4) compares the studied groups as regards the incidences of the common post-endoscopy symptoms encountered by the patients and shows that there were no significant differences as regards any of these symptoms.

Table (5) compares the studied groups as regards rate and causes of rebleeding and mortality rate and shows that there were no significant differences between them. Correlation between the rate of rebleeding and study parameters revealed that the rate of rebleeding has significant positive correlation with Child's score, PT, INR, grade of OV, presence of risky signs, number of units of blood transfused during resuscitation, amount of sclerosing agent and number of rubber bands used as shown in table (6).

Table (1): Demographic data, cause of cirrhosis and comorbidity

		Group I No=52	Group II No=52	Test value	P	Sig.
Age		50.1±12.1	49.7±10.6	**t**= 0.15	0.877	NS
Gender	Male	36(69.2%)	33(63.5%)	**X^2=0.388**	0.534	NS
	Female	16(30.8%)	19(36.5%)			
Diabetes		7(13.5%)	8(15.4%)	0.078	0.78	NS
Hypertension		4(7.7%)	6(11.5%)	0.443	0.506	NS
Cause of cirrhosis	HBV	5(9.6%)	6(11.5%)	0.102	0.750	NS
	HCV	45(86.5%)	45(86.5%)	0.000	1.000	NS
	others	2(3.8%)	1(1.9%)	0.343	0.558	NS
Positive bilharzial Ag		7(13.5%)	10(19.2%)	0.633	0.426	NS
Primary prophylaxis		9(17.9%)	10(19.2%)	0.064	0.800	NS

Table (2): Baseline sonographic, Doppler data and Child's score and laboratory data

		Group I No=52	Group II No=52	Test value	P	Sig.
Liver size	enlarged	2(3.8%)	2(3.8%)	0.877#	0.645	NS
	Average	14(26.9%)	10(19.2%)			
	shrunken	36(69.2%)	40(67.9%)			
Spleen size	average	3(5.8%)	5(9.6%)	0.542#	0.462	NS
	enlarged	49(94.2%)	47(90.4%)			
Portal vein diameter (cm) Mean ± SD		1.58 ± 0.21	1.53 ± 0.21	1.188•	0.238	NS
Portal vv velocity (cm/sec) Mean ± SD		13.27 ± 3.84	13.24 ± 4.11	0.299*	0.765	NS
Hepatic aa resistive index Mean ± SD		0.78 ± 0.07	0.77 ± 0.06	0.104*	0.917	NS
Child's grade	A	0.77 ± 0.06	10(19.2%)	X^2= 2.049	0.359	NS
	B	9(17.3%)	15(28.8%)			
	C	3057.7%)	27(51.9%)			
Hemoglobin (g/dl)		8.87 ± 1.53	9.05 ± 1.52	0.576•	0.566	NS
WBC's (cellx10^3/ml)		6.20 ± 3.86	6.30 ± 3.86	0.137*	0.891	NS
Platelet(x10^3)/ml		85.09 ± 33.87	92.34 ± 44.34	0.582*	0.560	NS
Albumin (g/dl)		2.54 ± 0.67	2.48 ± 0.66	0.368*	0.713	NS
Bilirubin (mg/dl)		2.34 ± 1.45	2.19 ± 1.51	0.973*	0.330	NS
GOT (IU/ml).		58.94 ± 33.13	61.17 ± 35.29	0.228*	0.820	NS
GPT (IU/ml)		50.03 ± 31.51	52.63 ± 38.46	0.085*	0.933	NS
PT (sec)		16.83 ± 3.42	16.72 ± 3.42	0.137*	0.891	NS
INR		1.49 ± 0.35	1.46 ± 0.32	0.251*	0.802	NS
APRI score		1.89 ± 1.08	1.83 ± 1.09	0.319*	0.750	NS

Chi-square •independent t test *Mann-Whitney U test, NS non significant

Table (3): Endoscopic findings in both groups at the beginning of the study and after two weeks

		Group I No.=52		Group II No.=52		X2	P	Sig.
		No.	%	No.	%			
Risky signs	Absent	11	21.2	8	15.4	0.58	0.446	NS
	Present	41	78.8	44	84.6			
No. of Oesophageal varices cords	2	18	34.6	17	32.75	0.57	0.449	NS
	3	27	51.9	27	51.9	0.73	0.394	NS
	4	7	13.4	8	15.4	0.06	0.811	NS
Oesophageal varices(OV) grade	OV I	0	0	0	0	0.04	0.847	NS
	OV II	16	30.7	15	28.8			
	OV III	24	46.2	26	50			
	OV IV	8	15.4	9	17.3			
Amount of EO (cc) Mean ± SD		10.2±4.3						
Number of rubber bands Mean ± SD				5.1 ± 0.9				
PHG grade	PHGI	4	7.69	3	5.76	0.29	0.593	NS
	PHGII	23	44.2	22	42.3	0.04	0.833	NS
	PHGIII	23	44.2	25	48.1	0.16	0.689	NS
Duodenopathy		16	30.7	18	34.6	0.24	0.628	NS

NS non significant

Table (4): Post endoscopy symptoms

	Group I No.=50		Group II No.=50		X2	P	Sig.
	No.	%	No.	%			
Dysphagia	35	67.3	32	61.5	0.17	0.68	NS
Epigastric pain	47	90.3	40	76.9	1.78	0.182	NS
Heart burn	40	76.9	38	73.1	0.23	0.629	NS
Odynophagia	29	55.7	33	63.4	0.68	0.409	NS
Retrosternal pain	47	90.3	41	78.8	1.82	0.186	NS
Dyspepsia	45	86.5	39	75	1.96	0.161	NS

NS non significant

Table (5): Rates of rebleeding and mortality

		Group I No=52		Group II No=52		X2	P	Sig.
		No	%	No	%			
Rebleeding	2 weeks	5	9.5	3	5.76	2.17	0.14	NS
	4 weeks	2	4.25	1	2.04	2.05	0.81	NS
	6 weeks	1	2.22	1	2.22	0	1	NS
	No	44	84.6%	47	90.4%	0.791	0.374	NS
	Yes	8	15.4%	5	9.6%			
Cause	Ulcers	5	62.5%	3	60%	0.008	0.928	NS
	PHG	2	25%	1	20%	0.043	0.835	NS
	OV	1	12.5%	0	0%	0.677	0.411	NS
	GV	0	0%	1	20%	1.733	0.188	NS
Mortality	Survival	41	79%	40	79.8%	0.210	0.647	NS
	Death	11	21%	10	19.2%			
Mortality after rebleeding	Survival	5	62.5%	3	60%	0.008	0.928	NS
	Death	3	37.5%	2	40%			

NS non significant

Table (6): Correlation between rebleeding and selected study parameters

	Group I (N=52)		Group II (N=52)		All studied cases (N=104)	
	r	**P (Sig.)**	**r**	**p(Sig.)**	**r**	**P (Sig.)**
Age	+0.016	0.910 (NS)	+0.148	0.296 (NS)	+0.048	0.625 (NS)
Sex (Male, Female)	+0.062	0.661 (NS)	+0.023	0.869 (NS)	+0.038	0.698 (NS)
Diabetes (No, Yes)	+0.144	0.308 (NS)	+0.042	0.769 (NS)	+0.093	0.347 (NS)
Hypertension (No, Yes)	+0.277	0.347 (NS)	+0.118	0.406 (NS)	+0.074	0.456 (NS)
Bilharzial antigen	+0.434	0.326 (NS)	+0.376	0.287 (NS)	+0.334	0.187 (NS)
Primary prophylaxis	-0.393	0.295 (NS)	-0.243	0.182 (NS)	-0.274	0.187 (NS)
Ascites(Absent, Mild,…)	+0.192	0.173 (NS)	+0.126	0.374 (NS)	+0.163	0.099 (NS)
Child classification (A,B, C)	+0.145	0.078 (NS)	+0.123	0.093 (NS)	+0.136	0.014 (S)
PT	+0.436	0.001 (HS)	+0.250	0.044 (S)	+0.350	<0.001 (HS)
INR	+0.526	<0.001 (HS)	+0.283	0.042 (S)	+0.419	<0.001 (HS)
OV grade(2 , 3 , 4)	+0.259	0.008 (HS)	+0.207	0.024 (S)	+0.233	0.001 (HS)
Risk signs	+0.221	0.007 (HS)	+0.139	0.045 (S)	+0.179	0.001 (HS)
Units of blood (0-5)	+0.377	0.006 (HS)	+0.393	0.004 (HS)	+0.387	<0.001 (HS)
Hepatic a. RI	+0.126	0.372 (NS)	+0.055	0.701 (NS)	+0.096	0.332 (NS)
PV velocity	-0.293	0.095 (NS)	-0.243	0.082 (NS)	-0.264	0.067 (NS)
PV diameter	+0.090	0.527 (NS)	+0.083	0.557 (NS)	+0.075	0.448 (NS)
Amount of EO injected	+0.329	0.017 (S)	---	---	---	---
Number of rubber bands	---	---	+0.245	0.039 (S)	---	---
APRI score	+0.634	0.526 (NS)	+0.326	0.744 (NS)	+0.389	0.697 (NS)

DISCUSSION

In our study, 104 patients with first attack of variceal bleeding were randomized in two groups; group I (52 patients were treated by endoscopic sclerotherapy, their mean age was 50.1 year, 36 male and 16 female) and group II (52 patients were treated by endoscopic band ligation), their mean age was 49.7 year, 33male and 19 female. There was no significant difference between both groups regarding age and sex. There was no statistically significant difference between the studied groups regarding the cause of chronic liver disease, the majority of patients in both groups have chronic HCV infection, and this is mostly because HCV is the leading cause of chronic liver diseases in Egypt **[15]**.

There was no statistically significant difference between the studied groups regarding post endoscopy symptoms (dysphagia, odynophagia, retrosternal pain, epigastric pain, heart burn and dyspepsia). Oesophageal membrane injuries (erosions or ulcerations) were found in all patients. This agrees with Gimson et al. who found that Complication rates were similar in the two groups **[16]**. But, this disagrees with Stiegmann et al. who found band ligation to have improved survival and fewer complications **[17]**. Also, this disagrees with Laine et al. who reported a significant reduction in local complications but no difference in rebleeding or mortality **[18]**. Moreover, Frequency of treatment induced complications in band ligation were significantly lower as compared with sclerotherapy, mild chest pain and transient fever were significantly more in sclerotheray as reported by Shafqat et al. **[19]**.

In this study, there was no significant difference between the two studied groups as regards rate of early rebleeding. This agrees with Lo et al. who reported that the rate of early rebleeding following EVL was between 9% and 19%, which is close to results of Xu et al. who stated that the incidence of early rebleeding following EVL was (7.6%). Lo et al. reported 17% rate of re-bleeding with band ligation vs. 33% with sclerptherapy, Villanueva et al., (2006), reported 12% incidence rate for re-bleeding for band ligaton versus 21% for sclerotherapy **[20-23]**.

Causes of early rebleeding in the sclerotherapy group were: sclerosant ulcer in 5 cases (62.5%), PHG in 2 cases (25%) and OV in one case. This agrees with Sauerbruch et al. who found that early rebleeding following sclerotherapy is caused by sclerosant ulcer in most patients **[24]**. While, causes of early rebleeding in the band ligation group were: post banding ulcer in 3 cases (60%), PHG in one case (20%) and GV in one case (20%).This result agrees with Vanbiervliet et al. who reported that cases of severe bleeding after EVL were all caused by early slippage of the rubber bands, leaving the unhealed ulcers. Usually, the bands slip spontaneously within the second week after EVL **[25]**.

Mortality among rebleeding cases in the sclerotherapy group was 37.5%, while mortality in the band ligation group was 40%. The mortality rates in the previous literature ranged between 8% and 25%. This lower mortality rates are related to the improvement in the endoscopy techniques and in the efficacy of vasoactive drugs and prophylactic antibiotics **[26-29]**.

After two weeks of follow up there was no significant difference between both groups as regards clinical, laboratory data and endoscopic findings. Most cases of early rebleeding occur during the first 2 weeks of follow up. Rebleeding was due to development of sclerosant or post banding ulcers (5 cases in the first group and 3 cases in the second group). This agrees with Xu et al. who found that post-EVL bleeding was most likely to occur between the 7th and 13th day following the procedure **[21]**. Also, this agrees with Akriviadi et al. who found higher incidence of sclerosant ulcer and rebleeding when endoscopy was repeated earlier, e.g., 70% at 1 week and 30% at two week intervals **[30]**. Also, Tabibian et al. found that most esophageal ulcers bleeding (28 of 32) occurred within 2 weeks after the latest endoscopic treatment **[31]**. This can be explained by the more complete healing of the ulcer 2 weeks after endoscopic treatment.

After 4 weeks of follow up there was no significant difference between both groups as regards clinical, laboratory data and endoscopic findings. Rebleeding occurs in 2cases in group I and one case in group II. The cause of rebleeding in both groups was due to severe portal hypertensive gastropathy (PHG). After 6 weeks of follow up there was no significant difference between both groups as regards clinical, laboratory data and endoscopic findings. Rebleeding occurs in one case in group I (due to bleeding OV) and one case in group II (due to bleeding gastric varix).

In our study, it was found that early rebleeding has significant positive correlation with child-Pugh grade. Also this agrees with Yang et al. (2007) who found that the Child-Pugh score for liver function was an independent risk factor of post-EVL rebleeding **[32]**. This also agrees with Benedeto-Stojanov et al. who stated that patients with the most severe hepatocellular dysfunction (Child's group C) have the shortest period between the first bleeding and rebleeding (mean 20.8 days) **[33]**. Our results agree with Berreta et al. who proved that Child-Pugh C was an independent risk factor of death from rebleeding **[34]**. Also, this agrees with Amitrano et al. who concluded that child class C was an independent predictor of recurrent bleeding; mortality was mainly related to the severity of liver failure. This can be explained by the general concept that

patients with hepatic decompensation bleed more severely than those without hepatic decompensation **[35,36]**.

But, this disagrees with Zhao JR et al. who found that child class was not correlated with the risk of rebleeding and mortality based on univariate analyses. This difference because he used another procedure in treating bleeding Oesophegeal varices: Percutaneous Trans hepatic variceal embolization (PTVE). During PTVE, the portal vein is catheterized by a percutaneous trans-hepatic approach and the gastric vein feeding the varix is embolized with ethanol, steel coils, or cyanoacrylate glue using multi-detector row computed tomography **[27]**.

Size and extent of esophageal varices seen at index endoscopy were also significantly positively correlated to the rebleeding. This result agrees with Benedeto-Stojanov et al. who found that primary variceal bleeding was present in 50% patients with medium and in 65.38% patients with large varices **[33]**. There was no bleeding in patients with small varices. Also, our result agrees with Xu et al. who found that the extent and size of varices are independent risk factors for early rebleeding. Varices that extend along the entire esophagus are much more dangerous than varices that are limited to the middle and lower part. Moreover, a greater extent of varices often means that more rubber bands are needed, increasing the possibility of rebleeding **[21]**. It also agrees with Varghese, et al. who stated that higher grades of varices, presence of cherry-red spots and fundal varices predict variceal bleed in patients with liver cirrhosis **[37]**. The only exception to this is a study done by Koch et al. who found that 35% of patients with small varices bled, while only 20% of patients with large varices also bled. This difference because of small sample size, most cases were child class A and longer duration of follow up (36 months) **[38]**.

In our study; there was significant positive correlation between rebleeding and presence of risky signs on varices. All early rebleeding cases in both groups had risky signs on varices at index endoscopy. This agrees with the study of the Northern Italian Endoscopic Club (NIEC) has shown that endoscopic finding of "red signs" is related to the variceal bleeding **[39]**. Also, Benedeto-Stojanov et al. has shown that endoscopic finding of "red signs" is related to the variceal bleeding. The "red signs" were found in 85% of large varices with bleeding **[33]**.

There was positive significant correlation between rebleeding and the amount of EO injected in sclerotherapy group and number of rubber bands used in band ligation group. This agrees with Xu et al. who found that the number of rubber bands was an independent risk factor for re-bleeding after EVL. Therefore, for varices which were in the mild to moderate class, it may not be reasonable to launch many rubber bands. For severe varices, however, it's usually unavoidable to use more bands **[21]**.

CONCLUSION

Sclerotherapy is associated with higher incidence of rebleeding than band ligation. Most cases of early rebleeding occur during the first 2 weeks of follow up and were due to development of sclerosant or post banding ulcers. Early rebleeding in both groups was correlated to child pugh classification grade (early rebleeding more in child class C>child class B>child class A), elevated coagulation parameters (elevation in PT, INR) among studied groups, grade of oesophegeal varices: Most cases of early rebleeding cases had esophageal varices grade IV and presence of risky signs on varices. No significant correlation between rebleeding and ascites, PV diameter and color Doppler studies could be detected. No statistically significant difference between endoscopic sclerotherapy and band ligation regarding post endoscopy complications could be detected. No significant differences between scleotherapy and band ligation as regards overall mortality or mortality after rebleeding.

Ethical approval: Approved.

Funding: None.

Conflict of interest: Authors declare no conflict of interest related to this article.

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Image Case: Gastric Corpus Angiodysplasias in 61 years old Egyptian Man Presented by Dysphagia

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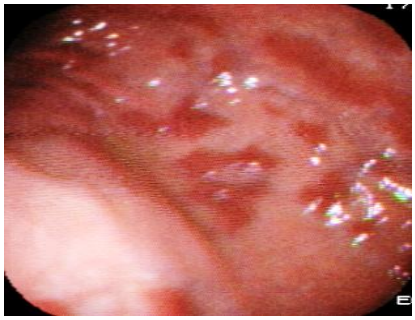
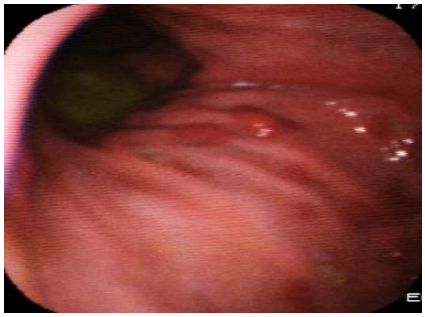
Key words:
Gastric;Angiodysplasias

Upper gastrointestinal endoscopy of 61 years old Egyptian man presented by dysphagia revealed : normal esophageal mucosa without masses, ulcers nor stricture; just sliding hiatus hernia. On examination of the stomach there were extensive angiodysplasias of the corpus of the stomach as shown in figures (1) and (2).



Fig(1):Extensive angiodysplasias of the corpus of the stomach.



Fig(2):Extensive angiodysplasias of the corpus of the stomach.

Value of Serum Neopterin Level in Evaluating Ulcerative Colitis Disease Activity

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Key words:
Ulcerative colitis (U.C.) activity, Neopterin

Background and study aim: Ulcerative colitis (UC) is a major type of inflammatory bowel disease (IBD). It is characterized by chronic inflammation of the large bowel occurring in genetically susceptible individuals exposed to environmental factors and typically, has a relapsing–remitting pattern. Neopterin serves as a marker of cellular immune system activation. This study aims to evaluate serum neopterin concentration as being a new biomarker for U.C. disease activity evaluation and to correlate it with some of the other markers of disease activity.

Patients and methods: This study included 80 subjects, twenty apparently healthy volunteers as a control group (Group I) that included (13 male and 7 female, mean age ± SD 36.0 ± 12.6 y) and sixty patients with UC disease as a patient group (Group II) that included (46 male and14 female, Mean age ± SD 35.5 ± 9.6 y) Group II was subdevided into 20 patients recently diagnosed as active U.C. disease, 20 patients clinically in relapse and 20 patients clinically in remission. Colonoscope and calculation of Simple Ulcerative Colitis Clinical Activity Index Score (SCCAIS) was done for patients with U.C. Laboratory investigations as complete blood count (CBC), erythrocytic sedimentation rate (ESR), Complete liver and kidney function tests, Prothrombin time (PT), partial thromboplastin (PTT), International Normalizing Ratio (INR) and determination of serum neopterin level were done for all subjects.

Results: Serum neopterin level for the U.C. patients was (Mean ± SD= 18.6 ± 5.79, range= 6 – 40) which is highly significant than the control subjects with (Mean ± SD = 5.9 ± 2.4, Range = 2.7 – 9.8). Serum neopterin concentration was positively correlated with SCCAIS (r = 0.77 and P-value <0.001) that indicates a high significant relation between serum neopterin level and clinical active U.C. than in those whose disease was in clinical remission. Serum neopterin concentration was positively correlated with ESR, TLC, platelet count and PT (r= 0.71, P-value <0.001), (r= 0.41, P-value <0.001), (r= 0.34, P-value <0.01) (r= 0.28, P-value <0.05) respectively, whereas negatively correlated with Hb. (r= -0.56, P-value = <0.001) and albumin (r= -0.35, P-value <0.01). There was statistically a high significant relation between serum neopterin level and endoscopic disease distribution (P-value <0.001) as serum neopterin level increases with the increasing of the U.C. disease extent.

Conclusions and Recommendations: Serum neopterin concentration can be used as a new biomarker for U.C. activity that could reliably distinguish between clinically active and inactive U.C., as well as, it can be a helpful tool in predicting the stage of the disease activity. Moreover, the degree of elevation in serum neopterin concentration may be in part related to location and extent of disease.

INTRODUCTION

Ulcerative Colitis (U.C.) is a chronic idiopathic inflammatory bowel disease (IBD) that differentiates itself by exhibition of a nontransmural, continuous and symmetrical pattern of inflammation limited to the colon with distal to proximal extension in disease progression. U.C. follows a chronic course, punctuated by clinical remissions and relapses **[1].**

Typically macroscopic lesions are mucosal ulcerations, with immune cell infiltration and cryptic abscesses at histology. U.C. usually manifests with bloody diarrhea, is associated with a number of extra-intestinal manifestations and may be acutely complicated by toxic megacolon **[2].**

Diagnosis of U.C. is based on clinical symptoms combined with radiological and endoscopic investigations **[3]**. Endoscopy remains an invasive, time-consuming, and expensive procedure. Hence, colonoscopy could not be reasonably repeated in the setting of a regular follow-up. Clinical indices are indirect and often inaccurate predictors of endoscopic activity **[4].**

The need for a diagnostic tool that would improve the conventional methods in IBD diagnosis directed the search towards potential immunological markers, since an aberrant immune response against microbial or endogenous antigens in a genetically susceptible host seems to be implicated in IBD pathogenesis **[5]**.

Neopterin, a stable pteridine derivative, is synthesised by macrophages upon stimulation with the pro-inflammatory cytokine and Interferon-gamma (IFN-γ) through induced transcriptional activation of the rate-limiting enzyme of pteridines biosynthesis (Oxenkrug et al., 2011). Neopterine is indicative of a pro-inflammatory immune status. It serves as a marker of cellular immune system activation **[6,7].**

This study aims to evaluate serum neopterin concentration as being a new biomarker for U.C. disease activity evaluation and to correlate it with some of the other markers of disease activity.

SUBJECTS AND METHODS

This case-control study was carried out in Internal and Tropical Medicine departments in collaboration with Clinical Pathology Department, Zagazig University Hospitals in the period from June 2014 to December 2015.

Subjects:
This study included 80 subjects. An informed consent was obtained from all individuals who shared in this study.

Subjects were classified into 2 groups:
Control group (group I):
This group included twenty apparently healthy volunteers (13 male representing 65.0 %, 7 female representing 35.0 %, Mean age ± SD 36.0 ± 12.6 y, age Range 19-60 y).

Patients group (group II):
This group comprised sixty patients (46 male representing 76.7%, 14 female representing 23.3 %, Mean age ± SD 35.5 ± 9.6 y, age Range 19-52 y) with U.C. disease. They were recruited from patients attending the Internal Medicine and Tropical Endoscopy Units and the IBD Outpatient Clinic of Zagazig University Hospitals. This group was further subclassified into three subgroups :

- Subgroup IIa : comprised 20 patients recently diagnosed as active U.C. disease.
- Subgroup IIb: comprised 20 patients clinically in relapse.
- Subgroup IIIc: comprised 20 patients clinically in remission.

Inclusion criteria:
- Age more than 18 years of both sexes.
- All patients with ulcerative colitis disease either recently discovered or previously discovered (i.e. ongoing remission and relapse).

Exclusion criteria:
- Age < 18 years.
- Patients refused to enter the study.
- Patients with other active autoimmune disorder (unrelated to IBD).
- Patients with cancer (current or past).
- Patients with apparent viral infection or any other septic focus.
- Patients on specific medications affecting the inflammatory response (i.e. NSAID, interferon, etc…).
- Patients with renal impairment.
- In addition for the control subjects, individuals with a known history of or first-degree relative with IBD were excluded.

Methods
All patients with U.C.were subjected to the following :

1- Full history.

2- Complete physical examination.

3- Abdominal ultrasound: Using GE LogiQ P5 Japan instrument.

4- Calculating the Simple Ulcerative Colitis Clinical Activity Index Score (SCCAIS) devised by Walmsley et al. [8]; U.C. was considered active if the (SCCAIS) was ≥5. Significant improvement in U.C disease activity is considered if there is a decrease of >1.5 points in the SCCAI (Table 1).

Table (1): Components of the SCCAI **[8]**

Symptom	Score
Bowel frequency (day)	
1—3	0
4—6	1
7—9	2
>9	3
Bowel frequency (night)	
1—3	1
4—6	2
Urgency of defecation	
Hurry	1
Immediately	2
Incontinence	3
Blood in stool	
Trace	1
Occasionally frank	2
Usually frank	3
General well being	
Very well	0
Slightly below par	1
Poor	2
Very poor	3
Terrible	4
Extracolonic features Arthritis, Uveitis, Erythema nodosum, Pyoderma gangrenosum	1 per manifestation

5- Colonoscope was done for patients, it was performed with a Pentax EC 3440F colonoscope after bowel preparation for an adequate exam and to decrease the risk for potential complications **[9].**

It was done as follow :

a. Discontinue iron-containing medications or constipating agents.

b. Stop free diets for 24 hours.

c. Beginning approximately 18 hours prior to the exam, 4L of specially balanced electrolyte lavage solution (e.g., polyethylene glycol electrolyte [PEG]) U.S. given orally. Administer at a rate of 1 to 2L per hour (8 oz every 10 minutes). Sugars should not be added to the gut lavage because this may cause sodium retention or lead to production of potentially explosive gases.

6- Laboratory investigations including :

- Complete blood count (CBC) by automated Celldyne 1700 machine.
- Erythrocytic sedimentation rate (ESR) by manual technique.
- Complete liver and kidney function tests by fully automated Chemistry Analyser (Mindray BS 800).
- Prothrombin time (PT), partial thromboplastin (PTT), International Normalizing Ratio (INR) were measured by an automated TECO analyser using Diamed chemicals.

7- Special investigations

- Quantitative determination of serum neopterin by ELISA technique: using neopterin ELISA kit REF59321; (Immuno-Biological Laboratories, Hamburg, Germany. 2014). Neopterin concentration was expressed as nanomol per liter (nmol/L).
- Serum neopterin less than 10 nmol/L is considered normal and high if it is more than 10 nmol/L.

Statistical Analysis

All data were entered and analyzed using Epi-Info version 6 and SPSS version 8 for Windows.

RESULTS

Table (2) Demographic data of the studied groups

	Controls (groupI) Number = 20		Cases (groupII) Number = 60		t	P-value
Age (years) Mean ± SD Range	36.0 ± 12.6 19 – 60		35.5 ± 9.6 19 – 52		0.21	0.83 **(NS)**
Gender	**Number**	**%**	**Number**	**%**	**X^2**	**P-value**
Male Female	13 7	65.0 **%** 35.0 **%**	46 14	**%**76.7 23.3 **%**	1.05	0.3 **(NS)**

Table (3): Serum neopterin level among the studied groups

Serum neopterin level	Group I (Controls)	Group II (Cases)	t	P-value
Mean ± SD **Range (nmol/L)**	5.9 ± 2.4 2.7 – 9.8	18.6 ± 5.79 6 – 40	9.5	**< 0.001**** **HS**

There was statistically a high significant difference of serum neopterin among the studied groups (P-value <0.001).

Table (4): Some clinical data and laboratory investigation of group II

Clinical data		Mean ± SD	Range	Normal value
SCCAIS		8.7 ± 3.3	3 – 16	Active ≥ 5
Whole blood count	**TLC**	11.6 ± 4.4	4.2 – 21.7	(4 – 11× 10^3/uL)
	Hb. conc.	12.0 ± 1.3	8.5 – 15	(12 - 16 g/dl)
	Hct. %	35.9 ± 3.95	25.2 – 44.5	(37 – 47 %)
	Platelet count	300.0 ±96.4	158 – 585	(150 - 400 × 10^3/uL)
Serum Albumin		3.6 ± 0.2	3 – 4	(3.5- 5.3 g/dl)
Serum Creatinine		0.9 ± 0.19	0.6 – 1.6	(0.6 – 1.2 mg/dl)
Coagulation profile	**PT (second)**	12.7 ± 0.5	12 – 13.5	(11- 14 sec.)
	PTT	33.6 ± 1.75	30 – 39	(26- 42 sec.)
	INR	1.13 ± 0.04	1.07 – 1.2	(0.8-1.2)
E.S.R.		45.3 ± 22.4	5 – 80	(2 – 7 mm/h)

TLC: Total leucocytic count **Hb:** Hemoglobin **Hct;** Heamatocrit **PT:** Prothrombin time
PTT: Partial thromboplastine **INR:** International Normalizing Ratio **E.S.R:** Etythrocytic sedimentation rate.

Table (5): Relation between serum neopterin level and different subgroups of group II patients

Clinical activity	Serum neopterin level		Significance
	Mean ± SD	Range (nmol/L)	
Subgroup IIa (Recently diagnosed)	22.97 ± 5.8	17.6 – 40	***HS
Subgroup IIb (Relapse)	19.7 ± 3.1	17.5 – 28	** HS
Subgroup IIc (remission)	13.2 ± 3.0	6.0 – 17.3	* HS

F = 28.2 P-value < 0.001**

Table (6): Least Significance Difference (LSD) of serum neopterin level in different subgroups of group II patients

Group II subgroups	Subgroup IIa Recently diagnosed	Subgroup IIb Relapse	Subgroup IIc Remission
Subgroup IIa (Recently diagnosed)		P-value < 0.05 **S**	P-value <0.001 **HS**
Subgroup IIb (Relapse)	P-value < 0.05 **S**		P-value <0.001 **HS**
Subgroup IIc (Remission)	P-value < 0.001 **HS**	P-value < 0.001 **HS**	

Table (7): Correlation between serum neopterin level and other parameters in group II

Parameters		r	P-value	Significance
Age		-0.15	> 0.05	NS
SCCAIS		0.77	< 0.001**	HS
Whole blood count	**TLC**	0.41	< 0.001**	HS
	Hb. conc.	-0.56	< 0.001**	HS
	Hct. %	-0.56	< 0.001**	HS
	Platelet count	0.34	< 0.01*	HS
Serum Albumin		-0.35	< 0.01*	HS
Serum Creatinine		0.12	> 0.05	NS
Coagulation profile	**PT**	0.28	< 0.05*	S
	PTT	0.04	> 0.05	NS
	INR	0.26	> 0.05	NS
E.S.R.		0.71	< 0.001**	HS

Table (8): Endoscopic disease distribution among the different subgroups of group II patients

Group II subgroups	Endoscopic disease distribution	
	Pancolitis	Left colitis
Subgroup IIa (Recently diagnosed)	18	2
Subgroup IIb (Relapse)	16	4
Subgroup IIc (Remission)	12	8
Total number of cases	46	14
%	76.7 %	23.3 %

For subgroup IIc (Remission), the endoscope was done with the onset of diagnosis

Table (9): Relation between serum neopterin level and endoscopic disease distribution

Endoscopic disease distribution	Serum neopterin level		Significance
	Mean ± SD	Range (nmol/L)	
Pan colitis	19.6 ± 5.4	10 – 40	** HS
Left colitis	15.3 ± 5.9	6 – 29	* HS

t = 2.52 P-value = 0.012*

DISCUSSION

Ulcerative Colitis is a major type of IBD. Typically, it has a relapsing–remitting pattern. Diagnosis of U.C. is based on clinical symptoms combined with radiological and endoscopic investigations **[10]**.

Endoscopy is accurate but is an invasive and expensive tool to follow up U.C. Hence, there has been a strong need to search for new biological markers of disease activity, which are simple to use in clinical practice, reliable and inexpensive. Hemoglobin, platelet count and ESR have been used for assessment of disease activity singly or in combination **[11]**.

This study included eighty subjects (twenty apparently healthy volunteers and sixty patients with U.C. [20 patients recently diagnosed as active U.C. disease, 20 patient's clinically in relapse and 20 patients clinically in remission]).

In this study, elevated ESR, decreased (Hb. level & Hct.%) and decreased serum albumin were correlating with disease activity, also there were trends toward an elevated TLC, platelet count, and PT being markers of disease activity. So, these markers may be helpful as indicators for disease activity.

The results of the laboratory tests is in concordance with Sachar and Walfish **[12]** as they had mentioned that laboratory tests should be done to screen for anemia, hypoalbuminemia, and electrolyte abnormalities in U.C. patients. Liver function tests should be done. Other possible laboratory abnormalities include leukocytosis, thrombocytosis, and elevated acute-phase reactants (eg, ESR).

The number of white blood cells increases during the acute phase response and is also influenced by the drugs utilized in IBD, such as glucocorticoids (increased) or azathioprine and 6-mercaptopurine (decreased). Albumin is a negative acute phase marker and decreased levels may be found during inflammation **[13]**.

Eng and Surawicz **[14]** had mentioned that laboratory evaluation may be helpful, but is never diagnostic. For example, an elevated white blood cell count can be seen with either acute self-limiting colitis or IBD. Anaemia, especially iron deficiency anaemia, suggests IBD, as the usual course in acute self-limiting colitis is too short for significant blood loss.

Also, Danese and Fiocchi **[15]** had mentioned that laboratory measurements are not diagnostic, but are helpful in assessing and monitoring disease activity and in differentiating U.C. from other forms of colitis. Blood counts and measurements of the ESR and the level of fecal lactoferrin or calprotectin help to determine the severity of the inflammation.

Regarding SCCAIS we can found that it is a predictive of U.C. disease activity, but are never diagnostic. This is in concordance with Higgins et al. **[16]** as they had mentioned that the SCCAI is the most vigorously validated index in U.C. and has good psychometric and performance validity. The SCCAI is also an adequate replacement for more objective disease activity measurements such as endoscopy and blood tests. The clinician-based SCCAI is able to categorize two types of patients: patients with inactive U.C. disease (SCCAI score <5) and patients with active U.C. disease (SCCAI score ≥5). These assessments require completion by the treating clinician, which makes them prone to bias, since the clinician gives an interpretation of the patient's response.So, this index gives an idea only about U.C. disease activity but not useful to detect the degree of the disease activity **[17]**.

Ciećko-Michalska et al. **[18]** have reported that measurement of neopterin concentration in serum may be a useful marker to assess disease activity in patients with IBD. They observed a positive correlation between increased concentrations of neopterin in serum and increased levels of (TNF-α and CRP), increasing the number of (leukocytes and platelets count) and the degree of disease activity in patients with both U.C. and Chron's disease (C.D).

In this study, we found that serum neopterin concentrations for all of the participating control subjects were within the normal value. In comparison to the control group (I), all of the three subgroups of group II U.C. patients interpret higher serum neopterin concentrations, which are above the normal value.

Results in this study found that serum neopterin concentration is greater in patients with active U.C. disease and also increases in pancolitis. As shown in our results serum neopterin level is greater in U.C. patients whose disease affecting the whole colon than those patients with the disease limited to left colon. The previous observation suggests that the serum neopterin level is directly proportional to the extent of the inflammed part of the colon. However, a larger study would be needed to confirm those observations.

Moreover, serum neopterin concentration was significantly greater in patients with clinically active U.C. than in those whose disease was in clinical remission and so; their results are going with the results of Husain et al. **[19]**.

CONCLUSIONS

- Serum neopterin concentration is increased in patients with clinically active U.C. when compared with controls, and therefore represents a new biomarker for disease activity that could reliably distinguish between clinically active and inactive ulcerative colitis
- The degree of elevation in serum neopterin concentration can be used as a helpful tool in predicting the stage of the disease activity. It may be in part related to location and extent of disease.
- Laboratory tests should be done to screen for anemia, hypoalbuminemia, electrolyte abnormalities, and other possible laboratory abnormalities as leukocytosis, thrombocytosis, and elevated acute-phase reactants (eg, ESR). These markers may be helpful as predictors for disease activity and severity, but are never diagnostic.

Ethical approval: Approved.
Funding: None.
Conflict of interest: Authors declare no conflict of interest related to this article.

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Peer reviewer: Walid Abdel-Dayem , Professor of Tropical Medicine and Hepatogastroenterology, Faculty of Medicine, Zagazig University, Egypt. **Editor: Tarik Zaher**, Professor of Tropical Medicine and Hepatogastroenterology, Faculty of Medicine, Zagazig University, Egypt

Value of Protein C and D-Dimer in Predicting Non Hepatocellular Carcinoma Portal Vein Thrombosis in Patients with Liver Cirrhosis

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Key words:
Liver cirrhosis, Portal vein thrombosis (PVT), Protein C (PC), D-dimer

Background and study aim: Patients with liver cirrhosis may develop serious changes of coagulation process. Protein C (PC) is synthesized in the liver and considered as the key component of an important natural anticoagulant pathway. D-dimer is a fibrin degradation product that represents an accurate marker of fibrinolytic activity. Advances in imaging techniques have resulted in 5–27% increase in patients with liver cirrhosis being diagnosed with portal vein thrombosis (PVT). The aim of this study was to estimate the value of plasma levels of protein C and D-dimer as predictors for diagnosis of PVT in patients with liver cirrhosis.

Patients and methods: This study included one hundred cirrhotic patients who underwent abdominal ultrasound and dynamic contrast enhanced computed tomography (CT) scans. Therefore, they were classified into two groups as following: Group 1: Included twenty six patients (22 males and 4 females, mean age 53.60±7.8 years) with PVT and Group 2: Included seventy four patients (65 males and 9 females, mean age 54.96±6.5 years) without PVT as a control group. Full history taking, complete physical examinations and laboratory investigations including liver function tests, complete blood count, prothrombin time (PT), activated partial thromboplastin time (aPTT), International Normalizing Ratio (INR) and determination the levels of plasma protein C and D-dimer were done for all cirrhotic patients.

Results: The levels of D-dimer were significantly higher in cirrhotic patients with PVT than in those patients without PVT, while the level of protein C were significantly lower in cirrhotic patients with PVT than the control group. Also, D-dimer was significantly increased from Child-Pugh class A to C, while PC was significantly progressively decreased. D-dimer cut-off values above 530 ng/L provided high sensitivity and negative predictive value (92.3% and 95.7%, respectively). Also, PC cut-off values below 71.6 % had a high sensitivity and negative predictive value (96.2 and 96.9%, respectively).

Conclusion: PVT formation in liver cirrhosis is closely associated with decreased plasma levels of PC, and increased D-dimer. So, they are considered risk factors in PVT formation in patients with liver cirrhosis. So, estimation of plasma levels of protein C and D-dimer are important for early detection of PVT in patients with liver cirrhosis.

INTRODUCTION

The liver plays a vital role in the coagulation process as it synthesizes and metabolizes the majority of fibrinolytic factors, as well as most proteins which favour and inhibit the process of coagulation and fibrinolysis **[1]**. Liver failure may disrupt the haemostatic system and decreased levels of most procoagulant factors, leading to severe bleeding or thrombotic complications **[2]**. However, decreased levels of the procoagulants are accompanied by decreases in levels of such naturally occurring anticoagulants as antithrombin III and protein C **[3,4]**.

Protein C (PC) is a major physiological anticoagulant that is synthesized in the liver, circulates in plasma and is considered a key component of an

important natural anticoagulant pathway **[5,6]**. It is vitamin K-dependent serine protease enzyme that is activated by thrombin into activated protein C (APC), and the treatment with vitamin K antagonists might further reduce the level of this naturally occurring anticoagulant in patients with liver cirrhosis, increasing the risk of thrombosis **[5]**.

D-dimer is a Fibrin degradation product (FDP) that represents an accurate marker of fibrinolytic activity **[7]**. D-dimer concentrations are routinely determined in the differential diagnosis of venous thromboembolism, including deep vein thrombosis and pulmonary embolism **[8,9]**. In addition, D-dimer concentrations increase with deteriorating liver function and may be associated with PVT **[10]**.

Portal vein thrombosis (PVT) is more commonly seen in end-stage liver disease **[11]** and may be associated with sclerotherapy, abdominal surgery or hepatocellular carcinoma (HCC) **[12,13]**. Life-threatening complications such as refractory ascites, upper gastrointestinal bleeding and intestinal ischemia and necrosis can occur in patients with PVT **[14]**. Therefore, it is necessary to develop efficient methods for detecting PVT, in order to allow earlier diagnosis, treatment and avoid serious complications.

The present study aimed to estimate the value of plasma levels of protein C and D-dimer as predictors for diagnosis of PVT in patients with liver cirrhosis.

PATIENTS AND METHODS

This study was conducted in the Tropical Medicine, Internal Medicine and Clinical Pathology Departments, Faculty of Medicine, Zagazig University Hospitals in the period from tune 2014 to July 2015. It included 100 cirrhotic patients. They underwent abdominal ultrasound and dynamic contrast enhanced computed tomography (CT) scans. Therefore, they were classified into two groups as following:

Group 1 (PVT group): It included twenty six cirrhotic patients (22 males and 4 females, mean age 53.60±7.8 years) were diagnosed with PVT.

Group 2 (control group): it included seventy four cirrhotic patients (65 males and 9 females, mean age 54.96 ± 6.5 years) without PVT.

Exclusion criteria:

- Patients suffering from malignancy or any blood diseases.
- Known and recent discovered patients with HCC by triphasic CT with and without elevated alpha fetoprotein.
- Patients receiving anti-coagulant or anti-platelet medications.
- Endoscopic therapy; splenectomy; autoimmune disease; liver cirrhosis due to autoimmune hepatitis or primary biliary cirrhosis.
- Patients having positive rheumatoid factor, trauma, pregnancy, recent surgery, infection or dehydration.
- Diabetes, hypertension, renal disorder, malabsorption.

All patients were subjected to:

- Thorough history taking
- Full physical examination
- Routine laboratory investigations as liver function tests (LFT), kidney function tests (KFT), complete blood picture.
- Prothrombin time (PT), activated partial thromboplastin time (aPTT) levels were determined by routine coagulation methods with the coagulation detector, using a Sysmex CA6000 automated analyser (Sysmex, Milton Keynes, UK).
- **Special investigations:**
  - **Estimation of plasma protein C:** The protein C antigen assay was done by a sandwich ELISA **[15]**.

  Reference ranges:

  - Protein C antigen values are generally expressed in relative percent as compared to pooled normal plasma. The reference range when normal plasma samples were tested by the Helena protein C antigen assay was 72-160% (mean, 110% and SD, 24%).
  - **Determination of D-dimer**: Using automated VIDAS D-dimer exclusion II **[16]**.

  Reference ranges: The normal range (up to 500 ng/ml)
- **Abdominal ultrasound:** Using Aloka SSD-200 (a 3.5 MHz transducer).
- **Dynamic contrast enhanced computed tomography (CT) scans :** To estimate the vessel patency within the portal venous system, including intrahepatic portal vein branches, main portal vein, superior mesenteric vein,

and splenic vein. PVT was appeared as a filling defect in the CT images **[17].**

- The diagnosis of liver cirrhosis based on the clinical examination, laboratory tests, imaging tests, and liver biopsy or fibroscan if present.
- Severity of the liver disease was scored according to Child-Pugh classification. (Table I), and patients were classified as class A, class B or class C, using parameter of serum bilirubin, serum albumin, prothormbin time (PT) or International Normalizing Ratio (INR), hepatic encephalopathy and ascites **[18]**.

Table (I): Child-Pugh-Turcotte criteria

	1 Point	2 Points	3 Points
Albumin (g/dl)	>3.5	2.8-3.5	<2.8
Bilirubin (mg/dL)	<2	2-3	>3
Ascites	None	Minimal	Moderate
Encephalopathy	None	Grade 1-2	Grade 3-4
PT (second prolonged) **INR**	<4 <1.7	4-6 <1.7-2.3	>6 >2.3

PT; prothormbin time INR, International Normalizing Ratio
Class A: 5-6 points; class B: 7-9 points; class C: 10-15 points

Statistical analysis

Continuous variables were expressed as the mean ± SD and the categorical variables were expressed as a number (percentage). Continuous variables were checked for normality by using Shapiro-Wilk test. Independent Student t-test was used to compare two groups of normally distributed data. Percent of categorical variables were compared using Pearson's Chi-square test. Receiver operating characteristic (ROC) curve analysis was used to identify optimal cut-off values of D-dimer and protein C with maximum sensitivity and specificity for prediction of portal vein thrombosis. Area Under Curve (AUROC) was also calculated, criteria to qualify for AUC were as follows: 0.90 – 1 = excellent, 0.80-0.90 = good, 0.70-0.80 = fair; 0.60-0.70= poor; and 0.50-0.6 = fail. The optimal cut-off point was established at point of maximum accuracy. All tests were two sided. P<0.05 was considered statistically significant. All data were analyzed using Statistical Package for Social Science for windows version 18.0 (SPSS Inc., Chicago, IL, USA) & MedCalc for windows version 13 (MedCalc Software bvba, Ostend, Belgium).

RESULTS

Results are shown in the following tables :

Table (1): Demographic data of studied groups.

Demographic data	PVT group (N=26)	Control group (N=74)	P-value
Age (years)	53.6 ± 7.8	54.9 ± 6.5	0.407*
Gender Male Female	 22 (84.6%) 4 (15.4%)	 65 (87.8%) 9 (12.2%)	0.737§
Child classification A (n=13) B (n=53) C (n=34)	 3 (11.5%) 14 (53.8%) 9 (34.6%)	 10 (13.5%) 39 (52.7%) 25 (33.8%)	0.967§

N=Total number of patients in each group; Quantitative data were expressed as mean±1SD; Qualitative data were expressed as a number (percentage); * Independent samples Student's t-test; § Chi-square test; P< 0.05 is significant.

Table (2): Levels of platelet, prothrombin time, activated partial thromboplastin time, D-dimer and protein C as regard Child class.

Parameters	Child-Pugh classification		
	A (N=13)	B* (N=53)	C* (N=34)
Platelet count (x10^3/mm^3)	106.4 ± 11.3	82.9 ± 12.3•	79.5 ± 21.2•
PT (seconds)	13.9 ± 0.9	16.9 ± 1.9•	18.9 ± 2.5•‡
APPT (seconds)	30.6 ± 3.1	31.7 ± 5.2	42.2 ± 12.2•‡
Protein C (%)	73.6 ± 16	65.9 ± 9.6•	64 ± 10.3•
D-dimer ((µ/ml)	436.6 ± 80	890.4 ± 103•	927.5 ± 165•

PLT, platelet; PT, prothrombin time; aPTT, activated partial thromboplastin time;
N=Total number of patients in each group; Quantitative data were expressed as mean±1SD; * Independent samples Student's t-test; P< 0.05 is significant; § P<0.05 versus group A, • P<0.001 versus group A, ♯ P<0.05 versus group B, ‡ P<0.001 versus group B.

Table (3): Comparison between the studied groups as regards levels of platelet, prothrombin time, activated partial thromboplastin time, D-dimer and protein C**.**

Parameters	PVT group (N=26)	Control group (N=74)	P-value
Platelet (PLT) count (x10^3/mm^3)	89.4 ± 28.7	91.2 ± 21.8	0.740*
PT (seconds)	16.1 ± 3.8	15.6 ± 3.7	0.557*
APPT (seconds)	35.4 ± 5.8	35.9 ± 5.4	0.691*
Protein C (%)	62.6 ± 7.2	72.3 ± 6.5	<0.001*
D-dimer ((µ/ml)	980.6 ± 112	534.2 ± 94.9	<0.001*

N=Total number of patients in each group; Quantitative data were expressed as mean±1SD; * Independent samples Student's t-test; P< 0.05 is significant.

Table (4): Levels of platelet, prothrombin time, activated partial thromboplastin time, D-dimer and protein C in PVT group versus control group in different Child class

Parameters	Child A			Child B			Child C		
	PVT group (N=3)	Control group (N=10)	P value	PVT group (N=14)	Control group (N=39)	P value	PVT group (N=9)	Control group (N=25)	P value
Platelet count (x10^3/mm^3)	99.6± 23.2	106.7± 26.5	0.685*	104.4± 14.2	109.7± 16.5	0.291*	59.3± 9.2	66.3± 12.5	0.046*
PT (seconds)	13.9± 0.3	14.0± 0.8	0.840*	16.4± 0.6	16.4± 0.8	1.000*	18.9± 0.6	18.4± 0.8	0.027*
APPT (seconds)	29.9± 3.3	30.1± 4.1	0.940*	31.9± 4.3	30.1± 3.1	0.099*	39.9± 3.2	40.1± 2.1	0.803*
Protein C (%)	69.9± 10.1	72.9± 8.2	0.605*	58.9± 9.1	66.6± 8.0	0.004*	60± 6.5	64.1± 4.0	0.013*
D-dimer ((µ/ml)	434± 28	415± 29	0.338*	875± 18	759± 19	<0.001*	980± 25	825± 21	<0.001*

N=Total number of patients in each group; Quantitative data were expressed as mean±1SD; * Independent samples Student's t-test; P< 0.05 is significant.

Table (5): Validity of D-dimer and protein C in prediction of portal vein thrombosis in all cirrhotic patients; ROC curve analysis

Cut-off values	SN % (95%CI)	SP % (95%CI)	PPV % (95%CI)	NPV % (95%CI)	Accuracy (95%CI)	AUROC (95%CI)
D-dimer > 530 ng/L	92.3% (73.8-100)	59.5% (25.5-93.5)	44.4% (10-78.8)	95.7% (81.6-100)	68.0% (35.7-100)	0.7
Protein C < 71.6%	96.2% (83-100)	41.9% (7.7-76.1)	36.8% (3.4-70.2)	96.9% (84.9-100)	56.0% (21.6-90.4)	0.6

ROC curve: Receiver Operating Characteristic curve; SN: Sensitivity; SP: Specificity; PPV: Positive Predictive Value; NPV: Negative Predictive Value; 95%CI: 95% Confidence Interval; AUROC: Area Under Receiver Operating Characteristic curve.

DISCUSSION

Portal vein thrombosis (PVT) is one of the severe complications of liver cirrhosis **[19,20]**. It is more commonly seen in end-stage liver disease and particularly in those who have HCC **[11]**. It may lead to hemorrhage of the gastrointestinal tract, intestinal ischemia or refractory ascites, and could even be life-threatening **[13]**. So, early diagnosis and treatment of PVT in patients with liver cirrhosis may save lives.

The prevalence of PVT in the present study was high (26%). However, many studies proved that the advanced imaging techniques have resulted in 5–27% of patients with liver cirrhosis being diagnosed with PVT **[10,21,22]** and others reported that prevalence of PVT ranges from 0.6% to 26% in liver cirrhosis **[19,20]**.

The liver plays an important role in the coagulation process as it synthesizes and metabolizes the majority of fibrinolytic factors, as well as proteins which favour and inhibit the process of coagulation and fibrinolysis. Liver failure may disrupt the haemostatic system, leading to severe bleeding or thrombotic complications **[1,2]**.

This study found that PT and aPTT were significantly prolonged with the deterioration of liver function from Child-Pugh class A to C, but did not correlate with the formation of PVT. The coagulation function of cirrhotic patients was generally suppressed due to their hepatic failure, which is usually reflected as decreased coagulants. So, they considered as important parameters in indicating liver dysfunction **[10,15]**.

The results showed significantly progressive decreasing of PLT that correlated with the stages of liver dysfunction. These results can be explained as PLT counts were decreased possibly from hypersplenism, decreased production of thrombopoietin synthesis in the liver **[10,23]**.

Protein C (PC) is a major physiological anti-coagulant. The thrombin–thrombomodulin complex activates PC, which inhibits the blood coagulation cascade by selective degradation of the procoagulant factors Va and VIIIa **[5,12]**.

In the current study plasma protein C concentrations were found to be decreased significantly with deteriorating liver function, such that both Child-Pugh class B and class C patients had significantly lower protein C concentrations than class A. So that protein C activity may be used as a sensitive marker of hepatocellular damage **[2]**.

Moreover, plasma PC levels in the PVT group were significantly lower than those in the control group. One of the underlying mechanisms may be due to the fact that hepatocytes fail to synthesize adequate amounts of PC under ischemic and hypoxic conditions. Also, the decrease in PC may be attributed to the endothelial cells damage caused by portal hypertension, which leads to the activation and subsequent consumption of PC in fibrolytic processes **[10]**.

D-dimer is a fibrin degradation product, a small protein fragment present in the blood after a blood clot is degraded by fibrinolysis **[7]**. It is a sensitive marker of coagulation and fibrinolysis. Hyperfibrinolysis in cirrhotic patients might represent a state of low grade disseminated intravascular coagulation **[24,25]**.

In this study, D-dimer levels were significantly increased from Child-Pugh class A to C so, it was found to have significant correlations with liver dysfunction. Also, D-dimer in the PVT group was significantly higher than this in the control group **[26]**.

The sensitivity, specificity, positive and negative predictive values for PVT of PC and D-dimer in liver cirrhosis patients was calculated. The D-dimer cut-off values were above 530 ng/L and PC cut-off values were below 71.6 %. Those cut-

off values provided a sensitivity of 92.3% and 96.2%, a negative predictive value of 95.7% and 96.9%, for D-dimer and PC respectively, so decreased PC and increased D-dimer values can suspect the presence of PVT **[10,26]**.

CONCLUSION

The previous data concluded that disturbed coagulation in patients with liver cirrhosis is directly related to the severity of cirrhosis and more importantly, it showed that decreased PC and increased D-dimer values were the risk factors in PVT formation. Therefore, they can suspect the presence of PVT and then specific imaging techniques should be done to confirm the diagnosis and initiate early treatment before the occurrence of serious complications.

RECOMMENDATIONS

Although both coagulation and anticoagulation systems in cirrhotic patients are suppressed as a result of the functional failure of the liver, the two systems may still be maintained. Therefore, there is no tendency for hemorrhage or thrombosis under stable conditions. When cirrhotic patients present under stress, such as sepsis, trauma or scheduled for operative interference, the balance between the two systems is disturbed and those patients should be screened for both coagulation and anticoagulation systems to be managed accurately.

Plasma transfusion (with its contents of coagulant and antithrombotic factors) might be used in particular postoperative decompensation in chronic liver disease patients.

Antithrombotic therapy can be used for recanalization of the portal vein in PVT patients, but evidence for the safety and efficiency of anticoagulants in PVT patients is still lacking and need proper study.

Ethical approval: Approved.
Funding: None.
Conflict of interest: Authors declare no conflict of interest related to this article.

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Peer reviewer: Mostafa Elshamy , Professor of Tropical Medicine and Hepatogastroenterology, Faculty of Medicine, Zagazig University, Egypt. **Editor: Tarik Zaher**, Professor of Tropical Medicine and Hepatogastroenterology, Faculty of Medicine, Zagazig University, Egypt