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

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Atef Eraky
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5- Global Impact Factor (Impact Factor for year 2015 is =0.544**) http://globalimpactfactor.com/afro-egyptian-journal-of-infectious-and-endemic-disease/**

6- Universal Impact Factor (Impact Factor for year 2013 is = 1.0599): http://www.uifactor.org/Search.aspx?q=2090-7184

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CONTENTS

Blood Ammonia level : Is it a Clue for the Presence of Oesophageal Varices in Cirrhotic Patients ?

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Key words: Oesophageal varices (OV); Blood ammonia level (BAL); Spleen longitudinal diameter(SLD); Portal vein diameter (PVD); Splenic vein diameter(SVD)

Background and study aim: Endoscopic screening of all patients with liver cirrhosis add major burden to endoscopic units. Non-invasive detection of Oesophageal varices (OV) help to reduce the necessity of endoscopic screening.The aim of this work is to assess the diagnostic utility of blood ammonia level (BAL) as noninvasive predictor for presence of OV and evaluate its correlation with variceal size in cirrhotic patients.

Patients and Methods: This prospective cross sectional study was conducted upon 100 cirrhotic patients who attended Hepatology, Gastroenterology and Infectious diseases department, Benha University Hospital. Fasting blood ammonia was measured and upper gastrointestinal endoscopy was done for all patients. Patients were divided according to presence of OV into two groups: Group I : included 30 patients with liver cirrhosis without OV. Group II: included 70 patients with liver cirrhosis with OV who were subdivided into four subgroups: Group IIa:included 11 patients with grade I O.V. Group IIb: included 21 patients with grade II O.V. Group IIc: included 15 patients with grade III O.V. Group IId: included 23 patients with grade IV O.V.

Results: The study showed that there was a highly significant increase in the mean values of BAL in cirrhotic patients with varices in comparison to those without varices. Also the study showed highly significant increase in the mean values of BAL in patients with large OV (grade III,IV) in comparison to patients with small and medium sized varices (grade I,II). By multivariate analysis, the presence of O.V. was independently associated with increased blood ammonia levels.

Conclusion: Blood ammonia level could be a non invasive predictor for the presence of OV and could be clinically useful, as it correlated with the size of OV.

INTRODUCTION

Liver cirrhosis is a leading cause of death worldwide. It is the end result of a long-lasting process, usually clinically silent and unnoticed by the patient and the physician for years **[1].** The clinical course of patients with advanced cirrhosis is often complicated by a number of important sequelae that can occur regardless of the underlying cause of the liver disease. These include portal hypertension and its consequences of gastroesophageal variceal hemorrhage **[2].** Portal hypertension is a progressive, inevitable sequelae of liver cirrhosis that leads to formation of portosystemic collateral veins, among them, oesophageal varices (OV) have the greatest clinical influence because their rupture results in variceal bleeding that can be fatal. OV can be diagnosed by Upper gastrointestinal (GI) endoscopy which is the gold standard and it is recommended by guidelines to screen all cirrhotic patients for OV at the time of diagnosis. Lack of detection of OV at the first endoscopic evaluation mandates repeat endoscopy annually in decompensated cirrhotic patients and every 2-3 years in patients with compensated cirrhosis **[3].** However, the majority of cases undergoing screening endoscopy either do not have varices or have

varices but do not require prophylactic therapy **[4]**. Patients without varices develop them at a rate of 8% per year and the progression from small to large varices occurs in 10 to 20% of cases yearly **[5]** Therefore, the identification of non endoscopic, non invasive methods that can accurately predict OV in cirrhotic patients, particularly those of large size, can help to identify patients at greatest risk and hence reduce the exigency of endoscopic screening **[6]**. In cirrhosis, the major portion of ammonia carried by portal blood is shunted by portosystemic collaterals into systemic circulation. This raised blood ammonia level (BAL), on the other hand, could be a good mirror of portosystemic collaterals as well as portal hypertension **[7].** The aim of this study was to evaluate the utility of blood amonia as a non invasive predictor of OV in cirrhotic patients regarding their presence and size and compared it with platelet count/ splenic diameter ratio that is one of the most important non invasive predictors of O.V.

PATIENTS AND METHODS

Study design

This cross-sectional study was carried out prospectively at the department of Hepatology, Gastroenterology and Infectious diseases, Benha University Hospital. The study was approved by ethical committee of Benha Faculty of Medicine and its University Hospitals. Before enrolling in the study, informed consent was obtained from each participant.

Patients

Hundred adult cirrhotic patients were included in this study. All patients met the diagnostic criteria of liver cirrhosis by clinical, biochemical and ultrasonographic findings. Patients were divided according to the results of the upper gastro-intestinal endoscopy into the following groups:

- **Group I:** Included 30 patients with liver cirrhosis with no endoscopic evidence of oesophageal varices.
- **Group II:** Included 70 patients with liver cirrhosis with endoscopic evidence of oesophageal varices, who were subdivided into four subgroups according to the **Modified Grading System [8]** :
  - ✓ **Group IIa:** Included 11 patients with grade I O.V.
  - ✓ **Group IIb:** Included 21 patients with grade II O.V.
  - ✓ **Group IIc:** Included 15 patients with grade III O.V.
  - ✓ **Group IId:** Included 23 patients with grade IV O.V.

Exclusion criteria :

Patients who received endoscopic variceal ligation (EVL) or sclerotherapy, surgery for oesophageal varices, patients with history of previous or current use of beta blockers, presence of hepatic encephalopathy, active or recent GI bleeding within 4 weeks, portal vein thrombosis on ultrasonography, hepatocellular carcinoma, serum creatinine of >1.3 mg/dl and patients in whom endoscopy is contraindicated were excluded from the study.

Patients were then evaluated by thorough history taking, complete clinical examination (to confirm signs of chronic liver disease or liver cell failure and signs of complications), Complete blood count (CBC), Liver profile tests including; Serum Albumin (gm/dl), Prothrombine time and activity, Alanine aminotransferase (ALT) (U/L), Aspartate aminotransferase (AST) (U/L), Serum bilirubin (total and direct) (mg/dl), Kidney function tests including; Blood urea (mg/dl) and Serum Creatinine (mg/dl), Blood levels of ammonia (NH4) **(**µmol/l**) :** measured by kinetic enzymatic method with glutamate dehydrogenase by using ammonia–liquizyme single reagent **[9]** provided by Bioassay system, Germany. Abdominal ultrasonographic examination was done with stress on portal vein diameter, spleen longitudinal diameter and exclusion of hepatocellular carcinoma and portal vein thrombosis. Upper GIT endoscopy was done by olympus video endoscopy at Hepatology, Gastroenterology and Infectious diseases department, Benha University Hospital. During endoscopy, presence of esophageal varices and its grades, gastric varices, portal hypertensive gastropathy were noted. Modified Child Pugh Score was calculated for all patients.

Statistical analysis :

The data collected were tabulated and analyzed by SPSS (statistical package for social science) version 22.0 on IBM compatible computer.

Two types of statistics were done:

I- Descriptive 1- Mean value (X) and Standard Deviation [SD]: for quantitative data. 2- Frequency and percenatage 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. Variables found to be associated with the dependent variable at univariate logistic regression at a probability threshold of less than 0.10 were entered into multivariate logistic regression models to avoid the effect of co linearity.

RESULTS

This prospective study was conducted upon 100 adult cirrhotic patients who were classified into two groups; group 1 included 30 cirrhotic patients without esophageal varices and group 2 included 70 cirrhotic patients with esophageal varices. The mean age of group 2 cases was 48.93±9.84 which was significantly higher than that of group 1 (Mean±SD=42.67±8.70). Male predominance was noted in group 2 ; 52 cases (74.29%) vs. 14 cases (46.67%) in group 1 with statistical significant difference between both groups. Regarding Child classification, most patients in group I were Child A (66.7%) and most patients in group II were Child B and C (74.29%). BAL was significantly higher and platelet count/splenic diameter ratio was significantly lower in group 2 than group 1 (p value =0.00 for each). On the same hand, BAL was significantly higher and platelet count/ splenic diameter ratio was significantly lower in large sized varices (grade III and IV) than small sized varices (grade I and II). BAL was the only predictor of presence of OV by multivariate logistic regression analysis. Spearman correlation revealed that there was highly significant positive correlation between BAL and size of varices (P value <0.001). BAL at cutoff value = 48 umol/L had 75.7% sensitivity, 100% specificity, 100% PPV, 63.8 % NPV and 93.2 % accuracy in detection of OV and at cutoff level 67 umol/L, it had 46.2% sensitivity, 97.7% specificity, 75% PPV and 92.4% NPV for detection of large OV (grade III and IV). Platelet count/splenic diameter ratio at cutoff level = 1073 had sensitivity 94.3%, specificity 56.7%, PPV 83.5% and NPV 81% for detection of OV and at cutoff level = 570 it had 76.9% sensitivity, 81.6% specificity, 38.5% PPV and 95.9% NPV for detection of large OV (grade III and IV). On Comparing blood ammonia level and platelet count/splenic diameter ratio as predictors for OV, no significant difference was detected between them.

Table (1): Blood ammonia levels and Platelet count/ splenic diameter ratio of the studied groups.

			T-test and chi-square	
	Group I Cirrhotics without O.V. (N=30)	**Group II Cirrhotics with O.V. (N=70)**	**Test value**	**P-value**
Ammonia (µmol/l)	5.98±39.17	9.76 ±54.84	-8.153	0.000
Platelet count/splenic diameter Ratio	322.43±1076.86	251.43 ±698.05	6.327	0.000

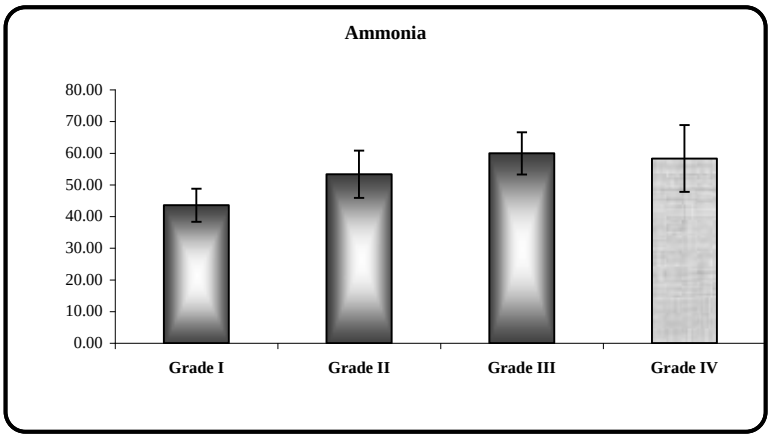

Figure (1): Blood ammonia levels among different grades of oesophageal varices

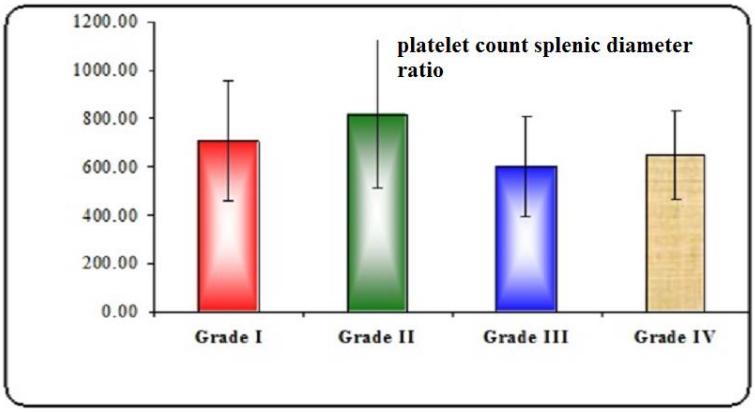

Figure (2): platelet count/splenic diameter ratio among different grades of oesophageal varices

Table (2): Multivariate logistic regression analysis for predictors of presence of oesophageal varices.

	B	S.E.	Wald	P-value	Odd	95% C.I. for Odd	
						Lower	Upper
Age	-0.052	0.072	0.527	0.468	0.949	0.825	1.092
Gender	0.551	1.191	0.214	0.644	1.734	0.168	17.884
INR	0.147	0.700	0.044	0.834	1.158	0.294	4.566
Prothrombin time	0.583	0.339	2.962	0.085	1.791	0.922	3.478
HB%	0.726	0.396	3.360	0.067	2.067	0.951	4.491
PLT	-0.092	0.117	0.615	0.433	0.913	0.726	1.147
Splenic diameter	1.274	1.073	1.408	0.235	3.574	0.436	29.298
P.V. diameter	0.632	0.608	1.078	0.299	1.881	0.571	6.199
Child class	-0.278	0.956	0.084	0.771	0.757	0.116	4.929
PHG	0.352	1.446	0.059	0.808	1.422	0.084	24.216
Blood ammonia	0.520	0.190	7.491	0.006	1.681	1.159	2.439
platelet count/ splenic diameter ratio	0.013	0.016	0.681	0.409	1.013	0.982	1.045

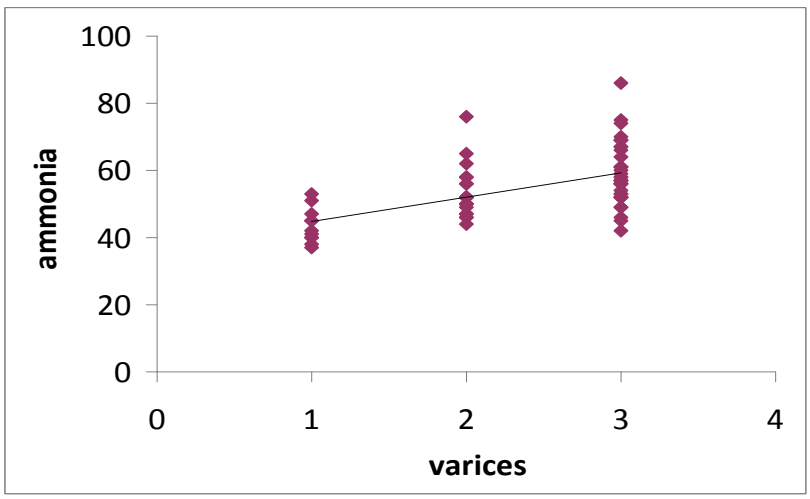

Figure (3): Correlation between blood ammonia levels and size of varices

Table (3) : Diagnostic performance of platelet count / splenic diameter ratio and blood ammonia level for diagnosis of oesophageal varices.

Cutoff	Sens.	Spec.	PPV	NPV	Accuracy
platelet count/ splenic diameter ratio = 1073	94.3	56.7	83.5	81.0	82.6
Blood ammonia level = 48	75.7	100.0	100.0	63.8	93.2

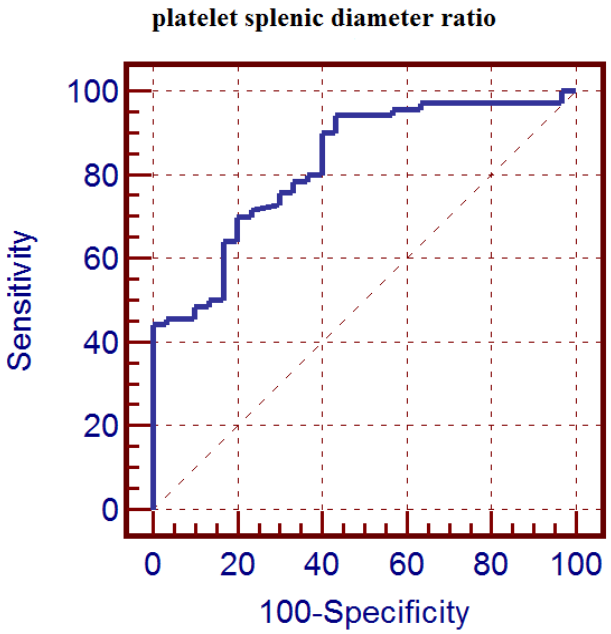

Figure (4): ROC curve of platelet count / splenic diameter ratio for diagnosis of eosophageal varices

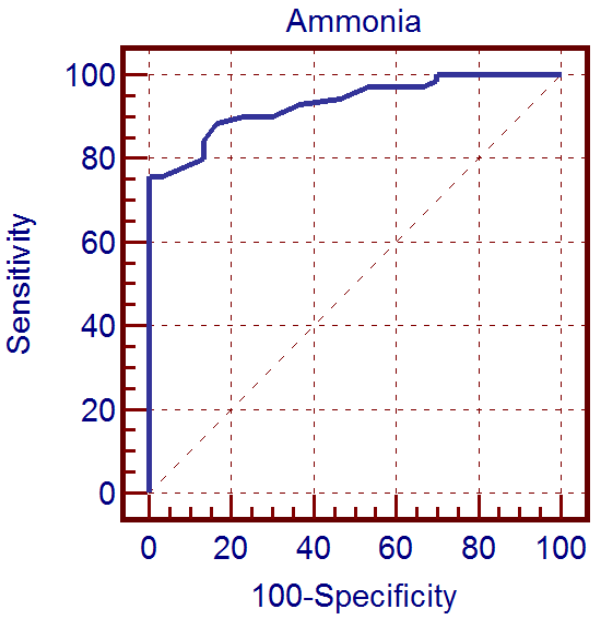

Figure (5): ROC curve of blood ammonia level for diagnosis of eosophageal varices

Table (4): Diagnostic performance of platelet count / splenic diameter ratio and blood ammonia level for diagnosis of large oesophageal varices (grade III and IV).

Cutoff	Sens.	Spec.	PPV	NPV	Accuracy
platelet count/splenic diameter ratio = 570	76.9	81.6	38.5	95.9	84.7
Blood ammonia level = 67	46.2	97.7	75.0	92.4	75.7

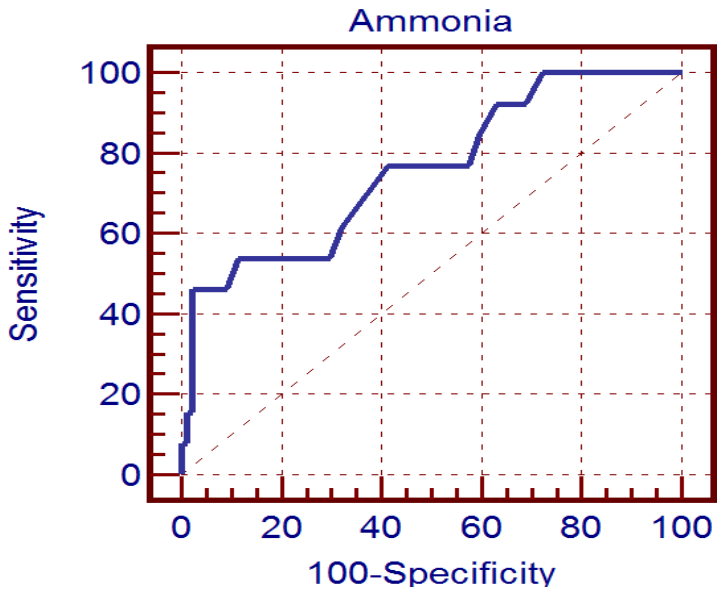

Figure (5): ROC curve of blood ammonia level for diagnosis of large oesophageal varices

Table (5): Comparison between blood ammonia level and platelet count/ splenic diameter ratio as predictors for varices

Ammonia &platelet count splenic diameter ratio			
Difference between areas	**Standard error**	**95% Confidence interval**	**Significance level**
0.092	0.085	-0.075 to 0.258	0.280

DISCUSSION

Oesophageal varices (OV) are the most important and critical portosystemic shunts that develop secondary to portal hypertension. Endoscopic prophylactic band ligation and non-selective beta blockers can minimize the risk of oesophageal bleeding by 50% [10]. Endoscopic screening of all cirrhotic patients would lead to a large number of unnecessary endoscopies and additional burden to endoscopic units [11]. The development of non-invasive methods for OV prediction could reduce the use of upper gastrointestinal endoscopy in variceal screening and also provide an alternative way to confirm the results of conventional endoscopic diagnosis [**12**]. A total of 100 adult cirrhotic patients were selected from those attending the department of Hepatology, Gastroenterology and Infectious diseases, Benha University Hospital during the period from January to June 2015. Regarding BAL, the current study showed significant difference between cirrhotic group with OV and that without OV (p = 0.000). The mean ammonia level in cirrhotics with OV was 54.84 µmol/l while it was 39.17 µmol/l in those without OV. In addition, BAL was also significantly high in patients with large sized varices (grade III and IV). These results came in agreement with the studies done by Tarantino et al. **[7]** and

Khondaker et al. **[13]** who reported that not only BAL increased in cirrhotic patients with OV but also with those who had large sized varices. BAL at cutoff value = 48 umol/L had sensitivity 75.7% and specificity 100% in detection of OV and at level = 67 umol/L, it had 46.2% sensitivity, 97.7% specificity in detecting large sized OV (grade III and IV) in cirrhotic patients of the current work. A study done by Tarantino et al. [**7**] reported that BAL at cutoff value = 42 umol/ L had sensitivity of 97% and specificity of 43% for detection of OV and Khondaker et al. [**13**] found that blood ammonia levels at ≥ 63 umol/l had sensitivity of 95% and specificity of 50% in detecting large OV in patients with cirrhosis suggesting its usefulness in identifying patients with large varices who need endoscopies. Also El-Hefny et al. **[14**] concluded that BAL at cutoff value 77.5 umol/L had sensitivity 100% and specificity 95% for detection of O.V. On the same hand, a study done by Montasser et al. **[15]** concluded that BAL at cutoff value 133 umol/L had sensitivity of 100% and specificity of 96% in detecting large varices. On contrary to this study, Ramzy et al. **[16**] concluded that ammonia alone can not predict the presence nor the grade of OV based on the fact, the BAL determination suffers from some limits in its measurements as the collection, handling, storage, and analysis of blood samples are all potential sources of error.

The present study detected significant decrease in the mean values of platelet count/ splenic diameter ratio in cirrhotic patients with varices in comparison to those without varices and moreover in cirrhotics with large varices (grade III and IV) compared to those with small varices (grade I and II). Also, our study found that platelet count/ splenic diameter ratio at cutoff value 1073 had sensitivity 94.3%, specificity 56.7%, PPV 83.5% and NPV 81% for detection of OV and at cutoff 570 had sensitivity 76.9%,specificity 81.6%, PPV 38.5% and NPV 95.9% for detection of large varices. This came in concordance with Abo-Alsoud et al. **[17]** who reported; significant decrease in the mean values of platelet count/ spleen diameter ratio in cirrhotic patients with varices in comparison to other patients without varices, and that the best cutoff value for detection of O.V was 638.9 with sensitivity 100%, specificity 97.5%, PPV 95.2% and NPV 100%. In contrast to our results, Qamar et al. **[18]** and Hassan,et al. **[19]** found that platelets/splenic longitudinal diameter (PLT/SLD) ratio did not show significant difference in patients with and without OV suggesting that these markers cannot predict the presence of varices. This conflict may be due to difference in severity of liver disease in our patients as 74.29 % of group 2 cases in our study were Child B &C and 66% of group 1 were Child A while the previous study included patients of Child "A" and early "B" liver cirrhosis who had less impairment of platelet count which may explain this disagreement. In the current study and according to the multivariate logistic regression analysis for predictors of OV, BAL was the only predictor of OV presence. This result was in agreement with Hassan,et al. **[19]**, who reported that BAL, PVD, SVD and SLD were good non-invasive predictors for the presence of OV in cirrhotics with the superiority of ammonia and PVD, and was in disagreement with Ramzy et al. **[16]**. Significant positive correlation was noted in our study between BAL and size of varices (r=.557 and P value <0.001) according to Spearman correlation analysis. This finding was comparable with that reported by Tarantino et al. [7] where r= 0.43 and P value was <0.001. On the same hand Hassan,et al**. [18]** reprted that among non-invasive parameters including BAL, PVD, SVD and SLD, only BAL positively correlated with the size of OV and Khondaker et al. **[13**] observed moderate but significant correlation between BAL and size of OV. when comparing the performance of blood ammonia with the PLT/SLD ratio in the current work, no significant difference was detected (p value= 0.280). A result that came in agreement with Tarantino et al. **[7]** who do not found dissimilar reliability between BAL and PLT/SLD in prediction of OV. Although the Plts/SLD ratio do not suffer from external confounding factors but its disadvantages are consistent with changes in platalet count either in the form of thrombocytemia that is sometime related to the auto-antibodies presence which turns out in falsely low count of PLTs. or falsely high count of PLTs, as in patients suffering from liver cirrhosis with hepatocelular carcinoma **[20]**.

CONCLUSION

Blood ammonia level could be a non invasive predictor for the presence of OV and could be clinically useful, as it correlated with the size of OV.

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

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Role of Serotonin in Development of Varices in Patients with Cirrhosis

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Key words: Serotonin- Varices in Patients with Cirrhosis- MELD score.

Background and study aim: Serotonin is a one of the monoamine neurotransmitters secreted by serotonergic nerve endings in multiple sites in the brain and gastro-intestinal tract wall. The presence of serotonin receptors on hepatic stellate cells can cause contraction of these cells closing the sinusoidal fenestrae and raising the portal vein pressure. The aim of this work is to study the relation between free serotonin level and the presence of varices in patients with cirrhosis.

Patients and Methods: This prospective case control study was carried out on 70 patients with liver cirrhosis attended or admitted to Hepatology Department in Shebein El-Kom Teaching Hospital within the period between May and October 2015. They divided into two groups: Group 1: 40 patients with varices diagnosed by upper GIT endoscopy. Group 2: 30 patients without varices. In addition, 20 healthy persons served as control group (Group 3). All subjects were subjected to full history taking, clinical examination and laboratory investigation including plasma free serotonin by Enzyme Linked Immunosorbant Assay (ELISA).

Results: serum serotonin level in group I was significantly higher than group II and control group. Serum serotonin level was significantly correlated to esophageal varices grade, Child, MELD and updated MELD scores. It was also clear that serotonin level rises significantly with higher grades of esophageal varices.

Conclusion: Free serotonin had a good power of prediction for development of varices and correlated well with severity of liver disease in patients with cirrhosis assessed by Child, MELD and updated MELD scores as well as OV grade.

INTRODUCTION

Portal hypertension which is considered as one of the most important complications of liver cirrhosis is associated with development of a hyperdynamic circulation and complications such as ascites, hepatic encephalopathy and oesophago-gastric varices. Patients with cirrhosis and gastro-oesophagealvarices have a hepatic venous pressure gradient during hemodynamic catheterization of at least 10-12mmHg **[1]**.

Gastroesophageal varices are present in approximately 50% of patients with liver cirrhosis. Their presence correlates with the severity of liver disease. Patients without varices develop them at a rate of 8% per year **[2]** and the progression from small to large varices occurs in 10 to 20% of cases yearly. The most important predictor of hemorrhage is the size of varices, with the highest risk of first hemorrhage occurring in patients with large varices, about 15% per year **[3]**.

Variceal bleeding is associated with a high morbidity and mortality. The mortality associated with each episode of variceal bleeding ranges from 17% to 57% **[4]**.

The incidence of bleeding can be reduced with nonselective beta-blockers **[5]**.

It is also suggested that prophylactic endoscopic variceal band ligation can decrease the incidence of variceal bleeding and mortality in patients with liver cirrhosis who have large varices **[6]**.

The prevalence of oesophageal varices among cirrhotic patients is variable, ranging from 24% to 80%. Therefore, endoscopic screening of all patients with liver cirrhosis would result in a large number of unnecessary additional burdens to endoscopic units **[7]**.

Therefore, the identification of non endoscopic, non invasive methods that can accurately predict esophageal varices, particularly large esophageal varices in cirrhotic patients and help to identify patients at greatest risk and thereby reduce the necessity of endoscopic screening **[8]**.

Several studies have examined how to identify patients with varices using non-invasive or minimally invasive methods to avoid endoscopy in patients with a low risk of varices. These studies include biochemical, clinical and ultrasound parameters, transient elastography, CT scanning and video capsule endoscopy **[9]**.

Serotonin is released from platelets at the site of injury in the liver to regulate the process of hepatic regeneration and fibrosis **[10]**. In the pathogensis of cirrhosis the hepatic stellate cells (HSC) are transformed into myofibroblasts under the influence of the inflammatory mediators secreted by the damaged liver cells **[11]**. There's also increased number of 5HT receptors on the HSC cell membrane enhancing the cell response to serotonin. This can cause contraction of these cells closing the sinusoidal fenestrae and raising the portal vein pressure **[12]**.

Rudic, et al. **[13]** found that mean plasma free serotonin was higher in patients with varices than in patients without varices ($P<0.05$). Also, they found that the correlation of plasma serotonin concentration and fundal varices was highly significant ($P<0.01$), and they proved that the mean plasma free serotonin level was much higher in patients who had esophageal and gastric fundal varices than in patients who had only esophageal varices ($P<0.01$).

Also, Abdelkader, et al. **[14]** concluded that plasma free serotonin level could possibly be used as a noninvasive predictive method for the presence of gastroesophageal varices.

The aim of this work was to study the association between free serotonin concentration in plasma and the development of varices in patients with cirrhosis.

PATIENTS AND METHODS

I- Patients:

This prospective case control study was carried out on 70 patients with liver cirrhosis attended or admitted to Hepatology Department in Shebein El-Kom Teaching Hospital within the period between May and October 2015.

They divided into two groups:

Group 1: 40 patients with varices diagnosed by upper GIT endoscopy. Group 2: 30 patients without varices. In addition, 20 healthy persons served as control group (Group 3).

The study protocol was approved by the scientific committee of Benha faculty of medicine.

Selection of patients

Inclusion criteria:

Patients with cirrhosis with or without varices, Cirrhosis was diagnosed by clinical manifestations, laboratory investigations and ultrasonography **[15]**. Varices were diagnosed by upper GIT endoscopy.

Exclusion Criteria :

Patients with primary and secondary liver tumors, patients with history of depression and patients receiving selective serotonin reuptake inhibitors (SSRIs) and other antipsychotics were excluded from the study.

II- Methods :

All patients were submitted to the following:

1- Full history taking and thorough clinical examination paying specific attention to the manifestations of liver cirrhosis e.g. palmer erythema, jaundice, ascites, lower limb edema, encephalopathy and splenomegaly.

2- Laboratory investigations including: Complete blood count (CBC)-ALT (Alanine amino-transferase)-AST (aspartate amino-transferase)-Serum bilirubin (total, direct) - Serum albumin - P.T. (Prothrombin time) - INR (international normalized ratio)- Serum creatinine-HCV-Ab (Hepatites C virus antibody) and HBs Ag (Hepatites B virus surface antigen).

3-Assessment of liver cirrhosis by:

- Child Turcotte Pugh (CTP) classification **[16]**.
- MELD score (Model for End Stage Liver Disease) **[17]**.

- Updated MELD score (uMELD) **[18]**.

4- **Abdominal ultrasonography for evaluation of:** To confirm presence of cirrhosis exclude focal lesions to rule out patients with primary and secondary liver tumors from the study, determine spleen size and to detect portal vein diameter and ascites.

5- **Upper gastrointestinal endoscopy and grading of esophageal varices:** According to Dancygier and Ragon **[19]**.

6- **Specific investigations:** Measurement of plasma free serotonin by ELISA.

Statistical analysis:
The data were coded, entered and processed on computer using SPSS (version 18).The results were represented in tabular and diagrammatic forms then interpreted. Mean, standard deviation, range, frequency, and percentage were use as descriptive statistics.

The following test was done: Chi-Square test X^2 was used to test the association variables for categorical data. Student's t-test was used to assess the statistical significance of the difference between two population means in a study involving independent samples. ANOVA (F test) for normally quantitative variables, to compare between more than two groups, and Post Hoc test (LSD) for pairwise comparisons

P value was considered significant as the following: *P>0.05: Non significant. *P≤0.05: Significant

RESULTS

The demographic features and characteristic of patients were summarized in table 1 and 2.

The study was carried out on 70 patients with liver cirrhosis attended or admitted to Hepatology Department in Shebein El-Kom Teaching Hospital divided into 2 groups according to presence of varices within the period between May and October 2015. In addition, 20 healthy persons served as control group. There was no statistically significant difference between groups as regards the age and gender; however varices tend to be more common in males than females. Varices were found in (57.14%) of patients with cirrhosis.

There was no statistically significant difference between the groups as regard to the residence and occupation, however patients with varices tend to be from rural areas and work as farmers.

Cases of varices have higher child score, when compared with cases without varices with statistical significance.

MELD and updated MELD scores were significantly higher in group I, when compared to group II with statistical significance.

Group I has higher serotonin level than group II and control group with statistical significance (Table 3 and Figure 1),

Patients with gastric fundal and esophageal varices have higher serotonin level than patients with esophageal varices only (Table 4).

Patients with higher grades of varices have higher serotonin level (Table 5 and Figure 2).

ROC curve analysis of serum free serotonin level revealed that, at a cut off value of 202 nmol/L; the sensitivity for detection of varices was 96.7%, specificity 80.0%, positive predictive value (PPV) was 78.37%, negative predictive value (NPV) was 80.0%; area under the curve was 92 denoting good predictive value of serotonin in prediction of varices (Table 6 and Figure 3).

There is significant correlation between serotonin level and serum albumin, total billirubin, INR, platelets count, MELD, updated MELD and Child classification indicating that the serum serotonin level is correlated to the severity of liver function decompensation in patients with cirrhosis (Table 7 and Figures 4,5,6,7).

Table (1) : Demographic features and characteristic of patients

Characteristic	Group I (patients with varices) N = 40(57.14%)	Group II (Patients without varices) N= 30(42.86%)	P-value
Age(years)			
Range	25-61	25-61	0.474
Mean±SD	44.65±9.55	43±9.42	
Gender			
Male	25(62.5%)	20(66.7%)	0.714
Female	15(37.5%)	10(33.3%)	
Residence			
Urban	14(35%)	9(30%)	0.654
Rural	26(65%)	21(70%)	
Occupation			
Farmer	26(65%)	22(73.3%)	0.457
Non-farmer	14(35%)	8(26.7%)	
Smoking	14(35%)	16(53.3%)	0.125

*= significant

Table (2) : Clinical characters of the studied patients

HCV	32(80%)	25(83.3%)	0.723
HBV	8(20%)	5(16.7%)	0.737
Severity of liver disease			
Child A	7(17.5%)	18(60%)	0.001*
Child B	18(45%)	9(30%)	
Child C	15(37.5%)	3(10%)	
MELD score Mean±SD	20.07±6.73	11.56±6.53	0.000*
uMELD score Mean±SD	4.13±.88	3.16±.80	0.000*

Table (3): Serotonin levels in the studied groups

	Group I (patients with varices) N = 40		Group II (Patients without varices) N= 30		Group III (Control) N= 20		P-value
	Mean	SD	Mean	SD	Mean	SD	
Serotonin (nmol/L)	220.18	48.40	125.57	22.37	38.75	6.72	Between (I and II) p=**.000*** Between (I and III) p=**.000*** Between (II and III) p=**.000***

* = significant

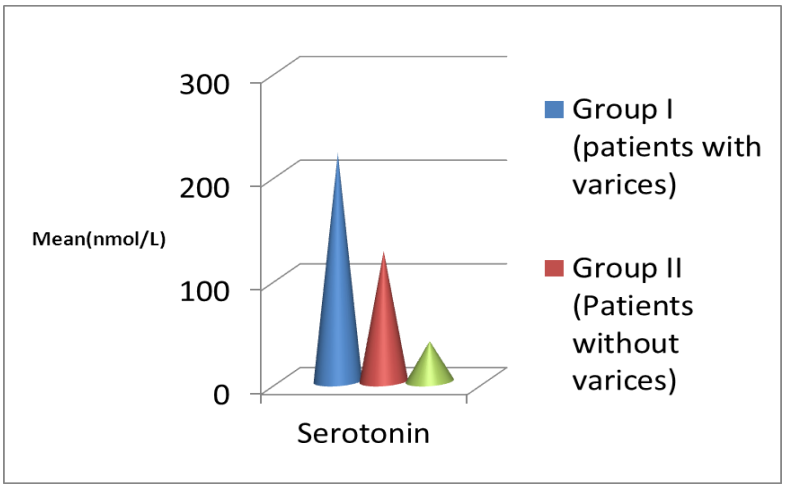

Figure (1): Serotonin levels in the studied groups

Table (4) : Comparison between patients with gastric fundal and esophageal varices and patients with only esophageal varices according to serum level of serotonin

	Esophageal varices with no gastric fundal varices N = 33		**Gastric fundal varices N= 7**		**P-value**
	Mean	**SD**	**Mean**	**SD**	
Serotonin (nmol/L)	206.51	39.36	284.5714	33.21073	**.000***

* = significant

Table (5) : Association between serotonin and grades of varices

	Grade I varices N = 10		**Grade II varices N= 10**		**Grade III varices N= 10**		**Grade IV varices N= 10**		**P-value**
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	
Serotonin (nmol/L)	197.4	22.95	210.4	38.99	231.5	51.7	241.4	64.08	**.000***

* = significant

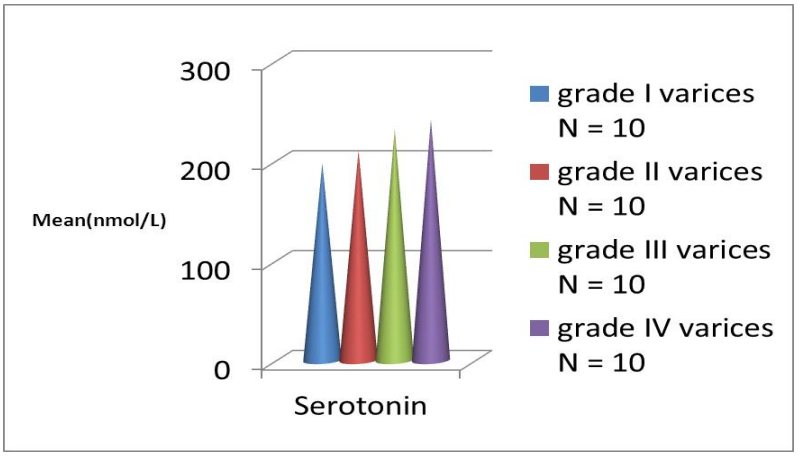

Figure (2): Association between serotonin and grades of varices

Table (6) : Serotonin as a marker for Oesophageal varices

Test	Cutoff	Sensitivity %	Specificity %	PPV%	NPV%	AUC	P value
Serotonin (nmol/L)	202	96.7	80	78.37	80	.92	**0.002***

* = significant

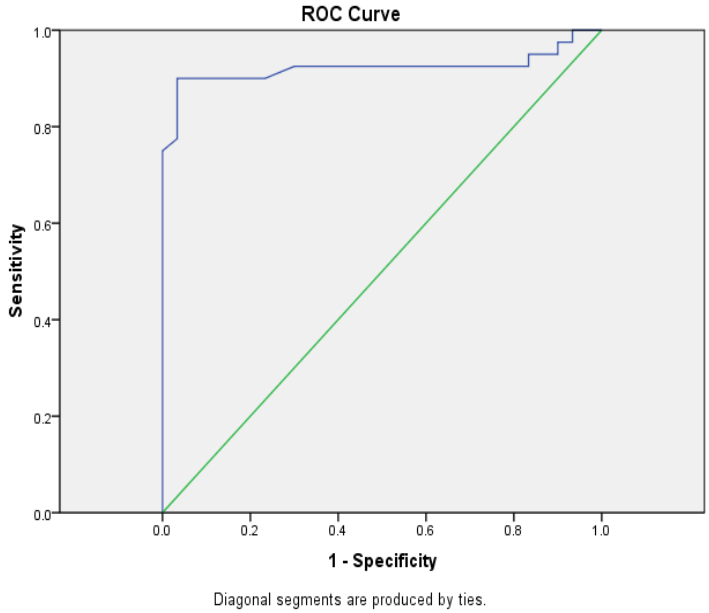

Figure (3): Receiver operative curve analysis of serotonin

Table (7) : Correlation between serotonin and other variables in studied patients

Variables \ Serotonin	Pearson correlation r	P value
Child score	.300	**0.012***
Creatinin	.171	0.158
Albumin	-.558	**0.000***
Platelets	-.739	**0.000***
Bilirubin	.412	**0.000***
INR	.630	**0.000***
MELD	.345	**0.003***
Updated MELD	.273	**0.022***
Esophageal varices grades	.358	**0.023***

* = significant

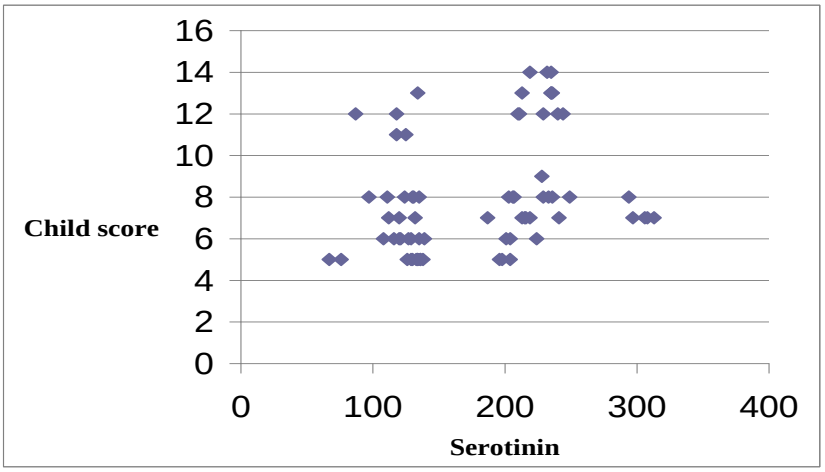

Figure (4): Correlation between serotonin and child score

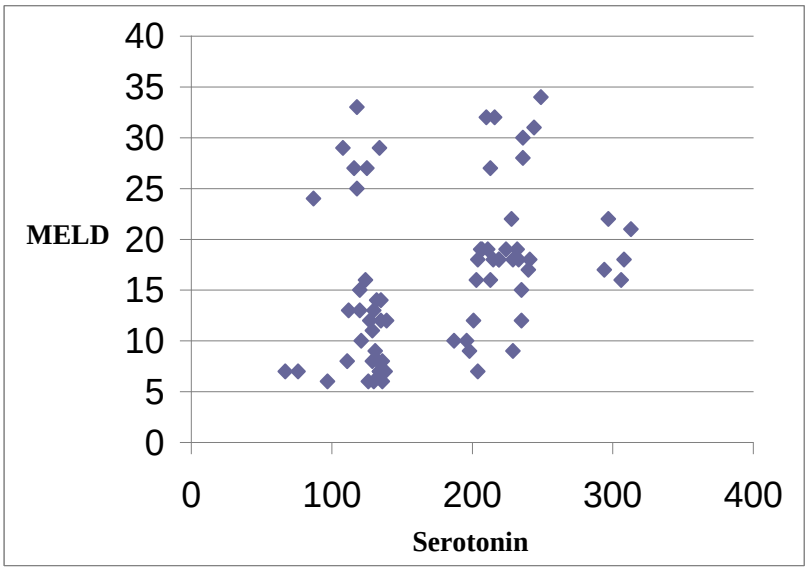

Figure (5): Correlation between serotonin and MELD

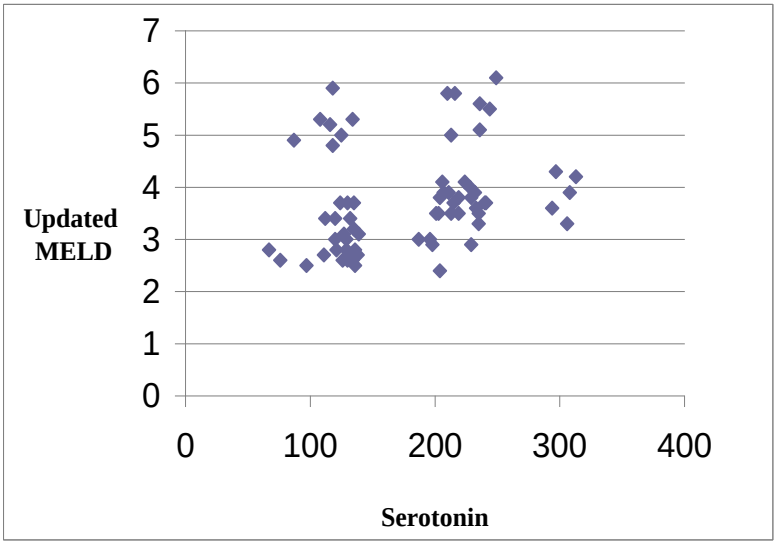

Figure (6): Correlation between serotonin and updated MELD

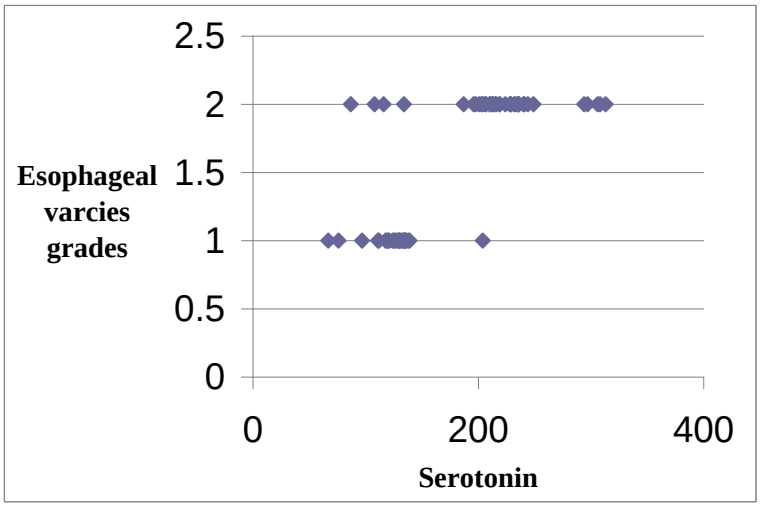

Figure (7): Correlation between serotonin and Esophageal varices grades

DISCUSSION

Portal hypertension is a serious condition with various severe life threatening consequences most important of which is varices development and bleeding. This study aims at expolring the correlation between serum serotonin level as a vasoactive amine and presence of varices.

Results of the present study revealed that, free serotonin level was significantly higher in patients with varices (mean= 220.18 ± 48.40nmol/L) than patients without varices (mean = 125.57 ± 22.37 nmol/L) and control group (mean= 38.75 ± 6.72 nmol/L) (P value=0.000). Also, Patients with gastric fundal and esophageal varices have higher serotonin level than patients with esophageal varices only (Table 4). This results agreed with the results of the study done by Rudic et al. **[13]** in which free serotonin level was investigated and they found that mean plasma free serotonin was higher in patients with esophageal varices than in patients without varices (P<0.05). Also, they found that the correlation of plasma serotonin concentration and fundal varices was highly significant (P<0.01), and they proved that the mean plasma free serotonin level was much higher in patients who had esophageal and gastric fundal varices than in patients who had only esophageal varices (P<0.01). Also, this result agreed with the results of the study done by Abdelkader et al. **[14]** in which they concluded that plasma free serotonin level could possibly be used as a noninvasive predictive method for the presence of gastroesophageal varices.

In the present study patients with higher grades of varices have higher serotonin level (Table 5 and Figure 2). This results agreed with the results of the study done by Abdelkader, et al. **[14]** in which they found that a highly significant stepwise progressive increase in the free serotonin level was recorded through grades of esophageal varices. This results agreed also with the results of the study done by Hammam et al. **[20]** in which they concluded that serum serotonin level is significantly correlated to the grade of esophageal varices in patients with viral hepatitis related cirrhosis. This result disagreed with the result of Rudic et al. **[13]** who found that no significant difference between the serotonin concentration and the size of esophageal varices, this difference may be due to small number of the studied patients (33) in their study.

ROC curve analysis of serum free serotonin level revealed that, at a cut off value of 202 nmol/L; the sensitivity for detection of varices was 96.7%, specificity 80.0%, positive predictive value (PPV) was 78.37%, negative predictive value (NPV) was 80.0%; area under the curve was 92 denoting good predictive value of serotonin in prediction of varices. These results are comparable to those reported by Abdelkader et al. **[14]** who concluded that plasma free serotonin level could possibly be used as a noninvasive predictive method for the presence of gastroesophageal varices (Table 6 and Figure 3).

In the present study, there is significant correlation between serotonin level and serum albumin, total billirubin, INR, platelets count, MELD, updated MELD and Child classification indicating that the serum serotonin level is correlated to the severity of liver function decompensation in patients with cirrhosis (Table 7).

CONCLUSION

This study concluded that Varices were found in 57.14% of cirrhotic patients. MELD and updated MELD scores were significantly higher in patients with varices. Free serotonin may have a role in the development of gastric fundal varices, indicating the clinical value of serotonergic receptor blockers in these patients. Free serotonin is significantly increased in cirrhotic patients with varices; it had a good power of prediction for development of varices. And correlated well with severity of liver disease in patients with cirrhosis assessed by Child, MELD and updated MELD scores as well as OV grade.

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

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Acute Brucellosis: Presentation and complications in Adults

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Key words:
Acute brucellosis, Presentation, Laboratory findings, Complications

Background and study aim: Brucellosis is one of the important bacterial zoonotic infectious diseases. The epidemiological zone of this infection includes the Arabian Peninsula. This study was conducted to describe the clinical presentation, laboratory findings and frequency of complications in adult brucellosis patients admitted in the hospital

Patients and Methods: This was a retrospective study conducted at the Infectious Diseases Hospital from May 2015 to May 2017. The patients included in this study were diagnosed as acute brucellosis. Diagnosis of acute brucellosis based on epidemiologic data, clinical manifestations, and laboratory investigations. Patients were treated according to the standard guidelines for the management of brucellosis and its complications.

Results: A total 192 patients were enrolled into the study. The main presenting symptoms were Fever (91.6%), night sweats (85.9%), arthralgia (76%), and low back pain (63.5%). The most common focal involvements observed were hepatic involvement (28.1%), Osteoarticular involvement (22.4%), Hematological involvement (17.1%) and orchiepididymitis involvement (9.4%).

Conclusion: Brucellosis considered an important medical problem. Diagnosis of the disease is not difficult but the diagnosis of its localized forms can occasionally be difficult because of misdiagnosis with other diseases. In humans, Eradication of the disease can be done by the public health education and the control of the disease in animals.

INTRODUCTION

Brucellosis is one of the important bacterial zoonotic infectious diseases caused by gram negative bacteria, brucella spp. It is a widespread disease with worldwide prevalence and more than 500 000 new cases annually **[1]**. The epidemiological zone of this infection includes Mediterranean basin, the Arabian Peninsula, Indochina, some parts of central Asia and South America **[2]**.

The brucellosis can be transmitted to human by consumption of infected meat from infected domestic animals e.g cattle, goat, sheep, camel, pigs… ect or close contact with the secretions of these infected animals **[3]**. The transmission also possible occurred by consumption of unpasteurized dairy such as ice cream, soft cheese, butter but yogurt and hard cheese have a lower risk of transmission than others due to lactic fermentation **[4]**.

The forms of the clinical course of brucellosis in humans are acute, sub-acute and chronic. Patients were classified into three groups according to their history, clinical picture and the time of clinical presentation: acute (0-2 months), sub-acute (3-12 months) and chronic (>1 year) **[5]**.

This study was conducted to describe the clinical presentation, laboratory findings and frequency of complications in adult brucellosis patients admitted in the hospital.

PATIENTS AND METHODS

This is a descriptive study conducted from May 2015 to May 2017at the Infectious Diseases Hospital (IDH), Kuwait. The patients included in this study were diagnosed as acute brucellosis. Diagnosis of acute brucellosis was clinically based on the history of contact with animals or consumption of unpasteurized dairy

and also, based on the presence of symptoms, e.g. fever, chills, night sweats, myalgia, anorexia, headache, joint pain and confirmed serologically by positive of brucella Immunoglobulin M (IgM) antibodies, standard tube agglutination titer (SAT) ≥1:160 or isolation of a Brucella spp. from blood cultures.

All patients were subjected to history taking and thorough clinical examination; tests for liver function test (LFT), kidney profile (KP), complete blood count (CBC), malaria film, erythrocyte sedimentation rate, blood glucose, rheumatoid factor (RF), C-reactive protein (CRP), Coagulation profiles, urine analysis and chest radiograph were performed on the day of admission and during stay in the hospital.

The patients were called for outpatient follow up after discharge from the hospital. The outpatient visits were every two weeks until full recovery. At each visit, LFT, KP, CBC, and CRP were investigated.

Regarding complicated brucellosis, diagnosis of osteoarticular involvement e.g arthritis, sacroiliitis, spondylitis, were determined by appropriate findings on physical examination and radiological investigations e.g X-ray and magnetic resonance imaging (MRI) if indicated. Diagnosis of genitorurinary system involvement e.g orchitis and/or epididymitis was defined by finding tenderness and swelling of scrotum, testis and epididymis and confirmed by ultra-sonography.

Diagnosis of Hepatic involvement was defined as the presence of more than five-fold of normal levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Diagnosis of brucella endocarditis was determined by cardiac murmur and confirmed by detection of vegetation using transthoracic echocardiography study. Diagnosis of neurobrucellosis was defined by neurological manifestations and confirmed by positivity of SAT and/or positivity of cerebrospinal fluid (CSF) culture and abnormal findings in cerebrospinal fluid analyses (protein >45 mg/dL, glucose <2:3 of the blood glucose level, >10 leukocyte/mm3).

Management was done as per standard guidelines for the management of brucellosis and its complications. The regimens included different combinations of antibiotics e.g oral doxycycline (100 mg/12h), oral rifampin (600 or 900 mg /24h), gentamicin infusion (240 mg/24h), intramuscular streptomycin (1 g/24 h), oral ciprofloxacin (500 mg/12 h), and co-trimoxazole (160/800/12 h). In neurobrucellosis patients, intravenous ceftriaxone (2 g/12h) was added to the regimen initially for 2-4 weeks. The treatment duration was 6-12 weeks in osteoarticular involvement, 12–24 weeks in neurobrucellosis, and 6–12 weeks for the other clinical forms.

The SPSS ver. 17.0 was used for the statistical analysis of data.

RESULTS

A total 192 patients were enrolled in this study, overall, the mean age of the patients included in the study was 36.16 ± 11.21 years (18-61 years), males outnumbered the females, 161 (83.9%) vs 31(16.1%). Of these, 62 patients (32.3%) were Indian, 21 (10.9%) Egyptian, 54 (28.1%) Bangladesh, 8 (4.2%) Seri lanka, and 47 (24.5%) Kuwaiti. Majority of the male patients were shepherds 144 (75%) and veterinaries 12 (6%), working in farms and had positive history of contact with the secretions of animals. The remaining of the patients 36 (19%) consumed of unpasteurized dairy.

Fever (91.6%), night sweats (85.9%), arthralgia (76%), and low back pain (63.5%) were the most common presenting symptoms; (Table 1). The most common clinical findings were fever (91.6%), peripheral arthritis (22.4%), hepatomegaly (25%), splenomegaly (21.3%) and scrotal swelling (9.4%), (Table 1).

C-reactive protein elevations (76.5%), Amino-transferase elevations (28.1%), Leukocytosis (11.9%) and anemia (9.98%) were the most frequent laboratory findings (Table 2). The Tubal agglutination test was positive in 100% of cases, with titers ranging from 1/160 to 1/2560. 106 patients revealed Brucella spp growth in their blood cultures (Table 2).

The focal involvement of infection occurred in about 42% percent of cases. The most common focal involvements were hepatic involvement (28.1%), Osteoarticular involvement (22.4%), Hematological involvement (17.1%) and orchid-epididymitis involvement (9.4%).The other focal involvements are shown in Table (3).

The osteoarticular involvement included arthritis (22.4%), sacroiliitis (14%), spondylitis (2.08%), and psoas abscess (0.52%). The most common joints involvements were wrist joints, knee joints and hip joints. The commonest hematological

abnormalities were leukocytosis (11.9%), anemia (9.98%), and thrombocytopenia (7.2%) (Table 3).

The numbers of relapsing cases (21 cases) were 10.9% percent of cases.

Table (1) : Clinical manifestations of 192 patients with acute brucellosis

Symptoms	NO (%)	Signs	No (%)
Fever	176 (91.6%)	Fever	176 (91.6%)
Night sweat	165(85.9%)	Splenomegaly	41 (21.35%)
arthralgia	146(76%)	Hepatomegaly	48 (25%)
Fatigue	102(53.1%)	Hepatoslenomegaly	31 (16.1%)
Anorexia	51(26.5%)	Lymphadenopathy	11 (5.7%)
Weight loss	41(21.3%)	Scrotal swelling	18 (9.4%)
Myalgia	75(39%)	Arthritis	43 (22.4%)
Low back pain	122(63.5%)	Heart murmur	2 (1.04%)
Nausea/vomiting	24(12.5%)	Neck stiffness	1 (0.52%)
Headache	28(14.5%)	Jaundice	0 (0.0%)
Scrotal pain	18(9.4%)	Rash	0 (0.0%)
Cough	9(4.6%)		
Shortness of breath	2(1%)		

Table (2) : Laboratory findings of 192 patients with acute brucellosis

Laboratory findings	NO (%)
ALT elevations > 5 folds (10 – 55 IU/L)	54 (28.1%)
AST elevations > 5 folds (5 – 34 IU/L)	54 (28.1%)
Total Bilirubin elevations > 20 umol/L	0 (0.0%)
Serum creatinin elevations > 115umol/L	2 (1.04%)
Anemia (HB <140 g/L)	19 (9.98%)
Leucopenia (WBCs < 3600)	16 (8.3%)
Leukocytosis (WBCs > 11200)	23 (11.9%)
Thrombocytopenia (PLT < 150.000)	14 (7.2%)
CRP elevation > 8mg/L	147 (76.5%)
ESR elevations > 20mm/hr	34 (17.7%)
Rheumatoid factor positive	0 (0.0%)
Tubal agglutination test (TAT)>1:160	192 (100%)
Blood culture for brucella	106 (55.2%)

ALT: Alanine aminotransferase; **AST:** Aspartate Aminotransferase; **CRP:** C-reactive protein; **ESR:** erythrocyte sedimentation rate

Table-(3) : Localized infection (complications) of 192 patients with acute brucellosis

Focal infection	NO (%)
Hepatitis	54 (28.1%)
Orchitis and epididymo-orchitis	18(9.4%)
Arthritis	43 (22.4%)
Sacroiliitis	27 (14%)
Spondylitis	4 (2.08%)
Psoas abscess	1 (0.52%)
Hematological abnormalities	33 (17.1%)
CNS involvement (Neuro-brucellosis)	1 (0.52%)
Cardiac involvement (endocarditis)	2 (1.04%)
Renal involvement (glomerulonephritis)	2 (1.04%)

DISCUSSION

Brucellosis is considered a major medical problem in many countries worldwide where brucellosis is endemic. Brucellosis is endemic in Kuwait **[2]**. The epidemiology of brucellosis in Kuwait was changed over the last decades, reported infection rates decreased from 68.9 per 100,000 population in 1985 reaching the level 2.1 per 100,000 population in 2006 **[6]**. This is may be attributed to eradication programs among animals, socio-economic changes and improved disease recognition.

Brucellosis can occur at any age but it is more prevalent in the adolescents and young adults **[7]**. In the current study, the age of patients ranged from 18 to 61 years and 76% of patients were between 18 and 44 years. Some studies reported that brucellosis is more frequently occurs in this age group **[8]** because this age group is the most productive group in the community and most of them working on farms with dairy production e.g shepherds and veterinaries. Brucellosis can occur in both genders **[9]**. In the present study, males outnumbered the females, 161 (83.9%) vs 31 (16.1%). Male were at higher risk of acquiring this disease than female because The male may be more likely to come into contact with infected animals and have more opportunities to drink raw milk than female. Also, in the current study, the prevalence of brucellosis is high among shepherds and veterinarians and this may be another reason why the prevalence is higher among males than females. This also leads to fact that brucellosis is considered an occupational disease.

Many routs have been implicated in the transmission of Brucellosis and its prevalence. In the endemic countries, the primary route of transmission is by the ingestion of raw or unpasteurized dairy products; whereas in the developed countries, infection can occur due to occupational exposure **[10]**. In the current study, the main routs of transmission were consumption of unpasteurized dairy products and contact with infected animals. The consumption of unpasteurized dairy products has been reported in 69 percent of cases in Kuwait by Mousa et al., **[11]**. Inter-human transmission of brucellosis has been reported in one female patient who probable got the disease from her husband by sexual contact. Similar to some reports that documented the sexual transmission of brucellosis **[12]**.

Brucellosis is a systemic infection which can affect any system, tissue or organ of the body. Organ involvement can be considered as a complication or as focal involvement. The present study showed that 42% percent of cases had focal involvement or complications of brucellosis. Hepatic involvement is seen as the most frequent complication of brucellosis in this study (28.1%) in the form of mild hepatitis and no cases were reported with acute liver failure. This is in accordance with T. Buzgan et al. **[13]** who detected 24.8 percent of the cases of brucellosis had Elevated transaminase levels. Rarely, Brucella can also cause splenic or hepatic abscesses, acute cholecystitis, colitis, pancreatitis and peritonitis **[14]** and none of these complications were recorded in this study.

Brucellosis is frequently associated with osteo-articular involvement **[15]**. Osteo-articular involvements include peripheral arthritis, sacroiliitis, spondylitis, Paravertebral abscess and psoas abscess **[16]**. Osteo-articular involvement occurs in 20–85% of cases **[17]**. Osteo-articular involvement was noted in 39% of acute cases in the present study. Infection of the joints is the most common localized osteo-articular complication of brucellosis and a frequent cause of infectious arthritis in countries where brucella is endemic **[15]**. In the present study, Peripheral arthritis had been reported in 22.4 percent of cases affecting mainly wrist, knee, hip joints and sacroiliitis had been reported in 14 percent of cases. Four cases (2.08%) of our 192 patients exhibited spondylitis. In accordance with Turan Buzgan et al. **[13]** we found that the lumbar spine was the most frequent site of spondylitis.

Genitourinary involvement has been noted in 2 to 10% of the patients with brucellosis and epididymoorchitis is the most frequent genitourinary complication. The incidence of epididymoorchitis in brucellosis is estimated at 2%-20% **[18]**. In the present study, epididymo-orchitis occurred in 9.4% of all patients with brucellosis which is in accordance with Khan **[19]** who investigated 100 patients with brucellosis in Saudi Arabia and found testicular involvement in 6%.

Hematological changes are frequent in brucellosis, but these hematological abnormalities are usually mild such as anemia, leukopenia and Thrombo-cytopenia **[20]** and serious hematological involvement is rare e.g Disseminated intravascular coagulation. In the current study, anemia was

found in 19 patients (9.98%), leukopenia in 16 (8.3%), thrombocytopenia in 14(7.2%) and Disseminated intravascular coagulation was not reported. This figure is lower than reported by Kdeniz et al. **[21]** who found that anemia was present in 128 patients (55%), leukopenia in 49 (21%), thrombocytopenia in 59 (26%) and pancytopenia in 18 (8%) patients.

Neurobrucellosis has been reported in 2% to 5% of the patients with brucellosis **[22]**. Meningitis or meningo-encephalitis is the most frequent neurological complications of brucellosis **[22]**. In our study, only one case (0.52%) was diagnosed with neurobrucellosis. This low incidence of CNS involvement in current study may be due to management of this especial group in general hospital and not refer to IDH.

In this study, two (1.04%) brucellosis patients exhibit renal involvement. one patient presented with Glomerulonephritis and another with interstitial nephritis and endocarditis. in some case reports, Glomerulonephritis and tubule-interstitial nephritis have been reported **[17]**.

Cardiovascular involvement of Brucellosis can result in endocarditis, myocarditis or pericarditis. Endocarditis is the most frequent presentation of cardiovascular involvement, which is documented in less than 2% of patients **[13]**. Endocarditis is a very severe complication of brucellosis. The aortic valve has been commonly involved, followed by the mitral valve alone or both valves concurrently. The aortic valve is affected in 75% of patients, and 50% of affected valves were previously healthy **[22]**. In the present study, endocarditis was found in 2 patients (1.04%) with aortic valve involvement. The valve replacement was done for the two patients.

In the present study, the small sample size of the studied patients and only one center where the study was conducted have limited the power to detect and describe all the clinical presentation, laboratory findings and complications of brucellosis.

CONCLUSION

Brucellosis is an endemic disease in Kuwait and considered an important health problem and it has a significant morbidity. The consumption of raw dairy products as well as occupational risks of contact with infected animal has been reported the main rout of transmission the disease in. Additionally, the disease affects primarily persons in their productive age.

Diagnosis of the disease is not difficult because it based on epidemiologic data, clinical manifestations, laboratory investigations and high degree of suspicion by the physicians. But the diagnosis of localized forms of brucellosis can occasionally be difficult because of misdiagnosis with other diseases.

There is no recommended protocol for management of complicated brucellosis. Further studies are needed to assess the most appropriate treatment choices and durations in complicated brucellosis. Eradication of the disease in humans can be done by the public health education and the control of the disease in animals.

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

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Parasitic Contamination of Commonly Consumed Fresh Vegetables and Fruits in Some Rural Areas of Sharkyia Governorate, Egypt

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Key words: parasites, vegetables, acetic acid, potassium permanganate, Sharkyia Governorate

Background and study aim: The healthy diet must basically contain fresh fruits and vegetables. Contaminated vegetables and fruits consumption is a way of getting people infected with intestinal parasites. This study aimed to detect the parasitic contamination in some common fresh vegetables and fruits in Sharkyia Governorate, Egypt and the effect of potassium permanganate (24mg/L) and acetic acid 5% upon these parasites.

Patients and Methods: The study examined 420 samples: lettuce, watercress, parsley, cucumber, carrots and strawberry. Their collection was done from some rural areas of Sharkyia governorate's markets. Samples were washed and the solution resulted from washing was filtered and centrifuged to concentrate the parasitic stages. Sediments and supernatants were microscopically examined using iodine and modified Ziehl–Neelsen stained smears. Then the positive samples were soaked separately in acetic acid 5% and potassium permanganate 24ml/L for 15 and 30 minutes. Then they were tested by 0.2% trypan blue stain to detect the viability of parasites.

Results: The parasites were determined in 164/420 (39%) samples. *Giardia lamblia* cysts were the most prevalent parasite (12.6%) followed by *Cryptosporidium spp.*oocysts (7.6%), *Entamoeba spp.* cysts (6.2%), *Blastocystis hominis* cysts (3.8%), *Hymenolepis nana* eggs (2.8%), *Ascaris lumbricoides* eggs (1.9%), various helminths larvae (1.6%), *Enterobius vermicularis* eggs (1.4%) and *Balantidium coli* cysts (0.9%). The highest contaminated one was watercress (55.7%) followed by lettuce (45.7%), parsley (44.2%), cucumber (31.4%), strawberry (30%) and carrots (27.1%). There was a reduction in the viability of the parasites after exposure to acetic acid 5% and potassium permanganate 24mg/L but the statistical difference between the percentages was insignificant.

Conclusion: The results of the study emphasized a possible role of contaminated raw vegetables and fruits in the spread of parasitic diseases in Sharkyia governorate, Egypt. Acetic acid 5% and potassium permanganate 24ml/L are considered effective disinfectants to reduce parasitic contamination of fresh vegetables and fruits.

INTRODUCTION

Maintenance of a healthy human diet can be achieved by vegetables and fruits as they contain many vitamins, carbohydrates and minerals **[1]**. Also, the risk of certain diseases as chronic diseases and cardiovascular diseases can be reduced by them **[2]**. In less developed countries, there are certain factors which contribute to the contamination of vegetables and fruits during the planting process including the usage of insufficiently treated wastewater for irrigation, soil contamination by the animal wastes and increased application of improperly composted manures to the soil. The bad hygienic practice by food handlers during the preparation of fresh vegetables and fruits can lead to high prevalence of contamination **[3]**. Certain new habits contribute to the increase in the occurrence of food borne illness linked to consuming fresh vegetables and fruits. These include the increase in consumption of raw or improperly cooked vegetables

and fruits in fast meals eaten in restaurants, canteens and from street sellers of food **[4].**

In the last years, the breaking off the barriers in between the countries leads to the widespread of parasitic infections all over the world. This is made by the export of contaminated vegetables from developing countries to developed ones **[5]**. Consumers are advised to wash fresh vegetables and fruits with running tap water before consuming them because the tap water contains a chlorine oxide which can kill the microorganisms which found on the surface of fruits and vegetables **[6].** This chlorination method is considered a traditional disinfection method but it isn't particularly effective in reducing helminths and some protozoan numbers to low levels **[7].** Nowadays, certain types of disinfectants are used worldwide such as acetic acid, potassium permanganate, Ozone, Ultraviolet irradiation and several chemicals **[8].**

In less developed countries including Egypt, there is a lack in the diagnosis of outbreaks caused by contaminated vegetables and fruits and no adequate previous surveys have been conducted to record the incidence of parasitic contamination in vegetables and fruits **[9].** So, this work aimed to investigate the prevalence of helminths and protozoa contamination of some selected types of raw vegetables and fruits commonly consumed and sold in Sharkyia governorate rural markets with an offer of reducing these contaminations by using common disinfectants like acetic acid 5% and potassium permanganate 24mg/l. So, this reduction may decrease the hazards upon the health.

PATIENTS AND METHODS

Study type:
A cross sectional study was performed during the period from January to December 2016. The practical work was done at the laboratories of Medical Parasitology Department, Faculty of Medicine, Zagazig University, Egypt. The study included (420) vegetables and fruits samples (70 samples from each type). Five types of vegetables and one type of fruits; (lettuce, watercress, parsley, cucumber, carrots and strawberry).

Sample collection:
Samples were collected from all available sales outlets (street vendors, commercial groceries and supermarkets) in some rural areas of Sharkyia governorate including (Faqous, Banayuos, Kafr El Hamam, Kafr Sakr, Abo Hammad, El Qenayat, Abbasa). Each sample was put with its root in a separate nylon bag and labeled.

Procedure for sample preparation and washing:
About two hundred grams was weighted from each sample, and then about one liter of physiological saline was used to immerse each vegetable and fruit's sample in aseparate sink. The samples were left soaked overnight for sedimentation to take place.The top layer was discarded and the remaining solution was sieved to remove debris after that, they were transferred to the tubes for centrifugation to be centrifuged at 2000 rpm for twenty minutes. The supernatant was transported to a test tube and the examination of the sediment was done **[10]**.

Detection of parasites:
The supernatant was examined by Zinc sulphate flotation method to detect light weight eggs and cysts of protozoa **[11].** Then, the sediment was examined microscopically after mixing it well by the following methods: simple smear and iodine stained smear for detection of parasitic eggs, cysts and larvae. In the unstained smear, the sediment's drop was placed on a clean slide and covered by a cover slip, then, examined under a light microscope using x10 and x40 objectives. In iodine smear, a drop of Lugol's iodine was added to the sample before being covered by the cover slip **[12].** To detect the oocysts of coccidian protozoa, we used Modified Ziehl–Neelsen stain smears. Then they were examined microscopically by oil immersion lens (x1000) **[13].**

Disinfection of vegetables:
The used disinfectants were acetic acid 5% and potassium permanganate 24mg/l. The positive samples for each parasite were collected separately in one container with 15 ml of saline. Each parasite suspension was divided in two tubes after mixing it well. Then, it was centrifuged at 1500 r.p.m for 5 minutes. Then, the following was done for each parasite: 5ml of 5% of acetic acid was added to the sediment and the supernatant of the 1st tube. 5ml of 24 mg/l potassium permanganate was added to the sediment and the supernatant of 2nd tube. After 15 minutes from exposure to these disinfectants. About 5 ml of the suspension from the two tubes were taken and centrifuged again. Then, they were exposed again to these disinfectants for 30 minutes **[14]**. These procedures were conducted at the room temperature (25°c).

Viability assay:
Preparation of slides smears from the solutions exposed to the disinfectants was done and stained

by 0.2% trypan blue and examined microscopically (x100, x400, and x1000). Viable parasites appeared clear with light blue color and showed dye exclusion activity, while dead parasitic stages appeared dark blue in color with some changes of their external structure **[15].**

Statistical Analysis:
The results were analyzed using chi-square tests of the SPSS software version 17. The significance's threshold is fixed at 5% level (P-value). P-values >0.05 were considered insignificant, while P-values <0.05 were considered significant and <0.001 highly significant.

RESULTS

As shown in tables and figures.

Table (1): Prevalence rate (percentages) of parasitic contamination of the examined raw vegetables and fruits

	Lettuce (70)		Water cress (70)		Parsley (70)		Cucumber (70)		Carrots (70)		Straw berry (70)		Total examined (420)		X^2	P
	N	%	N	%	N	%	N	%	N	%	N	%	N	%		
Positive	32	45.7	39	55.7	31	44.2	22	31.4	19	27.1	21	30	164	39	18.57	0.002
Negative	38	54.3	31	44.2	39	55.7	48	68.6*	51	72.8*	49	70*	256	60.9		
X^2	1.03		1.03		1.83		19.3		29.26		22.4					
P-value	0.31		0.31		0.1>		<0.001**		<0.001**		<0.001**					

*Significant P value (P < 0.05)

Table (2): The distribution of each parasitic contamination of examined raw vegetables and fruits

Parasite	Total No. recovered (out of 420)	% of +ve (164)	Lettuce (70)		Water cress (70)		Parsley (70)		Cucumber (70)		Carrots (70)		Straw berry (70)	
			+ve	%	+ve	%	+ve	%	+ve	%	+ve	%	+ve	%
Enterobius egg	6	3.6	1	1.4	3	4.2	2	2.8	0	0	0	0	0	0
H. nana egg	12	7.3	3	4.2	5	7.1	4	5.7	0	0	0	0	0	0
Ascaris egg	8	4.96	1	1.4	4	5.7	3	4.2	0	0	0	0	0	0
Helminths larvae	7	4.2	4	5.7	2	2.8	1	1.4	0	0	0	0	0	0
E. histolytica cyst	26	15.9	2	2.8	4	5.7	3	4.2	6	8.5	5	7.1	0	8.5
Cryptosporoidium spp. oocyst	32	19.5	8	11.4	7	10	6	8.5	5	7.1	4	5.7	2	2.8
B. hominis cyst	16	9.7	1	1.4	3	4.2	2	2.8	3	4.2	3	4.2	4	5.7
G. lamblia cyst	53	32.3	12	17.1	11	15.7	10	14.2	8	11.4	7	10	5	7.1
B. coli cyst	4	2.4	0	0	0	0	0	0	0	0	0	0	4	5.7
P-value		<0.001**		0.11		0.24		0.56		0.38		0.5		

No: total examined samples. %: percentage from total examined for each type of vegetables and fruits.

Table (3): Percentage reduction in viability of some parasites after exposure to acetic acid (5%) and potassium permanganate (KMno4) 24mg/L for 15 and 30 minutes**.**

Parasite	K permanganate		P1	Acetic acid		P1	P2	P3
	Loss of viability			Loss of viability				
	15 min (%)	30 min (%)		15 min (%)	30 min (%)			
Enterobius egg	16%	18%	0.68	20%	23%	0.6	0.4	0.38
H. nana egg	10%	12%	0.65	12%	10%	0.65	0.6	0.6
Ascaris egg	9%	12%	0.47	15%	18%	0.56	0.19	0.23
Helminths larvae	3%	6%	0.49	9%	12%	0.48	0.07	0.13
E. histolytica cyst	21%	25%	0.5	23%	27%	0.51	0.7	0.7
Cryptosporoidium spp. oocyst	19%	21%	0.72	25%	28%	0.6	0.3	0.24
B. hominis cyst	22%	25%	0.61	30%	32%	0.7	0.19	0.27
G.lamblia cyst	22%	26%	0.5	28%	31%	0.64	0.3	0.4
B. coli cyst	21%	25%	0.5	25%	28%	0.63	0.5	0.63

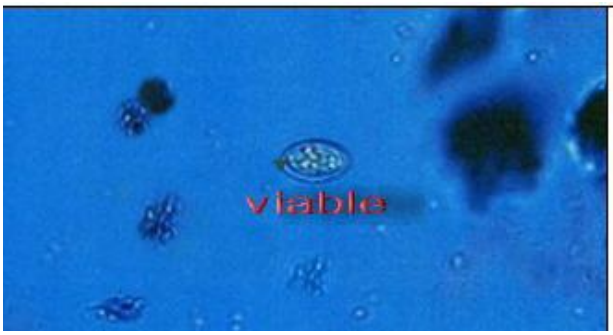

Figure (1): Viable *G. lamblia* cyst as detected by trypan blue 0.2% stain and appeared clear with light blue colour (X40).

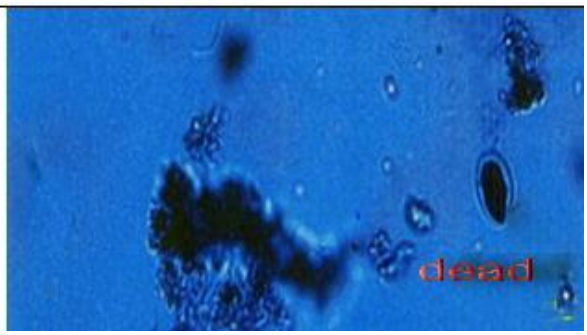

Figure (2): Non viable *G. lamblia* cysts as detected by trypan blue 0.2% stain and appeared dark blue and their structure could not be detected (X40).

The morphological changes of *Hymenolepis nana* egg due to exposure to potassium permanganate 24mg/L.

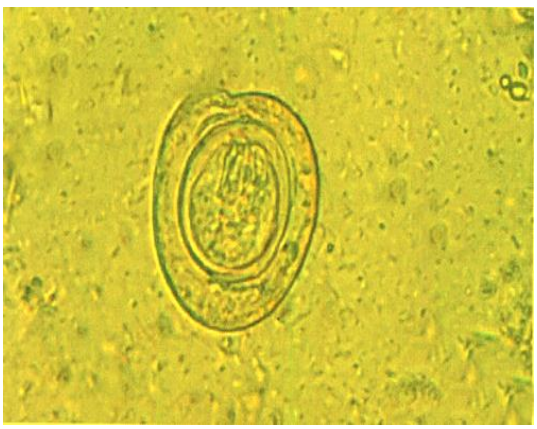

Figure (3): Dipping in the egg shell of *H. nana* egg after exposure to potassium permanganate 24mg/L for 15 minutes (X40).

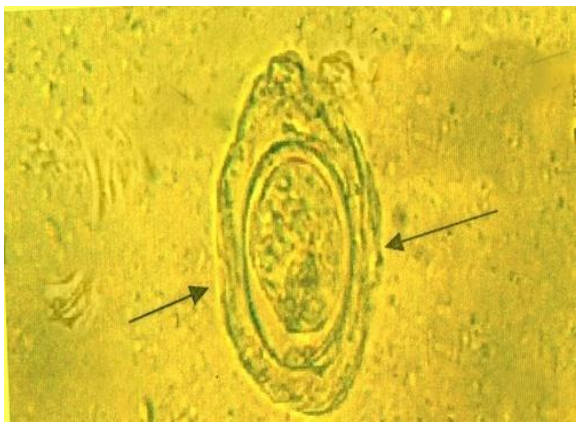

Figure (4): Marked damage in the egg shell of *H. nana* egg after exposure to potassium permanganate 24mg/L for 30 minutes (X40).

DISCUSSION

Vegetables and fruits are one of the most important sources of nourishment. But, they may be contaminated by intestinal parasites. They affect about 3.5 billion people **[16].** In less developed countries, there is a facilitation of transmission of parasitic infections to vegetables and fruits during cultivation by sewage contaminated irrigation water and untreated organic fertilizers carrying fecal helminths eggs and larvae. Also, the post-harvest handling, washing, transfer and storage contribute in their contamination **[17].** Consumption of the raw vegetables especially salads and fruits is almost daily in Egypt. Thus, it has been found that, there are high incidences of intestinal parasitic infections in raw vegetables consumed communities **[18].** Sharkyia governorate is mainly an agriculture community with large areas of agricultural fields where people mainly depend upon fresh raw vegetables in their food. Therefore, this study highlighted the most common types of vegetables and fruits which may transmit parasitic infection.

The current study showed a considerable high level of parasitic contamination of examined vegetables and fruits (39%) (Table 1). This result was nearly in agreement with Said **[19]** who reported that the rate of the parasitic contamination of vegetables was 31.7% in Alexandria, Egypt. In Nigeria and Ghana, Parasitic contamination of the vegetables was 36% **[20,21].** Another study recorded slightly lower levels of parasitic contamination as in Benha city, Egypt which reported a prevalence rate (29.6%) **[22].** Also, Abe et al. **[23]** showed that 37.5% of the studied samples of fruits and vegetables in Lafia's markets, Nigeria had some geohelminths contamination. Another study recorded slightly lower levels of parasitic contamination as in Ardabil city, Iran where a prevalence of 29% was recorded upon the contamination of garden vegetables with intestinal parasites **[24].** Also, Hassan et al. **[25]** reported 19.4% contamination in Alexandria, Egypt. Another study in Riyad, Saudi Arabia had a much lower level of vegetable contamination of parasitic infection (16.2%) **[26]**. But, the contamination of green vegetables was reported in a higher level in retail markets in Tripoli, Libya (58%) **[10]**. In Nigeria, 55% of the examined samples of vegetables were positive for different species of parasites **[27].** It was found that, the rate of parasitic contamination of collected samples of fruits and vegetables from local markets in Arab Minch town, Southern Ethiopia was 54.4% **[28].** Also, Nyarango **[29]** revealed (75.9%) contamination of the examined vegetables in Kenya. These findings 'differences are mainly due to the difference of the used techniques of investigations, the soil's type, type of water used for irrigation, climatic conditions, geographical locations and sanitary habits **[30].**

Table (1) showed the prevalence rate of parasitic contamination of the examined vegetables and fruits, where watercress samples were found to have the highest parasitic contamination (55.7%) followed by lettuce samples (45.7%), parsley (44.2%), cucumber (31.4%) and strawberry (30%). While carrots were found to be the least contaminated ones (27%). This variance of contamination is due to the variance in the shape and the surface area of the used vegetables and fruits. Lettuce, parsley and watercress have rough surfaces so that parasitic eggs, cysts and oocysts can attach easily to the surface of these vegetables. The pits on the surface of the strawberry are considered the chief factor leading to its contamination by protozoan stages. On the other hand, vegetables with smooth surface had less contamination because its smooth surface reduces the rate of parasitic attachment **[21].** These results were partially in accordance with a study in Khartoum state, Sudan, as lettuce recorded the highest level of contamination in fresh vegetable samples (36.4%) but cucumber wasn't contaminated **[31].** Similar results were reported in a study performed in Riyad, Saudi Arabia, as lettuce recorded with the highest rate of contamination (27.8%) followed by watercress (22.8%) **[26].** In Tripoli, Libya, watercress and lettuce samples were found to be contaminated more than other samples recording 100% and 96% respectively **[10]**. In Benha city, it was found that; lettuce had the highest parasitic contamination (45.5%), followed by watercress (41.3%) **[22].** Also, there was a high rate of lettuce contamination (40%) in Nigeria **[21].** In contrast, lettuce and watercress reported with lower rate of contamination (17%) than green onions (28%) in a study performed in Saudi Arabia 2006 **[32].**

Table (2) in the present study showed that *Giardia lamblia* cysts were the highest prevalent parasitic stage detected in the samples of raw vegetables and fruits (12.6%) followed by *Cryptosporidium spp*. oocysts (7.6%), *Entamoeba spp*. cysts (6.2%), *Blastocystis hominis* cysts (3.8%), *Hymenolepis nana* eggs (2.8%), *Ascaris lumbricoides* eggs (1.9%), different helminths larvae (1.6%), *Enterobius vermicularis* eggs (1.4%)

and finally *Balantidium coli* cysts were the least detected (0.9%).

In the present work, *G. lamblia* was the most prevalent parasite contaminating fruits and vegetables (12.6%), strawberry had a percentage (7.1%); lettuce had the highest percentage (17.1%). These findings are particularly like previous reports in Egypt, including the studies conducted in Alexandria, by Hassan et al. **[25]** who reported a prevalence rate of 8.8%. The prevalence of *G.lamblia* cysts was 23% of the examined samples of salad vegetables in Amman and Baqa'a in Jordan **[33].** Another study in Libya reported that *G.lamblia* cysts were found in 10% of the total examined vegetable samples **[10].** *G.lamblia* parasite was the third parasite that was detected in 1.6% of the samples in a study performed by Matini et al. **[30]**. On the other hand, *Giardia* cysts were detected in a higher rate in green vegetables in Riyad, Saudi Arabia **[26].** This high prevalence of *G. lamblia* in our study may be attributed to the long periods of survival *of Giardia* under cool and moist conditions and due to its resistance to the used chlorine in drinking water **[34].**

Cryptosporidium was the second prevalent parasite causing contamination of the examined samples (7.6%). *Cryptosporidium* was found in 4% of samples of fresh vegetables in Norway **[35].** This widespread of *Cryptosporidium* is mainly associated with the used water for irrigation and the use of the human excreta as manure. These findings agree with the study performed in Alexandria which detected a high rate of contamination of irrigation water in El Mahmoudeya canal by *Cryptosporidium* oocysts **[36]**.

The third prevalent parasite detected in this study was *Entamoeba spp*. cysts (6.2%). These results agree partially with a study in Shahrekord, Iran as cysts of *Entamoeba spp.* were detected in 9.2% of vegetable samples **[18].** In Sudan, it was found that *E. histolytica* was the most predominant parasite in fresh vegetables samples (42.9%) **[31].** *E. coli* was the most abundant parasite (2.6%) found in the survey done by Matini et al. **[30].** Much higher rates of the prevalence of *E. coli* cysts (11.2%) were recorded by Hassan *et al.* (2012) from a study in Alexandria, Egypt **[25]**. In Gaza, Palestine, the prevalence rate of vegetable samples contamination was 37.5% for *E. histolytica* **[37].** On the other hand, less rate of the prevalence of *E. histolytica* (0.6%) was recorded in Philippines **[38].** Detection of *E. histolytica* indicates the possibility of the contamination of the vegetables and fruits by human feces since the organism only lives in the human intestine **[18].**

H. nana eggs were detected in 2.8% of the examined samples. This level was like that recorded by Said (2012) as the prevalence of *H. nana* was 2.6% of vegetables samples in Alexandria, Egypt **[19].** *H. nana* eggs were 2.4% of vegetable samples in a previous study in Libya **[10],** 5% in another study in Zahedan, Iran **[39].** On contrast, other studies showed higher findings as in Riyad, Saudi Arabia in which *H. nana* eggs were found to contaminate 14.5% of vegetables samples **[26].** In Arba Minch town, Thousern Ethiopia, *H. nana* eggs were detected in 15.56% of examined vegetables samples **[28].** Lower rate was detected in Qazvin, Iran, as *H. nana* eggs were detected in 0.5% only from the parasite contaminated green vegetables **[40].** The observed difference might be due to difference in the climatic conditions and geographical location **[41].**

In the present study, *A. lumbricoides* eggs were detected in (1.9%) of the examined samples. Similar rate of contamination of vegetables samples with *A. lumbricoides* eggs was recorded (1.8%) of total examined samples in Turkey **[42].** Also, the rates of contamination with *Ascaris* eggs in vegetables were detected in Iran, as it was found to be 2.5% in Jiruft **[43],** 2.3% in Kazvin **[40].** Our results disagree with other studies that showed higher findings as in Alexandria governorate where *Ascaris* eggs were detected in 20.3% of the examined samples **[19].** The occurrence of *A. lumbricoides* (56.31%) among the examined fruits and vegetables was recorded in a study performed in rural areas of Zamfara States, Nigeria **[44].** A higher rate of contamination (68%) of fresh vegetables used in making the salad in Tripoli, Libya was recorded **[10].** A very high percentage of *A. lumbricoides* (89.33%) was recorded by Abe et al. **[23]**. The presence of *Ascaris* eggs in the vegetables is attributed to the usage of untreated night soil. This parasite's ubiquitous distribution could be attributed to the resistant nature of the eggs that enables them to survive under unfavorable conditions being unaffected by desiccation for several weeks.

In this study, helminths larvae were detected in 1.6% of the examined samples**.** These findings disagree with the higher rates of helminths larvae which were recorded in imported vegetable 6% and in native vegetable 7% in Tabriz, Iran **[45]**. On the other hand, Ezaptour et al. **[46]** reported

that helminths larvae were detected in (40%) of total vegetable samples in a study performed in Khorramabad, Iran. The increase in the prevalence of helminths larvae in some regions is due to subsequent silting of local rivers causing deposition of sandy loam top soils and increased soil moisture that might promote the emergence of these larvae **[47].**

In this study, *E. vermicularis* eggs were detected in 1.4% of vegetables samples. Nearly similar results were reported in Nigeria as the contamination rate of vegetables by *Enterobius spp*. was 0.8% **[48].** In Turkey, *Enterobius spp.* rate among the examined vegetables was 0.9% **[42].** On the other hand, higher levels of *E. vermicularis* (4.5%) were detected in a study conducted on vegetables in Philippines **[38].** Al-Binali et al. **[32]** recorded a rate (6.3%) of *E. vermicularis* eggs of vegetables samples in South Western Saudi Arabia. In Iran, *E. vermicularis* eggs were detected in 5.1% of the examined vegetables samples in Khorramabad **[46],** 8.1% in Zahedan **[39].** The predominance of *E. vermicularis* is associated with the socio-economic and environmental conditions and the bad hygienic practice of the vegetables handlers **[42].**

Table (2) showed the distribution of each parasitic contamination in various examined raw vegetables and fruits, where *G. lamblia* cysts were the most prevalent on lettuce samples (17.1%) followed by watercress (15.7%), parsley (14.2%), cucumber (11.4%), carrots (10%) and finally strawberry (7.1%). These results agree with a study conducted in Amman, Gordan **[33]** where lettuce samples were being the most contaminated (63%). In contrast, it was found in Libya that only 4% of lettuce was contaminated by *G. lamblia* cysts **[10].**

The results showed that *Cryptosporidium spp.* were most prevalent upon lettuce samples (11.4%) followed by watercress (10%), parsley (8.5%), cucumber (7.1%), carrots (5.7%) and finally strawberry (2.8%). These results disagree with a study performed by Said **[19]** who recorded that *Cryptosporidium spp.* oocysts were found in 2.5% of lettuce samples.

The results showed that *E. histolytica cysts* were most prevalent upon cucumber and strawberry samples (8.5%) followed by carrots (7.1%), watercress (5.7%), parsley (4.2%) and finally lettuce (2.8%).

As regards *H. nana* eggs, watercress samples were the most contaminated (7.1%), followed by parsley (5.7%) and lettuce (4.2%). But cucumber, carrots and strawberry samples were not contaminated**.** In our study, *A. lumbricoides* eggs were higher in watercress (5.7%) followed by parsley (4.2%) and finally lettuce (1.4%)**.** These results agree with a study performed in Tripoli, Libya which recorded that *Ascaris* eggs were detected in a high percentage in watercress [**10].**

This study showed that watercress was the highest contaminated vegetables by *E. vermicularis* eggs (4.2%), followed by parsley (2.8%) and then lettuce (1.4%). As regards *B. coli* cysts, strawberry is the only one of the examined samples which was contaminated by it (5.7%). This result may be due to the close contact of the mammillated fruits with soil.

Comparing these results with a previous study in Alexandria, Egypt, records were found as follows: watercress (46.7%), lettuce (45%) and parsley (36.7%) while leek (16.7%) and green onion (13.3%) **[19].** another study in Tripoli, Libya, revealed that watercress had the highest rate of contamination (100%) followed by lettuce (96%) while tomato had the least rate of contamination **[10].** In Benha, Egypt, lettuce (45.5%), watercress (41.3%), parsley (34.3%), green onion (16.5%) and leek (10.2%) **[22].** Also, in Riyad, Saudi Arabia, the highest rate of contamination was detected in 27.8% of lettuce, followed by watercress (22.8%) and parsley (17.4%) **[32].** these differences in the percentage among different kinds of vegetables are attributed to the nature of the building of the vegetables and fruits. As the smooth surface reduce the parasitic attachment **[49].**

Table (3) showed the comparison between the effects of two common disinfectants for vegetables as acetic acid (5%) and potassium permanganate (24mg/L) upon the viability of the parasites after exposure for 15 and 30 minutes. As regards *E. histolytica* cysts, the percentage of loss of viability was 21% after 15 minutes exposure to potassium permanganate, and then it reached 25% after 30 minutes exposure. Upon exposure to acetic acid 5%, the reduction in viability reached 23% after 15 minutes and extension of exposure up to 30 minutes decreased the viability to 27%.

Table (3) showed that the percentage of loss of viability of *G. lamblia* changed from 22% after 15 minutes to 26% after 30 minutes when exposed to potassium permanganate. While, on exposing to acetic acid (5%) for 15 and 30 minutes it changed from 28% to 31% respectively. This

difference was statistically insignificant. A study on the giardiacidal effect of lemon juice and vinegar on *G. lamblia* cysts was done by Sadjjadi et al. **[50]** revealed that the giardiacidal activity at 4°C after 3 hours for lemon juice and vinegar was 18.9% and 28.4% and at 24°C 28.3% and 40.6% respectively.

As regards *Cryptosporidium spp.* oocysts, the percentage of loss of viability was 19% after 15 minutes exposure to potassium permanganate, and then it reached 21% after 30 minutes exposure. Upon exposure to acetic acid (5%), the reduction in viability reached 25% after 15 minutes and extension of exposure up to 30 minutes decreased the viability to 28%. But in the last protozoal one (*B. coli)*, the percentage of loss of viability was 21% when exposed to potassium permanganate for 15 minutes and became 25% when extended to 30 minutes of exposure. While, on exposing to acetic acid (5%) for 15 and 30 minutes it changed from 25% to 28% respectively.

The explanation of the effect of the used disinfectants upon the different protozoa stages in this study may be that the acidic disinfectants act by breaking the nucleic acids and precipitating proteins bonds. Also, it may work by changing the pH of the environment making it unsuitable to these parasites **[51].** Abuladze *et al.* (2009) explained in his study how the exposure of the protozoa to potassium permanganate to the oxidation of the cell membrane phospholipids which led to membrane dysfunction and cell death **[8].**

According to this study, the percentage of loss of viability in *E. vermicularis* eggs changed from 16% after 15 minutes to 18% after 30 minutes when exposed to potassium permanganate. While, on exposing to acetic acid (5%) for 15 and 30 minutes it changed from 20% to 23% respectively.

H. nana eggs, the percentage of loss of viability was 10% after 15 minutes exposure to potassium permanganate, then it reached 12% after 30 minutes exposure. Upon exposure to acetic acid (5%), the reduction in viability reached 12% after 15 minutes and extension of exposure up to 30 minutes decreased the viability to 10%.

About the helminths larvae, the percentage of loss of viability was doubled from 3% after 15 minutes to 6% after 30 minutes when exposed to potassium permanganate. While, on exposing to acetic acid (5%) for 15 and 30 minutes it changed from 9% to 12% respectively.

In the case of *Ascaris* eggs, the percentage of loss of viability was 9% after 15 minutes exposure to potassium permanganate, and then it reached 12% after 30 minutes exposure. Upon exposure to acetic acid (5%), the reduction in viability reached 20% after 15 minutes and extension of exposure up to 30 minutes decreased the viability to 23%.

Considering these results, it was found that the effect of both potassium permanganate and acetic acid upon different helminths stages was less than upon protozoa stages. This may be attributed to the nature of the structure of helminths eggs **[52].** In general, *H.nana* eggs are very resistant to chemicals such as acetic acid and kept viable for several months and this is due to having an onchosphere covered by three basic layers which form the egg shell making an obstacle to the chemicals to reach the infective stage **[53].** *E. vermicularis* eggs have five membranes: one inner, lipoid layer, three middle layers called membrane Lucida and an outer, albuminous membrane which coats the egg. This membrane makes the eggs sticky leading to increase in the resistance of the eggs to disinfection **[54].**

Our study reported that, the percentage of the loss of viability of most of the examined parasites by using acetic acid (5%) is more than that by using potassium permanganate (24mg/L), however this difference was found to be insignificant. This effect appeared under the light microscope different magnifications by some morphological changes in the form of deformity, damage of the egg shell and shrinkage of the cyst wall. After exposure of raw vegetables and fruits in this study (lettuce, parsley, watercress, cucumber, carrots and strawberry) to both acetic acid (5%) and potassium permanganate (24 mg/L) for 15 and 30 minutes, they showed nearly normal taste, smell, consistency without color changes or softening in the vegetable leaves.

CONCLUSION

The results obtained from this research showed high contamination levels of raw vegetables and fruits with different helminths and protozoa stages. Thus, the consumption of raw vegetables and fruits may be one of the sources of parasitic infections among the public. The usage of certain types of disinfectants as potassium permanganate (24mg/L) and acetic acid (5%) is one of the effective ways of reducing fruits and vegetables borne parasitic infections. This study calls for the

need of strict hygienic measures in the areas where the vegetables and fruits are cultivated, sold, prepared and consumed. This is properly achieved by treatment of soil, manure and water used for cultivation of vegetables and fruits and disinfecting them before consumption. In addition, the surveys must be done in different governorates in Egypt to evaluate the levels of parasitic contamination in raw vegetables and fruits in both rural and urban areas.

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

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Effect of Health Promotion Program on Compliance of Patients with Hypertension toward Therapeutic Regimen

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Key words: Hypertension, Compliance, Health Promotion Program

Background and study aim: Hypertension is a global health issue among the adult population. Compliance with therapeutic regimen is an effective step for better control of blood pressure and preventing the risk of complications. The aim of study was to evaluate the effect of health promotion program on compliance with therapeutic regimen for patients with hypertension.

Subjects and Methods: A quasi experimental design was used in this study. The study was conducted in outpatient clinic, department of cardiology. A purposive sample of 80 adult patients suffering from hypertension divided into two groups study and control were included. The study lasted from the beginning of May 2016 to the end of May 2017. Four tools were used for collection of data. A structured interview questionnaire tool for patient consisted of personal characteristics of patients and patient's knowledge regarding hypertension, Compliance assessment scale, Observation checklist and Factors affecting patient's compliance.

Results: The study finding revealed that, there was a significant improvement in knowledge, practice and compliance of patients with hypertension in study group in post and follow up program phase P=0.00. Also, the patients showed high improved level regarding lifestyle habits. In addition to there was significant decrease in systolic, diastolic blood pressure for patients in study group.

Conclusion: It can be concluded that application of health promotion program for adult patients with hypertension showed an improvement in patients' knowledge which can reflect an improvement on their practice and compliance. It is recommended to increase awareness of patients about risk factors of hypertension to empower and motivate them to adopt healthy lifestyle to prevent the disease. This can be achieved through mass media and health education programs about the disease process and the importance of adopting healthy lifestyle.

INTRODUCTION

Hypertension is remains a major global public health challenges that has been identified as the leading risk factor for cardiovascular morbidity and mortality. Hypertension is defined as a systolic blood pressure equal to or above 140 mmHg and/or diastolic blood pressure equal to or above 90 mmHg. Normal levels of both systolic and diastolic blood pressure are particularly important for the effective function of vital organs such as heart, brain and kidneys as well as for overall health and well-being **[1]**. The prevalence of hypertension and burden of disease caused by high blood pressure (BP) is increasing in the world **[2]**. The World Health Organization **[3]** estimates that One-quarter of the world's adult population has hypertension, affects more than 26%, approximately one billion of the adult people all over the world and this is likely to increase to 29% to a total of 1.56 billion by 2025 will have hyper-tension. In Eastern Mediterranean Region, the prevalence of hypertension was 29% and it was affected approximately 125 million individuals **[4]**. Egyptian National Hypertension Project showed that hypertension is common among Egyptians. The

prevalence of hypertension is 26.3%. The problem is complicated by the low awareness rates, only 38% of hypertensive Egyptians aware of having high blood pressure **[5]**.

Compliance with therapeutic regimen is considered the main theme in the management of the patient with hypertension. Compliance is defined as the extent to which a patient's behaviour (taking medication, following a diet, modifying habits or attending clinics) coincides with healthcare giver advice **[6]**. Uncontrolled hypertension is caused by non-compliance to the therapeutic regimen. For patients with hypertension, compliance depending on compliance with dietary management, drug regimen, Lifestyle habites and clinic visits every 1-3 months. patient's knowledge, patients' beliefs and motivation towards the therapy help the patients to improve their compliance, thus will prevent the complications of hypertension which are debilitating and if not prevented can increase the burden of a disease that is already on the increase **[7]**.

Health promotion is defined as the "science and art of helping people change their lifestyle to move toward a state of optimal health, which is a balance of physical, emotional, social, spiritual, and intellectual health. There are different theories and models that are used to explain health promotion and incorporate it into practice. The most common model is the Health Belief Model **[8]**. Health Belief Model is concerned with what people perceive, or believe, to be true about themselves in relation to their health. The HBM was also widely used to explain a range of health behavior. There are six constructs to the HBM: perceived susceptibility, perceived severity, perceived benefits, perceived barriers, self-efficacy, and cues to action. The way persons relate themselves to each of these areas is predictive of how likely they are to engage or not engage in a certain behavior. In hypertension the HBM bases on studying compliances with lifestyle modification and antihypertensive medication **[9]**.

The aim of the study was to evaluate the effect of health promotion program on compliance with therapeutic regimen for patients with hypertension.

SUBJECTS AND METHODS

A quasi experimental design was utilized in the study. Study was conducted in outpatient clinics at Zagazig University Hospitals. Field work of this study was executed in 12 months, starting from May 2016 to May 2017.

Subjects:
A purposive sample of 80 adult patients with hypertension. They were divided randomly into two groups "study" and "control" (40 patients for each group).

Tools for data collection: Four tools were used for data collection**:**
Tool I: A Structured interview questionnaire tool for patients was designed by the researcher after revising of related literature and opinions of expertise for content of validity and included the following four parts:

- **Part 1:** Demographic characteristics of patients e.g. (age, sex, marital status, occupation, level of education ….etc)
- **Part 2:** Medical history of patients e.g. (chronic illness, family history, and disease duration)
- **Part 3:** Life style habites of patients (Pre/ Post/follow up test). It included Nutritional habits, Physical activity, Weight control .etc).
- **Part 4:** Patient's knowledge regarding hypertension (Pre/ Post / follow up test). It included meaning of a blood pressure reading, normal value of Bp for adults, hypertension is chronic disease, primary hypertension is the most common type ….etc.

Tool II: The Compliance assessment scale (Pre/ Post/ follow up test): It was used to assess the compliance of patients with hypertension toward therapeutic regimen **[10]**.

Tool III: Observation checklist (Pre/ Post/ follow up test): A structured observational checklist was developed by the researcher to evaluate patients ' practice It included: BP measurement, Breathing exercises, Progressive Muscle Relaxation, Meditation and Guided Imagery as guided by Lynn & LeBon **[11]**, Sue, DeLaune & Ladner **[12]**, Dave & Makwana **[13]** and Madhava & Deepa **[14]**.

Tool IV: Factors affecting patient's compliance (Pre, Post and follow up test): a Structure Interview Questionnaire sheet, It was designed by the researcher based on Health Belief model, to measure factors affecting compliance with antihypertensive therapy. It included six constructs: Perceived severity, Perceived susceptibility, Perceived benefits, Perceived barriers, Self- Efficacy and Cues to Action.

Health promotion program:
It was designed by the researcher after revising of related literature. It consisted of theoretical and

practical parts. Theoretical part implemented through five sessions. Each session took 30 minutes. It included definition of hypertension, classification, manifestations, risk factors, complications, and treatment…..etc. Practical part implemented through nine sessions. Each session took 30- 45 minutes. It included Blood pressure measurement, Breathing Exercises and Progressive Muscle Relaxation exercises ….etc. Patients were grouped; each group included 4-5 patients.

Content validity and Reliability:

Content validity was used for the modified tools and the designed booklet to determine whether the tools covered the aim or not. It developed by a jury of 5 experts four professors from faculty of Nursing, Zagazig University and one lecturer of cardiology from the Faculty of Medicine, Zagazig University. Reliability was done by using Cronbach test and retest **[15]**. It was used to examine whether the observation checklist, compliance assessment scale had internal consistency or not. The test was done and the agreement percentage was 97%.

The implementation phase for data collection started as following: The selection of the patients, the collection of data, and the implementation of the Health promotion program lasted over a period of 12months, starting from May 2016 to May 2017, Two months for pre- test (from beginning May, 2016 to the end of June, 2016), Six months implementation the program and posttest (from beginning of July 2016 to end of December 2016), 3 months after posttest follow up test was done which lasted 2 months (from beginning of April to end of May 2017). The questionnaire sheet was designed by the researcher. Data used was collected four day from the outpatient clinics of cardiology, from 9:00 am to 1:00 pm where the program was implemented. Patients were grouped; each group included 4-5 patients. It was necessary for the researcher to introduce herself for the patients and explain the purpose of the study. The data was collected in a simplified Arabic language. The study program consisted of 16 sessions; one session of them to identify the objective and the importance of the program. One third of the sessions (5) were theoretical, and two thirds (9) were practical and one session for revision.

Administrative and Ethical considerations:
The study was ethically approved from the dean of the faculty of Nursing, the manager of Zagazig University Hospitals, the head of outpatient clinics of cardiology, ethics committee at the faculty of nursing and from Ethical committee of faculty of medicine.

Statistical Design:
All collected data were organized, categorized, tabulated, entered, and analyzed by using SPSS (Statistical Package for Social Sciences); a software program version 14, 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), Paired sample t test (T test), Pearson chi-square test (X2) and Pearson Correlation (r) to detect the relation between the variables.

RESULTS

The first part of our results was the Demographic characteristics and medical history for patients with hypertension in both study and control groups including; gender, age, residence, marital Status, education, occupation and income (Table 1).

The second part of our results was concerned with the Patients' behaviour toward Lifestyle habites in both patients groups throughout the study phases, It clarified that Mean scores for total and all dimensions of lifestyle habites such as nutritional habites, physical activity, weight control and stress management were increased (improved) significantly between pre / post and pre / follow up program phase in the study group P= 0.00 compared to the smoking habites there was no improvement for patients in both study and control group throughout the study and follow up phases (Table 2).

The third part of our results was concerned with the Patients' knowledge about hypertension in both patients groups throughout the study phases. It revealed that the patients in study group had satisfactory level of knowledge about hypertension, diet, and treatment regimen between pre/post and pre/follow up program phase (95.0%, 82.5%) respectively, While no significant differences occurred in the control group. The table showed statistical significant difference in study group throughout the study phases P= 0.00 (Table 3).

The fourth part of our results was concerned with the Patients' compliance toward therapeutic regimen obtained by patients in both groups throughout the study phase. It represented that the patients in study group were good compliant

toward therapeutic regimen regarding diet regimen, treatment regimen and comply with lifestyle habites between pre/post and pre/follow up program phase (80.0%, 70.0%) respectively While no significant differences occurred in the control group. The table showed statistical significant difference in study group throughout the study phases P= 0.00 (Table 4).

The fifth part of our results was concerned with the Patients' practice obtained by patients with hypertension in both groups throughout the study phase. It showed that, the patients in study group had satisfactory practice related to BP measurement, breathing exercises, Progressive Muscle Relaxation, meditation, guided Imagery between pre/post and pre/follow up program phase P=0.001 compared to control group none of patients had satisfactory practice throughout the study phases (Table 5).

The sixth part of our results was concerned with the Factors affecting compliance with therapeutic regimen for patients based on the HBM in both patients groups throughout the study phases. It demonstrated that, positive changes in perception were observed in the study group for all HBM constructs except, cues to action between pre/ post and pre/follow up program phase P= 0.001, 0.002, 0.003, 0.004. While no significant differences occurred in the control group (Table 6).

The seventh part of our results demonstrated that there was statistical significant relation between the patients' knowledge and their demographic characteristics, only among their educational level in study group in post program phase ($p<0.05$), while, there was statistical significant relation between the patients' compliance and their demographic characteristics, among their age and income in study group in post program phase (p-value <0.05), also there was statistical significant relation between total compliance score and HBM among perceived severity, perceived benefits and Self-Efficacy in study group in post phase (p-value <0.05), there was statistically positive correlation between patients' knowledge and practice in post and follow up program phase, there was statistically positive correlation between patients' practice and compliance in post program phase. Also there was statistically positive correlation between patients' knowledge and compliance in post program phase and versus (Tables 7, 8, 9, 10).

Table (1): Demographic Characteristics for patients in both groups (n= 80)

Demographic characteristics	Group				MCP
	Study (n= 40)		Control (n= 40)		
	No	%	No	%	
Age in years					
25-50	15	37.5	17	42.5	
51-65	25	62.5	23	57.5	0.397
Range	25-65		25-65		
Mean ± SD	54.1 ± 11.0		53.9 ± 10.5		
Median	54.5		53.5		
Gender					
Male	10	25.0	13	32.5	0.483
Female	30	75.0	27	67.5	
Residence					
urban	8	20.0	7	17.5	0.776
Rural	32	80.0	33	82.5	
Marital Status					
Married	30	75.0	29	72.5	0.345
Single	8	20.0	6	15.0	
Widow	2	5.0	5	12.5	
Education					
Illiterate	26	65.0	30	75.0	0.857
School education	10	25.0	9	22.5	
University	4	10.0	1	2.5	
Occupation					
Work	2	5.0	2	5.0	0.840
Don’t work	38	95.0	38	95.0	
Income					
Enough	12	30.0	10	25.0	1.317
Not enough	28	70.0	30	75.0	
Crowding index					
< 2	13	32.5	14	35.0	0.259
≥2	27	67.5	26	65.0	

MCP: Mont Carlo exact probability * P < 0.05 (significant)

Table (2): Mean scores of Lifestyle habites for patients with hypertension in both groups (n= 80)

Lifestyle items	Study group (n=40)			Control group (n= 40)		
	Pre	Post	Follow Up	Pre	Post	Follow Up
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Nutritional habites	2.17±0.27	2.61±0.23	2.58±0.22	2.27±0.35	2.33±0.31	2.31±0.35
T-test (P1)	17.059 (0.000)*			3.240 (0.072)		
T-test (P2)	15.715 (0.000)*			2.202 (0.083)		
Physical activity	1.68±0.46	2.21±0.42	2.24±0.36	1.73±0.46	1.77±0.49	1.76±0.49
T-test (P1)	12.611 (0.000)*			2.449 (0.069)		
T-test (P2)	12.969 (0.000)*			2.138 (0.078)		
weight control	2.76±0.31	2.94±0.24	2.91±0.25	2.81±0.23	2.83±0.22	2.80±0.24
T-test (P1)	6.773 (0.000)*			1.704 (0.096)		
T-test (P2)	5.877 (0.000)*			0.650 (0.519)		
Smoking habites	2.77±0.22	2.78±0.21	2.79±0.22	2.05±0.3	2.02±0.34	2.02±0.34
T-test (P1)	2.077 (0.107)			1.262 (0.214)		
T-test (P2)	2.254 (0.081)			1.320 (0.118)		
Stress management	2.12±0.35	2.47±0.24	2.50±0.21	2.22±0.30	2.26±.29	2.25±0.29
T-test (P1)	11.343 (0.000)*			3.812 (0.063)		
T-test (P2)	11.720 (0.000)*			3.716 (0.071)		
Total lifestyle score	11.45±1.5	12.75±1.24	12.64±1.1	11.84±1.5	12.01±1.6	11.97±1.5
T-test (P1)	24.633 (0.000)*			5.050 (0.933)		
T-test (P2)	19.875 (0.000)*			3.374 (0.882)		

* P < 0.05 (significant) P1: Pre/post P2: Pre/ FU

Table (3): Patients' knowledge in both groups throughout the study phases (n= 80)

Knowledge	Group											
	Study (n=40)						Control (n=40)					
	Pre		Post		Follow Up		Pre		Post		Follow Up	
	No	%	No	%	No	%	No	%	No	%	No	%
knowledge about disease												
Satisfactory	2	5.0	39	97.5	31	77.5	2	5.0	2	5.0	2	5.0
Z-test (P1)	6.083 (.000*)						.000 (1.000)					
Z-test (P2)	5.745 (.000*)						.000 (1.000)					
knowledge about diet regimen												
Satisfactory	3	7.5.0	37	92.5	33	82.5	5	12.5	5	12.5	5	12.5
Z -test (P1)	5.831 (.000*)						.000 (1.000)					
Z -test (P2)	5.568 (.000*)						.000 (1.000)					
knowledge about treatment regimen												
Satisfactory	3	7.5	38	95.0	36	90.0	3	7.5	3	7.5	3	7.5
Z -test (P1)	5.916 (.000*)						.000 (1.000)					
Z -test (P2)	5.578(.000*)						.000 (1.000)					
Total knowledge												
Satisfactory	3	7.5	38	95.0	33	82.5	4	10.0	4	10.0	4	10.0
Z -test (P1)	5.604 (.000*)						.000 (1.000)					
Z -test (P2)	5.477 (.000*)						.000 (1.000)					

* P < 0.05 (significant) P1: Pre/post P2: Pre/ FU

Table (4): Patients' compliance score in both groups throughout the study phases (n= 80)

Compliance	Group											
	Study (n=40)						Control (n=40)					
	Pre		Post		Follow Up		Pre		Post		Follow Up	
	No	%	No	%	No	%	No	%	No	%	No	%
Compliance with diet regimen												
Good compliance	3	7.5	28	70.0	24	60.0	3	7.5	3	7.5	3	7.5
Z -test (P1)	4.600 (.000*)						.000 (1.000)					
Z -test (P2)	2.985 (.003*)						.000 (1.000)					
Compliance with treatment regimen												
Good compliance	5	12.5	37	92.5	33	82.5	8	20.0	8	20.0	8	20.0
Z -test (P1)	5.048 (.000*)						.000 (1.000)					
Z -test (P2)	4.600 (.000*)						.000 (1.000)					
Compliance with Lifestyle habites												
Good compliance	2	5.0	34	85.0	30	75.0	1	2.5	1	2.5	1	2.5
Z -test (P1)	5.916 (.000*)						.000 (1.000)					
Z -test (P2)	5.568(.000*)						.000 (1.000)					
Total compliance												
Good compliance	3	7.5	32	80.0	28	70.0	4	10.0	4	10.0	4	10.0
Z -test (P1)	5.578 (.000*)						.000 (1.000)					
Z -test (P2)	5.385 (.000*)						.000 (1.000)					

* P < 0.05 (significant) P1: Pre/post P2: Pre/ FU

Table (5): Patients' practices obtained by patients with hypertension in both groups throughout the study phases (n=80)

Patients' practices	Group											
	Study (n=40)						Control (n=40)					
	Pre		Post		Follow Up		Pre		Post		Follow Up	
	No	%	No	%	No	%	No	%	No	%	No	%
BP measurement												
Satisfactory	3	7.5	36	90.0	35	87.5	0	0.0	0	0.0	0	0.0
P (P1)	0.001*						-					
P (P2)	0.001*						-					
Breathing exercises												
Satisfactory	2	5.0	40	100.0	39	97.5	0	0.0	0	0.0	0	0.0
P (P1)	0.001*						-					
P (P2)	0.001*						-					
Progressive Muscle Relaxation												
Satisfactory	0	0.0	40	100.0	36	90.0	0	0.0	0	0.0	0	0.0
P (P1)	0.001*						-					
P (P2)	0.001*						-					
Meditation												
Satisfactory	0	0.0	39	97.5	32	80.0	0	0.0	0	0.0	0	0.0
P (P1)	0.001*						-					
P (P2)	0.001*						-					
Guided Imagery Satisfactory	2	5.0	40	100.0	39	97.5	0	0.0	0	0.0	0	0.0
P (P1)	0.001*						-					
P (P2)	0.001*						-					
Total practice Satisfactory	2	5.0	38	95.0	34	85.00	0	0.0	0	0.0	0	0.0
P (P1)	0.001*						-					
P (P2)	0.001*						-					

P: Friedman test for repeated measures * P < 0.05 (significant) P1: Pre/post P2: Pre/ FU

Table (6): Perception level pertaining of patients with hypertension in both groups throughout the study phases (HBM) (n=80)

Health Belief Model items	Study (n=40)			Control (n=40)		
	Mean ± SD			Mean ± SD		
	Pre	Post	Follow Up	Pre	Post	Follow Up
Perceived severity	11.5±1.3	16.9±0.9	16.9±0.9	12.1±2.1	11.9±1.8	11.9±1.8
P+(P1)	0.001*			0.328		
P+(P2)	0.001*			0.328		
Perceived susceptibility	5.0±0.6	10.4±0.9	10.4±0.9	6.5±0.8	6.6±0.9	6.5±0.8
P+(P1)	0.001*			0.488		
P+(P2)	0.001*			0.530		
Perceived Benefits	2.0±0.5	11.5±0.4	11.5±0.4	2.2±0.5	3.1±0.6	3.1±0.6
P+(P1)	0.001*			0.851		
P+(P2)	0.001*			0.851		
Perceived Barriers	9.5±1.7	12.7±1.5	11.6±1.4	7.6±1.1	7.5±1.2	7.5±1.2
P+(P1)	0.003*			0.920		
P+(P2)	0.002*			0.920		
Self-Efficacy	3.5±0.9	5.2±1.0	4.5±1.6	3.7±0.8	3.6±0.9	3.5±1.2
P+(P1)	0.004*			0.907		
P+(P2)	0.002*			0.899		
Cues to Action	6.0±1.2	6.3±1.1	6.2±1.4	6.7±1.3	6.7±1.4	6.7±1.4
P+(P1)	0.114			0.150		
P+(P2)	0.954			0.150		

P+: P value of Paired t-test * P < 0.05 (significant) P1: Pre/post P2: Pre/ FU

Table (7): Relation between total satisfactory knowledge score about hypertension and Demographic characteristics for patients in both groups throughout the study phases

Demographic characteristics	Study						Control					
	Pre		Post		Follow Up		Pre		Post		Follow Up	
	Total knowledge		Total knowledge		Total knowledge		Total knowledge		Total knowledge		Total knowledge	
	Satisfactory		Satisfactory		Satisfactory		Satisfactory		Satisfactory		Satisfactory	
	X^2	P value	X^2	P value	X^2	P value	X^2	P value	X^2	P value	X^2	P value
Age in years												
25-50	.140	.708	1.177	.278	.667	.414	.775	.379	.775	.379	.775	.379
51-65												
Gender												
Male	.208	.648	.024	.877	.667	.414	3.120	.077	3.120	.077	3.120	.077
Female												
Residence												
Urban	.037	.815	.004	.950	.027	.868	.531	.082	.531	.082	.531	.082
Rural												
Marital Status												
Married												
Single	5.531	.063	3.257	.196	1.091	.580	.048	.826	.048	.826	.048	.826
Widow												
Education												
Illiterate	.240	.808	.957	.025*	.000	1.000	.171	.679	.171	.679	.171	.679
School education												
University												
Occupation												
Work	.048	.826	.112	.738	1.253	.263	.048	.826	.048	.826	.048	.826
Don't work												
Income												
Enough	.171	.679	.263	.608	.811	.368	.208	.648	.208	.648	.208	.648
Not enough												

* $P < 0.05$ (significant)

Table (8): Relation between total good compliance score and Demographic characteristics for patients with hypertension in both groups throughout the study phases

Demographic characteristics	Study						Control					
	Pre		Post		Follow Up		Pre		Post		Follow Up	
	Total Compliance		Total Compliance		Total Compliance		Total Compliance		Total Compliance		Total Compliance	
	Good		Good		Good		Good		Good		Good	
	X^2	P value	X^2	P value	X^2	P value	X^2	P value	X^2	P value	X^2	P value
Age in years												
25-50	1.091	.580	6.790	.034*	4.270	.118	3.210	.087	3.210	.087	3.210	.087
51-65												
Gender												
Male	.024	.877	2.667	.102	.667	.414	.531	.082	.531	.082	.531	.082
Female												
Residence												
Urban	1.430	.232	.740	.857	.027	.868	.710	.825	.710	.825	.710	.825
Rural												
Marital Status	1.177	.278	.296	.586	.000	1.000	.354	.535	.354	.535	.354	.535
Married												
Single												
Widow												
Education												
Illiterate	.775	.379	.102	.749	.667	.414	.103	.780	.103	.780	.103	.780
School education												
University												
Occupation												
Work	1.310	.125	.440	.507	3.683	.055	1.430	.232	1.430	.232	1.430	.232
Don't work												
Income												
Enough	3.120	.077	.867	.035*	.360	.548	.024	.877	.024	.877	.024	.877
Not enough												

* $P < 0.05$ (significant)

Table (9): Relation between total good compliance score and HBM for patients in both groups throughout the study phases

HBM	Study						Control					
	Pre		Post		Follow Up		Pre		Post		Follow Up	
	Total Compliance		Total Compliance		Total Compliance		Total Compliance		Total Compliance		Total Complianc	
	Good		Good		Good		Good		Good		Good	
	X^2	P value	X^2	P value	X^2	P value	X^2	P value	X^2	P value	X^2	P valu
Perceived												
Severity	1.92	.909	2.15	.025*	1.65	.583	1.87	.640	1.22	.520	1.32	.612
Perceived												
susceptibility	.394	.778	.452	.683	.574	.153	2.23	.760	3.88	.570	3.88	.570
Perceived												
Benefits	3.03	.952	3.27	.036*	2.45	1.13	.207	.884	.076	.487	.057	.466
Perceived	2.39	.763	1.39	.952	.782	.202	2.23	.352	2.44	.644	2.44	.644
Barriers												
Self-Efficacy	2.14	.848	3.59	.012*	1.74	.765	2.17	.382	2.17	.382	2.17	.382
Cues to Action	2.38	.747	1.75	.458	.832	.222	2.84	.846	2.84	.846	2.84	.846

* P < 0.05 (significant)

Table (10): Correlation coefficient between knowledge, practice and compliance of study group throughout the study phases (n=40)

Item	Knowledge		Practice		Compliance	
	r	p	r	p	r	p
Pre knowledge	-	-	.217	.179	.257	.170
Pre practice	.217	.179	-	-	.861	.369
Pre compliance	.257	.170	.861	.369	-	-
Post knowledge	-	-	.473	.002**	.344	.030*
Post practice	.473	.002**	-	-	.668	.001*
Post compliance	.344	.030*	.668	.001*	-	-
FU knowledge	-	-	.319	.045*	.292	.068
FU practice	.319	.045*	-	-	.223	.236
FU compliance	.292	.068	.223	.236	-	-

*r: Spearman correlation coefficient * P < 0.05 (significant)

Interpretation of *r:

Weak (0.1-0.24) Intermediate (0.25-0.74) Strong (0.75-0.99)

DISCUSSION

Hypertension is often an asymptomatic disorder characterized by persistent elevation of the SBP at a level of 140 mmHg or higher and DBP at a level of 90 mmHg or higher **[16]**. High BP is a major risk factor for heart disease, congestive heart failure, stroke, impaired vision, and kidney disease. Clinical manifestations will become apparent, and patients will eventually complaint about persistent headaches, fatigue, dizziness, palpitations, flushing, blurred or double vision, or epistaxis **[17]**.

Health Belief Model (HBM) is widely used intervention programs aiming to change behaviors, especially those related to dietary practices, infectious disease, smoking cessation and high blood pressure screening. The model assumes that a person's beliefs about health are determinants of the possibility of an individual to make changes in the lifestyle behaviors. Therefore, health education utilizing HBM is a crucial factor in preventing illness, provision of information will help them in taking care of themselves and their family **[18]**.

Discussion of the results will cover these areas in the following sequence; demographic characteristics and medical history of adult patients with hypertension under the study, patients' behaviour toward life style habites, patients' knowledge about hypertension, compliance of patients with hypertension toward therapeutic regimen, patients' practice regarding Blood pressure measurement, relaxation exercises, Factors affecting patient's compliance based on Health Belief model and relation and correlation between different variables.

Demographic characteristics and medical history for patients with hypertension in both study and control groups including; gender, age, residence, marital Status, education, occupation and income were matched and this help to control other variables that may affect the outcome of the study**[6]**. The present study clarified that none the patients in both study and control groups had satisfactory level of knowledge regarding hypertension, in preprogram phase. This finding was agree with Elaskary **[19]** who reported that there was a significant increase in knowledge level post program and in follow up after three months compared to preprogram. This could be explained by the fact that patients didn't receive enough information from health care providers or/ and the health professionals didn't find the time to provide them with enough information and also the majority of the studied patients were illiterate. After implementation of the health promotion program, the results of the study showed that there was a statistical significant increase in patients' level of knowledge regarding hypertension, in study group. This finding agrees with Al-Wehedy Abd Elhameed and Abd El-Hameed **[20]** who illustrated that lifestyle modification sessions improved the knowledge scores of the study group of hypertensive patients with highly statistical significant difference between study and control. Ambaw, Alemie, Yohannes and Mengesha **[21]** concluded that right knowledge about hypertension and its treatment creates a clear understanding and avoids confusion about the treatment and the disease condition. Knowledge about hypertension and its treatment was found to be positively associated with adherence behavior. Patients with better awareness were more likely to adhere to their treatment.

In this study there was marked improvement in compliance score related to hypertension after the educational program. This approach is also supported by Ahmed **[22]** who reported that patient education plays a fundamental role in successful management of hypertension. Consequently, the failure to establish an effective communication with patients is associated with non-adherence and poor BP control. These mean that the provision of more detailed information about hypertension was associated with better compliance to treatment and BP control.

It was observed that patients had unsatisfactory level of practice about Blood pressure measurement, relaxation exercises preprogram, which significantly improved after patients' involvement in the education sessions. This finding was in the same line with Ez Elregal **[23]** in Ain Shams University who reported in a study about “Promoting Health behaviors of Clients with Hypertensive Kidney Disease by Using Health Promotion Model” that there was improvement of practice about Blood pressure measurement, breathing exercises and Progressive Muscle Relaxation after program. It might be due to that the patient required enough instructions about practices that help in minimizing occurrence of stress.

The Health Belief Model, which is widely used to study health behaviour, formed the theoretical framework for this study. The core components of the Model are perceived susceptibility, perceived severity, perceived benefits and perceived barriers. The Model postulates that health behaviour

towards a disease or treatment is succinctly influenced by the extent to which individuals believe they are susceptible to the disease and how severe they believe the disease is, the benefits they stand to gain by adopting the required health behaviour and the barriers standing in the way of adopting the required health behaviour. The expanded version of the Model also includes variables such as self-efficacy and cues to action **[24]**. The finding of the present study was expected in relation to HBM model constructs, that there were statistical differences for all constructs except for cues to action post intervention among the study group compared with the control group. Similar finding was reported by Elaskary **[19]** who reported that positive changes in the beliefs of patients in the intervention group in comparison to the control group for all HBM constructs except for cues to action.

The current study illustrated that there was statistical significant relation between total knowledge score and educational level in study group in post program phase. This finding is in contrast with Ikea, Aniebueb and Aniebuec **[25]** who showed that there was no statistical difference regarding knowledge score and educational level. The result of the study showed that there was statistical significant relation between total knowledge score and duration of disease in study group. Those individuals who had more years of illness showed a greater improvement in knowledge with their medical regimen after completion of the educational program. This could be explained as, with increasing duration of illness, the patients recognize that their disease is lifelong one and not curable. For these reasons, the patients' knowledge improved with duration of disease. This finding agrees with EDO **[26]** who found that there was a statistical significant relation between knowledge and duration of disease.

The result of the current study indicated that there was statistical significant relation between the patients' compliance and their age in study group in post program phase. This finding is in the same line with Ahmed **[21]** who reported that age of patients was a significantly correlated with compliance rate. These may be explained by the fact that older patients tend to be more scared of disease; consequently, they have fear of death than younger ones, so they comply with the medical regimen imposed by the disease. Also this contradiction may be explained as these studies included different age categories younger and older age. In addition, younger participants were more non-adherent to their treatment regimen possibly due to ignorance of the true nature of hypertension or denial of the existence of the disease.

According to relation between the patients' compliance and their income the current results showed that, there was statistical significant relation between the patients' compliance and their income in study group in post program phase. In investigator opinion this may be due to the patients with insufficient income can't adhere with therapeutic regimen because they don't have the money and cannot afford the cost of medications and transport costs to health centers. They often have to barter the pressing need to provide food for the family rather than procure medications. This finding was in accordance with Abd Allah **[27]** who found that financial support for patients improved their compliance.

According to relation between patients' compliance and HBM, the current results showed that, there was statistical significant relation between patients' compliance and perceived severity in study group in post phase. This finding matched with Mahrous **[28]** who also found that patients complying with their treatment depend on the degree to which they perceive themselves to be susceptible to the disease or its complications and their perceptions of the severity of the condition. This could be attributed to the persons who perceive hypertension to be a serious disease would be more compliant with medication and lifestyle modifications than those who do not hold this perception.

Concerning to relation between patients' compliance and HBM, the present study revealed that, there was statistical significant relation between patients' compliance and Self-Efficacy in study group in post phase. This finding was in accordance with Hu, Li & Arao **[29]** who found self-efficacy has been recognized as a major predictor of self-care behavior for chronic disease management as adopting healthy diet and regular exercise, reported better health status, and lower psychological distress. This may be due to the fact that most hypertension patients already know what actions they should take, such as weight loss, smoking cessation or participating in exercise activities, but knowledge is insufficient to stimulate actions. Patients need to believe in their capability and have confidence to perform the expected behaviour.

As regard to the relation between patients' knowledge and compliance, the present study revealed that there was positive correlation

between total knowledge & total compliance for patients in study group in post phase. This finding is supported by Al-Jbour, Abu Kamel & Barhoom **[30]** who stated that increasing patients' knowledge about the disease can achieve the goal of treatment, empower patients to make decision about their treatment, and can empower their motivation and intention to compliance with the treatment regimen. Also finding go in line with Duncan, Howe L, Manakusa & Purdy **[31]** who also found that Providing information about the disease and treatment regimen appears to be sufficient for compliance, and non-compliance is often attributed to inadequate knowledge.

While this finding was in contrast with Hassan **[32]**, Ahmed **[33]** who reported that there was no relation between knowledge about HTN and compliance with therapeutic treatment to control BP. This is may be justified by knowledge was not enough to achieve compliance and changing in lifestyle because knowledge is not the only component to achieve the goal, but also positive attitude and behaviors.

On summary, the results of this study support the hypothesis that the knowledge, compliance and practice scores of patients who received health beliefs model were higher than that of a control group. There was a significant improvement in knowledge, compliance and practice of patients with hypertension post and follow up phase of program in study group.

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

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Brucellosis mimic SLE

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Key words: Brucellosis, SLE, prozone phenomenon

INTRODUCTION

Brucellosis is an infectious disease with articular involvement. Brucellosis and rheumatologic disorders are difficult to discriminate between them in regions endemic to brucellosis.

The autoantibody level in patients with brucellosis has been discussed in a few studies. In one study showed autoimmune processes in the pathogenesis of brucellosis **[1]**.

The purpose of this paper is to report a case of brucellosis that presented with positive autoimmune markers while negative *Brucella* agglutination test. Also the effectiveness of prednisolone in an acute phase.

Case presentation:

18-year-old Saudi female patient not known to have any medical illness before brought by her family to the hospital complaining of fever and arthralgia for 10 days duration. She was not complaining of any medical illness till 10 days prior admission when she abruptly developed fever, the fever was subjective on and off relieving by paracetamol associated with rigors mostly in afternoon time relived spontaneously. Her fever was severe enough to prevent her going to institute. Associated with hair loss and loss of appetite. Not associated with night sweat, dyspnea, weight loss, headache, urinary tract or GI symptoms. She had a history of row milk ingestion. No previous history of same attack, not known to have any medical illness, she did one surgery five years prior admission which was appendectomy. She takes no medication currently except for paracetamol.She is single and lived in Makkah city with her Family, institute student, not smoker or alcoholic.

On examination at the presentation, she was ill-appearing, conscious, oriented, average body built, breathing smoothly, no jaundice, pallor or cyanosis.

Vital signs were temperature 39°C, blood pressure 95/55, heart rate:130, respiratory rate: 18, O2 saturation: 100% in room air.

The rest of examination was unremarkable.

Investigation:

Complete blood count shows anemia (hemoglobin: 9,4g/dl), WBC was 6.02 10^3/UI and platelets was 140 10^3/UI, coagulation profile was within normal limit but lactate dehydrogenase was elevated (LDH: 502 IU/L). Renal function tests and liver function tests were normal. Urinalysis was normal except for 24 hours urine collection was found to have proteinuria 333 mg.

Chest X-ray no obvious abnormality, ultrasound abdomen shows minimal free fluid in the pelvis.

Erythrocyte sedimentation rate was 22 mm/h, the C-reactive protein was within normal range.

Serology :

Brucella serology was negative also *Salmonella* and *Dengue* profile was negative, HIV and Hepatitis profile was negative.

Autoimmune markers :
Autoantibody profile including Antinuclear antibody (ANA) titer and Anti dsDNA titer were positive. C3 ,C4: within normal range.

Treatment :
After this laboratory result according to SLICC criteria the most likely the diagnosis was Systemic Lupus Erythematous (SLE).So we started the Prednisolone.At this time we have had already sent blood to culture. After that, she showed good clinical improvement on prednisolone and became a febrile and taking orally well. Two days after sending the blood culture, it became positive for gram-negative coccobacilli *Brucella melitensis. Brucella* titer requested again and it was negative. So we have a case of brucellosis with positive autoimmune markers. While *Brucella* titer is negative. We started antibiotics for brucellosis: doxycycline, streptomycin and rifampicin and we stopped prednisolone gradually.

Then we diagnosed the case as brucellosis and discharged home on oral antibiotics, and the she came in the clinic for follow up after two weeks where she is healthy.

DISCUSSION

In our case the final diagnosis was brucellosis in spite of implementing SLICC criteria for the diagnosis of SLE (proteinuria, arthritis, alopecia, ANA and Anti-Ds DNA high positive), also in another case published in 2014 found a close finding **[2]**. Also in another article, they found that it is possible to have a positive autoantibody markers in brucellosis **[3]**.

Prozone phenomenon is false negative agglutination test of *Brucella* **[4]**. So in our case, we found a false seronegative agglutination test of *Brucella* while there was culture positive for *Brucella*.

In this study, there was moderate proteinuria, proteinuria a common feature in brucellosis, but heavy proteinuria in brucellosis has rarely been reported **[5]**.

Corticosteroids should be considered in a patient has neurological symptoms due to brucellosis to reduce inflammation and improve neurologic outcome, like in our case prednisolone was started when the patient had severe symptoms then she improved dramatically.

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Role of Zinc Deficiency in Development of Minimal Hepatic Encephalopathy among HCV Induced Compensated Cirrhotic Patients

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Key words:
Minimal hepatic encephalopathy, zinc deficiency and zinc supplementation

Background and study aim: Minimal hepatic encephalopathy is a term used to describe cirrhotic patients who are clinically normal but show abnormalities of neurophysiological variables and/or cognition. Implicated theories of pathogenesis are similar to those of overt hepatic encephalopathy. Hypozincaemia was studied as a theory for the pathogenesis of overt hepatic encephalopathy. The aim of the present study is to evaluate the role of hypozincaemia in development of minimal hepatic encephalopathy among HCV induced compensated cirrhotic patients.

Patients and Methods: 30 patients with HCV induced compensated liver cirrhosis with minimal hepatic encephalopathy were studied versus 30 patients with HCV induced compensated liver cirrhosis without minimal hepatic encephalopathy. Diagnosis of minimal hepatic encephalopathy was based on positivity of both Number Connection Tests A and B. Serum zinc was evaluated for all subjects. Zinc deficient minimal hepatic encephalopathy patients were supplemented with zinc sulphate heptahydrate 220 mg daily for 1 month before HCV treatment and scores of Number Connection Tests A and B were redetermined.

Results: Serum zinc is significantly lower in patients with minimal hepatic encephalopathy than in those without minimal hepatic encephalopathy. Scores of Number Connection Tests A and B among zinc deficient patients with minimal hepatic encephalopathy were significantly improved after zinc supplementation.

Conclusion: Zinc deficiency may play a role in development of minimal hepatic encephalopathy among patients with HCV induced compensated liver cirrhosis. Zinc supplementation is recommended for the treatment of minimal hepatic encephalopathy among those patients.

INTRODUCTION

Zinc is an important trace element and is a constituent of many metal-protein complexes such as metalloenzymes. It has a pivotal role in the regulation of protein metabolism, immune responsiveness and membrane integrity. A group of disorders have been reported in patients with chronic liver disease which may reflect hypozincaemia. These abnormalities include taste and smell abnormalities, photoreceptor dysfunction, immune dysfunction and hypogonadism **[1]**. Hypozincaemia affects nitrogen metabolism by impairing the activity of urea cycle enzymes in liver **[2]** and by reducing the activity of glutamine synthetase in muscles **[3]**. It has also been reported that hypozincaemia may have a role in the pathogenesis of overt hepatic encephalopathy as serum zinc concentrations are decreased in patients with this condition and are inversely correlated with arterial concentrations of blood ammonia **[4]**.

Minimal hepatic encephalopathy is a term used to describe cirrhotic patients who are clinically normal but show abnormalities of neurophysiological variables and/or cognition **[5]**. It negatively affects health related quality of life and the ability to perform complex tasks such as driving **[6]**. It also increases the risk of developing overt hepatic encephalopathy **[7]**.

Impaired psychometric performance defines the presence of minimal hepatic

encephalopathy in cirrhotic patients who appear clinically normal. Patients with minimal hepatic encephalopathy show deficits in attention, fine motor skills and working memory while other cognitive abilities are relatively preserved. Patients with overt hepatic encephalopathy show additional disturbances in psychomotor speed, executive function and concentration **[8]**.

Promising results have been obtained using a group of paper and pencil neuropsychological tests; namely Number connection tests A and B, Figure connection test, Line racing test, Circle dotting, Serial-dotting test and Digit symbol test. This battery of tests is called the Psychometric Hepatic Encephalopathy Score (PHES) and is used to assess the required domains of attention, concentration, memory, visual perception, visuospatial orientation and visuoconstructive abilities. They are easily applicable and have been shown to have a high specificity for the diagnosis of minimal hepatic encephalopathy **[5,8]**.

PATIENTS AND METHODS

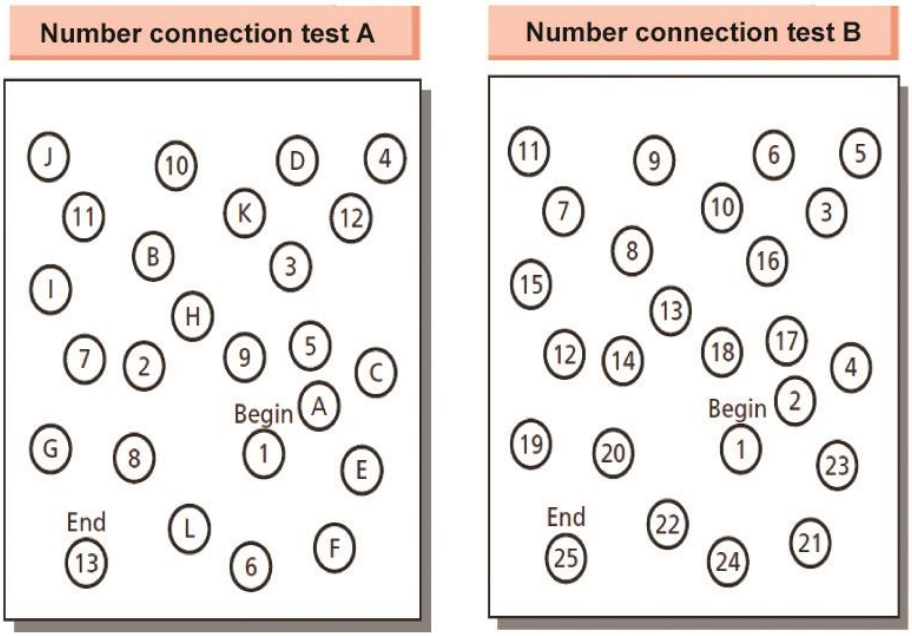
This case-control study was conducted in Tropical Medicine and Clinical Pathology departments, Zagazig University hospitals in the period from January to June, 2017. Cases and controls were recruited from the HCV treatment unit affiliated to Tropical Medicine Department, Zagazig University hospitals. The study included 60 patients classified into two groups; first group (GI) of 30 patients with HCV induced compensated liver cirrhosis with minimal hepatic encephalopathy and second group (GII) of 30 patients with HCV induced compensated liver cirrhosis without minimal hepatic encephalopathy. Categorization of patients into either group was based upon the scores of both Number Connection Tests A and B. Positivity of both tests defines MHE. All participants gave a written consent to be included in the study.

All compensated cirrhotic patients in G I and G II had no evident clinical abnormalities, were on average dietary constitution with no intentional protein restrictions, had no constipation and not intentionally taking medical, herbal nor dietary laxatives and were not taking diuretics.

Patients with history of overt hepatic encephalopathy, those taking alcohol, those with history of a neurologic disease (eg, previous stroke), those with extremes of BMI and those with history of diuretic intake within one week prior to estimation of their serum zinc were excluded from the study.

All study participants were subjected to complete history taking and clinical examination. Routine laboratory investigations in the form of CBC, LFT, PT and INR and pelviabdominal ultrasonography were done for all study participants. Diagnosis of liver cirrhosis was based upon laboratory parameters (low platelet count, elevated AST/ALT ratio) and sonographic parameters (small right lobe, enlarged caudate lobe, undulated surface, coarse echopattern, irregular hepatic veins, dilated portal vein, collaterals and splenomegaly) **[9]**. Diagnosis of compensation was based upon clinical (absent symptoms and signs of liver disease), laboratory (normal serum albumin, bilirubin and prothrombin concentrations) and sonographic (absent ascites) grounds.

Two psychometeric tests (Number Connection Tests A and B) were applied to all cirrhotic patients included in the study to detect minimal hepatic encephalopathy and to give a score for each patient **[10]**. Minimal hepatic encephalopathy among cirrhotic patients was diagnosed by positivity of both Number connection tests A and B; both of which are tests of visuo-spatial orientation and psychomotor speed. The subject is given a sheet of paper with 25 circles which are randomly spread over the paper. In NCT-A, the task is to connect the circles 1-A-2-B-3-C and so on until L-13 as quick as possible. In NCT-B, the task is to connect circles 1 through 25 as quick as possible. Numbers and letters are written in arabic and the patient is shown a demonstration. Test result is the time needed by the patient to complete the test including error correction time. In this work, NCT is considered abnormal when the time taken by the patient to complete the test is greater than mean + 2SD from that of healthy controls (more than 47 seconds).



Serum zinc level estimation was performed for all study individuals by colorimetric method using commercially available kit (zinc assay kit, Segma-Aldrich, Germany). Minimum of two ml of venous blood was collected by venipuncture on a sterile red-capped vacutainer (Becton Dickinson Laboratories, Franklin Lakes, NJ, USA). Blood was incubated at 37°C for 10-20 min till complete clot formation. Serum was separated in blood samples by centrifugation at 1800–2000 rpm for 15 minutes. Serum was deproteinized using 7% TCAA. Aliquots from supernatant were stored at −70°C for later analysis. Normal serum zinc is 80-150 µg/dL. Zinc deficiency was defined when serum zinc concentration <80 µg/dL.

Zinc deficient patients with HCV induced compensated liver cirrhosis and minimal hepatic encephalopathy were supplemented with zinc sulphate heptahydrate 220 mg/day equivalent to 50 mg/day of elemental zinc divided into two doses for one month before HCV treatment and Number Connection tests A and B were repeated and test scores were compared with those before zinc supplementation.

Data were checked, entered and analyzed using SPSS version 19 for data processing and statistics. Continuous data were expressed as mean ± SD and categorical data were expressed as number (percentage). Continuous data were checked for normality by using Kolmogorov-Smirnov test. Student t-test was used to compare two groups of normally distributed continuous data and Mann-Whitney U (MW) test was used to compare two groups of non-normally distributed continuous data. Categorical data were compared using the Chi-square ($\chi 2$) test. For the previously mentioned statistical tests, the threshold of significance was fixed at 5 % level (P value). P value of >0.05 indicates non-significant results. P value of <0.05 indicates significant results. P value of <0.001 indicates highly significant results. The smaller the P value obtained, the more significant are the results.

RESULTS

Table (1): Baseline characteristics of the studied groups

	G1 Patients with MHE	G2 Patients without MHE	P value	Significance
Age	54.5 ± 9.6	55.8 ± 9.2	0.6	NS
Sex (M / F)	19 / 11	18 / 12	0.92	NS
BMI	27.1 ± 3.5	26.7 ± 2.8	0.61	NS
Hb (gm / dL)	12.8 ± 2.7	13.1 ± 2.5	0.77	NS
WBC (x 10^3 cells/ mL)	6.3 ± 1.8	6.1 ± 1.9	0.71	NS
Plt (x 10^3 cells/ mL)	92 ± 37	85 ± 40	0.8	NS
ALT (U / liter)	69 ± 20	68 ± 18	0.82	NS
AST (U / liter)	70 ± 22	67 ± 20	0.65	NS
Albumin (g / dL)	3.4 ± 0.4	3.7 ± 0.4	0.11	NS
Bilirubin (mg / dL)	1.38 ± 0.4	1.4 ± 0.4	0.89	NS
Prothrombin time (seconds)	12.5 ± 1.6	12.1 ± 1.5	0.85	NS
Liver span (cm)	16.4 ± 2.3	16.5 ± 2.1	0.9	NS
Splenic axis (cm)	15.1 ± 3.5	14.8 ± 3.1	0.78	NS
Portal vein diameter (mm)	13.6 ± 0.8	13.5 ± 0.7	0.9	NS
Portosystemic collaterals	18 / 30 (60 %)	5 / 30 (16.7 %)	0.04	S
NCT-A score (seconds)	70 ± 5	41 ± 6	< 0.001	HS
NCT-B score (seconds)	64 ± 11	35 ± 10	< 0.001	HS
Serum zinc (mcg / dL)	66 ± 5	85 ± 4	< 0.001	HS

Table (2): Number of zinc deficient patients in both groups

	G1 Patients with MHE (30 patients)	G2 Patients without MHE (30 patients)	P value	Significance
Low serum zinc	23 / 30 (76.7 %)	4 / 30 (13.3 %)	< 0.001	HS
Normal serum zinc	7 / 30 (23.3 %)	26 / 30 (86.7 %)	< 0.001	HS

Table (3): Number Connection Test results before and after zinc supplementation in zinc deficient patients (23 patients) with HCV induced compensated liver cirrhosis and minimal hepatic encephalopathy

NCT	Score before zinc supplementation (seconds)	Score after zinc supplementation (seconds)	P value	Significance
A	69 ± 4	50 ± 5	< 0.001	HS
B	60 ± 7	45 ± 6	< 0.001	HS

DISCUSSION

Minimal hepatic encephalopathy is not uncommon complication of liver cirrhosis. Dhiman et al. **[11]** reported that the prevalence of minimal hepatic encephalopathy among compensated cirrhotic patients varied between 22% and 74% depending on the time and the number of psychometric tests used and the severity of the liver disease.

Many studies proposed the role of zinc deficiency in the pathogenesis of overt and minimal hepatic encephalopathy **[12,13,14]**. Zinc acts as a coenzyme for urea cycle enzymes and may be deficient in patients with liver cirrhosis especially if associated with protein malnutrition, maldigestion and malabsorption **[2]**.

Previous studies assessing the role of zinc deficiency on development of minimal hepatic encephalopathy were performed on patients not specifically complicating chronic HCV infection and included cirrhotic patients of all grades of liver cirrhosis. The aim of the present work is to study the role of zinc deficiency in development of minimal hepatic encephalopathy among a group of patients with HCV induced compensated liver cirrhosis to assess non-traditional factors of the pathogenesis helping to give a chance for a new line for treatment of such a problem which negatively affects the quality of life of cirrhotic patients and puts them at risk of accidents and of developing overt hepatic encephalopathy.

In this study, there was a highly significant statistical difference between both studied groups as regard scores of Number Connection Tests A and B. This reflects that selection of patients in either group was based upon scores of both tests. We adopted positivity of both tests to define MHE. Weissenborn et al. **[15]** reported that combination of both NCT A and B is a sensitive method for diagnosis of early HE.

Mean age of patients with MHE is 54.5 years compared with a mean age of 55.8 years for patients without MHE with a non-significant difference between both means. This result indicates the fact that age is not an included risk factor for the development of MHE.

In this study, 19 patients with MHE were males and 11 were females while 18 patients without MHE were males and 12 were females. This non-significant difference in male-to-female ratio between both groups indicates that gender is not an included risk factor for the development of MHE. Male-to-female ratio of HCV induced liver cirrhosis is approximately 1.5:1. This is in agreement with Abd El Wahab et al. **[16]** who stated a ratio of 1.25:1. These figures reflect the fact that males are more commonly exposed to infection than females and to the fact that females may clear viremia better than males **[17]**.

As regard to routine laboratory parameters, there was a non-significant difference between both groups as regard CBC and liver function tests. Serum albumin was higher in the group of HCV induced compensated liver cirrhosis without MHE compared with those with MHE but the statistical difference did not reach the level of significance. The relative hypoalbuminemia among patients with MHE reflects protein malnutrition among these patients. This is in agreement with Van der Rijt et al. **[18]** who reported a similar state of protein malnutrition among a group of patients with overt HE.

As regard to sonographic parameters, there was a non-significant difference between both groups as regard liver span, splenic axis and portal vein diameter. This non-significant difference can be explained by the fact that patients in both groups were HCV induced compensated liver cirrhotic patients. As regard to portosystemic collaterals, there was a significant difference between both

groups being more common among the group of MHE. This finding is in agreement with Tarantino et al. **[19]** who reported a strong relationship between portosystemic collaterals and ammoniacal encephalopathy.

The difference in both serum zinc concentration and number of zinc deficient patients between the two groups are statistically highly significant. Serum zinc concentration in the group of compensated liver cirrhosis with MHE is lower than that in the group of compensated liver cirrhosis without MHE (mean 66 µg/dL vs 85 µg/dL). Number of zinc deficient patients in the group of compensated liver cirrhosis with MHE is higher than that in the group of compensated liver cirrhosis without MHE (23/30 vs 4/30).

Postulated pathogenetic mechanisms of hypozincaemia in a sector of cirrhotic patients include inadequate intake of zinc-rich protein diet, alcohol-induced malabsorption and the influence of cytokines espicially interleukin-6 which is well known to decrease zinc metabolism **[20]**. Increase urinary zinc excretion in patients with cirrhosis seems related to the impairment of albumin synthesis. The decrease in serum albumin and the increase of free amino acid concentration in cirrhosis cause displacement of zinc bound from the macromolecular ligand that result in the increase of zinc filtration in the renal glomerulus **[21]**.

In 2004, Yang et al. **[13]** studied the role of hypozincaemia on development of subclinical hepatic encephalopathy among 20 non-alcoholic cirrhotic patients most of them were HBV induced and some of them had decompensated liver disease. Their findings agree with the results of the present study namely the presence of a correlation between hypozincaemia and development of MHE among HCV induced compensated cirrhotic patients.

On supplementing zinc-deficient minimal hepatic encephalopathy patients with zinc sulphate heptahydrate 220 mg/day (equivalent to 50 mg / day elemental zinc), mean scores of NCT A and B improved with a highly significant statistical difference. This finding is similar to that obtained by Mousa et al. **[22]** who supplemented a group of compensated and decompensated liver cirrhotic patients with zinc and antioxidants for 3 months and found a significant improvement of scores of psychometric tests they adopted after supplementation.

In conclusion, the lowered serum zinc among HCV induced compensated cirrhotic patients with minimal hepatic encephalopathy and the improvement of scores of NCT A and B of zinc deficient patients after zinc supplementation suggest a role of hypozincaemia in development of minimal hepatic encephalopathy.

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

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Study of Frequency of Prediabetes in Patients with Chronic Hepatitis C Virus Infection

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Key words: hepatitis C virus, Homeostasis Model Assessment of Insulin Resistance, insulin resistance

Background and study aim: Egypt has the highest prevalence of hepatitis C virus (HCV) infection in the world and is facing an epidemic of type 2 diabetes mellitus. The objective of this study was to assess the frequency of prediabetes in patients with chronic HCV infection.

Subjects and Methods: A cross-sectional study was performed on 60 HCV patients. Twenty healthy persons were taken as controls. Full history taking, clinical examination, routine laboratory and radiological investigations were done Body mass index (BMI), Waist Hip ratio, serum fasting glucose and fasting insulin were determined. IR was calculated by the Homeostasis Model for Assessment of Insulin Resistance (HOMA-IR), where values less than 2 has been considered completely normal and higher than 2 as a prediabetic state.

Results: Serum fasting glucose, fasting insulin and HOMA-IR levels were significantly higher among HCV group compared with control group. The frequency of prediabetic (HOMA-IR values >2) among non-diabetic chronic HCV patients is 63.33%.

Conclusion: Chronic HCV patients should be screened regularly for insulin resistance to avoid the double burden of diabetes mellitus and HCV.

INTRODUCTION

Hepatitis C virus (HCV) infection is one the main causes of chronic liver disease worldwide. The number of chronically infected persons worldwide is estimated to be about 160 million, but most are unaware of their infection **[1]**. Approximately 700,000 persons die each year from HCV related complications, which include cirrhosis, hepatoceullar carcinoma (HCC) and liver failure **[2]**. The most recent epidemiological survey done in Egypt revealed that 10% of the population had HCV antibody and 7% had positive HCV-RNA **[3]**.

Number of studies have demonstrated a strong association between HCV infection and insulin resistance (IR), providing a possible link between this infection and diabetes mellitus **[4]**. Prediabetes mellitus is defined as a state of abnormal glucose homeostasis in which deficiency or resistance to insulin is the hallmark. Prediabetes mellitus precedes the development of overt type 2 diabetes mellitus (T2DM). It is associated with increased mortality and morbidity, and thus fits well with the criteria of a disease condition **[5]**. The gold standard for the assessment of IR is the euglycemic hyperinsulinemic clamp technique. Another more practicable and also well-accepted method of measuring systemic IR is the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR). It is calculated by the following formula: fasting glucose (mmol/L) × fasting insulin (μU/L)/22.5 **[6]**. In previous reports, HOMA less than 2 has been considered 'completely' normal and higher than 2 as prediabetic state **[7]**. The progression of prediabetes to T2DM has been examined in a number of populations with varying results. In general, epidemiological studies indicate

that approximately 25% of individuals progress to T2DM in 5 years, whereas about 50% remain prediabetic and about 25% revert to normal **[8]**. The aim of this study was to assess the frequency of prediabetes assessed by (HOMA-IR) in patients with chronic HCV infection.

SUBJECTS AND METHODS

Subjects :

This study was conducted on 80 subjects attending or admitted to the Department of Hepatology, Gastroenterology and Infectious Diseases and outpatient clinic at Benha University hospital during the period from May 2016 to April 2017 after approval of Benha University ethical committee. They were classified into 3 groups. Group I: included thirty patients with chronic HCV with no evidence of cirrhosis, group II: included thirty patients with HCV related cirrhosis and group III: twenty apparently healthy volunteers of matched age, sex and BMI were included as control group. **Exclusion criteria** included clinical evidence of diabetes mellitus, family history of T2DM, non HCV related liver disease, e.g (HBV, alcohol abuse, hepatic schistosomiasis, autoimmune hepatitis, hemochromatosis, Wilson disease, hepatocellular carcinoma, any endocrinal disorder, diabetogenic drugs e.g. (steroids), severe systemic diseases (cardiac, respiratory or renal diseases), obese patients (BMI>30) & waist hip ratio (for men above 0.9 and for women above 0.85).

Materials :

All the patients and controls were subjected to full history taking, complete clinical examination including (1) BMI: measured as weight in Kg/ height m^2 and categorized following World Health Organization classifications. BMI≤ 18.5: underweight & BMI of 18.5-24.9: healthy range & BMI of 25-29.9: overweight & BMI ≥30: obese **[2]**. (2) Waist Hip Ratio (WHR):- measured as waist circumference (measured the circumference of the waist at its smallest points)/ hip circumference (measured the circumference of the hips at their widest point). World Health Organization stated that abdominal obesity is defined as a WHR above 0.9 for males and above 0.85 for females **[2]**.

The following laboratory investigations were done:

Sampling:

Six milliliters of venous blood were withdrawn under aseptic precautions after fasting for 10 -12 hours and distributed as follows:

a- 2 milliliters whole blood was put in EDTA vacutainer (violet cap) and mixed up & down gently which was used to measure CBC.

b- 2 ml on Na Fluoride serum test tubes, centrifuged at (1500 rpm for 10 minutes). The separated serum is used for the assay of fasting blood sugar.

c- 2 plain test tubes without anticoagulant. The plain test tubes were left till coagulation. After coagulation, samples were centrifuged (at 1500 rpm for 15 minutes). The separated serum was used for the assay of Bilirubin, albumin and Insulin.

Routine Laboratory Investigations:-

1. **CBC** was done for all samples using a fully automated cell counter**, Mythic 18 (Orphee) from Switzerland.**
2. **Liver Function Tests:** Alanine Transaminase, Aspartate Transaminase applying kinetic method, serum albumin, Prothrombine time and activity, Alkaline phosphatase- Bilirubin (total and direct).
3. **Fasting blood sugar** applying glucose enzymatic colorimetric method All biochemical tests were done using Biosystem A15 auto-analyzer

Specific Laboratory Investigations :

1- **Fasting insulin level :** using ELISA kit supplied from BioTina GmbH. Alter Weg 18, 79112 Freiburg, Germany according to manufacturers' instructions.

2- **HOMA- IR** (Homeostasis Model Assessment of Insulin Resistance) it was calculated using the equation HOMA-IR = fasting glucose (mmol/L) x fasting insulin (μU/L)/22.5. Cutoff point to define insulin resistance is ≥ 2.

-- **Child–Pugh score**: To assess the degree of hepatic decompensation of the patients using biochemical measures (serum bilirubin, INR and serum albumin level) and clinical measures (ascites and hepatic encephalopathy) **(Pugh et al., 1973)**

Statistical analysis :

The statistical analysis was conducted using STATA/SE version 11.2 for Windows (STATA corporation, College Station, Texas). The collected data were summarized in terms of mean± Standard Deviation (SD) and range for quantitative data and frequency and percentage for qualitative data. Comparisons between the different study groups were carried out using the Chi-square test (χ^2) and Fisher Exact test (FET) to compare proportions as appropriate. The Student t-test (t)

was used to detect difference in the mean between two parametric data, while the Mann-Whitney test (z) was used to compare two non-parametric data. Oneway Analysis Of Variance (ANOVA; F) and the Kruskal Wallis test (χ^2) were used to compare differences between more than two groups regarding parametric and non-parametric data respectively, followed by post-hoc test using the Bonferroni method to detect differences in pairs. The person correlation coefficient (r) and the Spearman Correlation coefficient (rho; ρ) were used to test for the correlation between estimated parameters as appropriate.

RESULTS

Table 1 showed that there was no significant difference between the studied groups regarding age, sex, BMI and waist Hip ratio (P =1.00, 0.43, 0.46, 0.14, respectively). The mean value of fasting blood glucose was significantly higher in both of group I (4.9±0.5) and group II (5±0.5) than controls (4.7±0.3) mmol/L with P value (0.04). The mean value of fasting insulin level was significantly higher in both of group I (12.71±7.89) and group II (12.86±7.71) than controls (mean=4.12±1.48) with P value (<0.001). The mean value of HOMA-IR was significantly higher in both of group I (2.94±1.81) and group II (2.9±1.81) than controls (0.28-1.41) with P value (<0.001). Table 3 shows that pre diabetes was significantly higher in both group I (63.33%) and group II (63.33%) than controls (0.00%) (P<0.001). Child–Pugh score classification of group II showed that 15 patients (50%) were Child A, 6 patients (20%) were Child B and 9 patients (30%) were Child C. Table 4 showed that there was no significant difference between Child score classification of group II regarding fasting glucose level, fasting insulin and HOMA-IR (P = 0.5, 0.36 and 0.41, respectively).

Table (1): Distribution of the studied groups regarding their sex, age, BMI and Waist/Hip ratio

Variable		Group I (no.=30)		Group II (no.=30)		Group III (no.=20)		X^2	P
		No	%	No	%	No	%		
Sex	Female	15	50.0	15	50.0	10	50.0	0.00	1.00
	Male	15	50.0	15	50.0	10	50.0		
Age (yrs)	Mean ±SD; (range)	44.4±9.11; (28-59)		46.57±8.82; (28-59)		43.2±10.3; (24-59)		F= 0.86	0.43
BMI (kg/m^2)		25.57±1.53	23-29	25.92±1.67	23.5-29.3	26.13±1.71	23.8-29.7	0.77	0.46
Waist/Hip ratio		0.81±0.02	0.79-0.89	0.82±0.03	0.79-0.89	0.83±0.03	0.79-0.89	2.00	0.14

X^2: Chi-square test
F: Oneway Analysis of Variance (ANOVA)
FET: Fisher Exact Test
P: Probability

Table (2): Distribution of the studied groups regarding fasting blood sugar, fasting insulin level and HOMA-IR

Variable	Group I (no.=30)		Group II (no.=30)		Group III (no.=20)		F	P
	Mean ±SD	Range	Mean ±SD	Range	Mean ±SD	Range		
FBS (mmol/L)	4.9±0.5	3.9-6	5±0.5	3.9-5.9	†‡4.7±0.3	3.9-5.2	3.40	0.04 (S)
Insulin (IU/ml)	12.71±7.89	1.4-26.5	12.86±7.71	1.63-26.5	†‡4.12±1.48	1.46-6.45	12.15	<0.001 (HS)
HOMA-IR	2.94±1.81	0.29-5.91	2.9±1.81	0.28-5.9	†‡0.85±0.33	0.28-1.41	12.82	<0.001 (HS)

F: One way Analysis Of Variance (ANOVA)
S: Significant (P<0.05)
HS: Highly Significantly (P<0.001)
†: Significant differences compared to Group I
‡: Significant differences compared to Group II

Table (3): Frequency of prediabetes among the studied groups

Prediabetes (HOMA-IR>2)	Group I (no.=30)		Group II (no.=30)		Group III (no.=20)		X^2	P
	No	%	No	%	No	%		
Yes	19	63.33	19	63.33	0	0.0	24.13	<0.001 (HS)
No	11	36.67	11	36.67	20	100		

X^2: Chi-square test

Table (4): Correlation between Child score & FBS, fasting insulin and HOMA-IR in group II

variable	Child A (no.=15)		Child B (no.=6)		Child C (no.=9)		F	P
	Mean ±SD	Range	Mean ±SD	Range	Mean ±SD	Range		
FBS (mg/dl)	92.53±9.64	71-105	89.5±11.64	77-106	87.89±7.97	75-99	0.70	0.5
Insulin (IU/ml)	11.77±7.52	2-23	10.97±7.3	3.1-21	15.95±8.21	1.63-26.5	1.06	0.36
HOMA-IR	2.71±1.84	0.4-5.9	2.37±1.58	0.68-4.24	3.56±1.9	0.28-5.88	0.92	0.41

F: One way Analysis Of Variance (ANOVA)

DISCUSSION

Hepatitis C virus (HCV) infection is one the main causes of chronic liver disease worldwide. The number of chronically infected persons worldwide is estimated to be about 160 million, but most are unaware of their infection **[1]**. Since HCV has been discovered in late 1980, chronic hepatitis C (CHC) has become a complex multifaceted disease with several extra hepatic manifestations **[9]**.

Numbers of studies have demonstrated a strong association between HCV infection and insulin resistance (IR), providing possible link between this infection and diabetes mellitus **[4]**. IR is strongly connected with chronic hepatitis C (CHC) and IR can be developed early in the course of CHC **[10]**. Type II diabetes mellitus (T2DM) is more common in patients with chronic HCV than in the overall public and chronic hepatitis B patients **[11]**. IR has a major role in development of T2DM and it is the best predictor for the development of T2DM, and it assumes an essential part in development of T2DM **[12]**. HCV advances the progression of IR directly by affecting insulin signaling pathway at the cellular level. In addition, IR may play a role in the progression of the liver disease. In euglycemic individuals the estimation of HOMA-IR level helps to quantify IR **[13]**. Diabetes can adversely affect the course of CHC **[14]**. In this study we aimed to assess the frequency of prediabetes assessed by (HOMA-IR) in patients with chronic HCV infection.

In this study, statistical analysis revealed significant difference between the studied groups as regard fasting blood glucose; it was higher in both of group I and group II than controls, and there was no significant difference between group I and group II. In addition, in this study statistical analysis revealed significant difference between the studied groups as regard fasting insulin level; it was higher in both of group I and group II than controls and there was no significant difference between group I and group II. In addition, statistical analysis revealed significant difference between the studied groups as regard HOMA-IR; it was higher in both of group I and group II than controls and there was no statistically significant difference between group I and group II. This was in agreement with Desouky et al. **[15]** & Ali et al. **[16]** & Souza et al. **[17]** and Jason et al. **[18]** who had demonstrated a strong association between HCV and IR and proved that IR has a high prevalence among patients of chronic HCV and found that HCV patients had significantly higher levels of all markers of IR, including fasting glucose, fasting insulin and HOMA-IR**.**

In this study, 63.33% of group I were prediabetics (HOMA-IR>2) and also 63.33% of group II were prediabetics while none of the control group was prediabetic, so the frequency of prediabetes

among non-diabetic chronic HCV patients was 63.33%. This high prevalence is consistent with Desouky et al. **[15]** & Ali et al. **[16]** and Delgado-Berrego et al. **[19]** who found that the prevalence of prediabetes among HCV patients was (63.8%, 64% and 62% respectively), however it was higher than a previous Pakistani study where 51% of their chronic HCV patients had IR **[20]**.

IR occurs in HCV patients through different mechanisms. One of these mechanisms is interfering with insulin signaling pathway in hepatocytes and increasing the inflammatory response with production of cytokines such as TNF-a and interleukin 6 and increasing oxidative stress **[21]**.

HCV infection also promotes the expression of glucose 6 phosphatase (G6P) and phosphor-enolpyruvate carboxykinase 2 (PCK2) leading to increased glucose production and enhancement of IR **[22]**. Another mechanism which is triggered by HCV is down regulation of the expression of glucose transporter 4 (GLUT4), which is necessary for the uptake of glucose. Thus, glucose uptake is decreased leading to an increase in plasma glucose and the development of IR**.** Another explanation of IR could be the expression of HCV core protein, which initiates IR through alterations in signaling in the insulin receptor substrate-1 pathway **[23]**.

This study also showed that there was no significant difference between Child score classification of group II regarding fasting glucose level, fasting insulin and HOMA-IR and this ensured that in this study IR was not connected with the severity of liver disease. This is consistent with Ali et al. **[16]** & Mohamed et al. **[24]** and Li-fen et al. **[25]** and Hui et al. **[26]** who demonstrated that prediabetes in HCV is not connected with the severity of liver disease, and no correlation between liver fibrosis and HOMA-IR values and IR is a direct vial feature. It agreed also with Lecube et al. **[27]** who studied groups of patients with CHC and chronic hepatitis B matched by age, sex, BMI and fibrosis stage, HOMA index was found higher in hepatitis C patients.

On the other hand these results disagreed with Jason et al. **[18]** who assessed the extent of IR in relation to the severity of liver disease and hepatic fibrosis and found that, increased HOMA-IR values was associated with a higher rate of fibrosis progression and more advanced stages of hepatic fibrosis. Also theses results disagreed with Furutani et al. **[28]** who concluded that IR was connected with impaired glucose tolerance and the severity of the liver disease in non-diabetic patients with HCV infection.

CONCLUSION

This study showed that the frequency of prediabetes among non-diabetic chronic HCV patients was 63.33%. In addition, prediabetes in chronic HCV patients was not connected with the severity of liver disease and insulin resistance is a direct viral feature. Also this study concluded that HCV patients should be assessed for IR and prediabetes in their routine evaluation, to avoid the double burden of DM and HCV.

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

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Relationship between Interleukin 12 Levels and Suppressed CD4 Counts in HIV Patients

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Key words:
Human immunodeficiency virus, CD4 T cells, Interleukin 12, Highly active antiretroviral therapy.

Background and study aim: Interleukin 12 (IL-12) increases T cell proliferation, elevates natural killer (NK) and cytotoxic T cell activity, and induces the production of interferon gamma (IFN-γ). The aim of this study was to assess the potential relation of serum interleukin 12 levels in the suppression of CD4+ T-cell count in HIV patient in spite of low viral load after Highly Active Anti-Retroviral Therapy (HAART).

Patients and Methods: Thirty sero-positive HIV male patients were selected with low viral load after HAART. They were divided into two groups according to their immunological response. The first group included 15 male patients with low CD4 counts. The second group included 15 male patients with high CD4 counts. All patients were investigated for complete blood count (CBC), liver function test (LFT), kidney profile (KP), estimation of the levels of TNF-α, IFN-γ, IL-10, IL-12.

Results: Serum levels of IL-12 were significantly higher in Group II than in Group I (mean±SD IL-12 levels of 11.91± 2.8 versus 6.9±2.9, $p<0.05$). The serum levels of IL-12 were positive correlated with CD4 counts ($r = 0.514$; $p<0.05$). Similarly, a positive correlation between IL-12 levels and IFN-γ levels were noted ($r=0.602$, $p<0.01$). No significant correlations were observed between IL-12 levels and viral loads and also, no correlation between serum levels of IL-12 and serum levels of IL-10 and TNF-α.

Conclusion: Reduction of the levels of IL-12 in immunologic non-responders HIV-infected patients may play a role in impairment of immunological recovery following HAART. Although, we found that IL12 production was correlated with IFN-γ, the mechanism by which the reduced production of IL-12 in immunologic non-responders HIV-1-infected patients remains poorly understood.

INTRODUCTION

Human immunodeficiency virus (HIV) is a lentivirus (a member of the retrovirus family) that results in *acquired immunodeficiency syndrome* (AIDS) **[1]**. HIV primarily infects vital cells in the immune system of human such as helper T cells ($CD4^+$ T cells), dendritic cells and macrophages. Cell-mediated immunity is impaired, and the body becomes progressively more susceptible to opportunistic infections When CD4+ T cell numbers decline below a critical level **[2]**.

Highly Active Anti-Retroviral Therapy (HAART) keeps the levels of HIV in the body at a low level, so that the immune system is able to recover and work effectively. HAART in HIV-1-infected patients has a broad spectrum of clinical outcomes. In the majority of patients, CD4+ T-cells increase over time and the plasma viral load becomes undetectable. However, in a number of subjects, a discrepancy between CD4+ T-cell recovery and plasma viral load is noted. CD4+ T-cell count can increase despite persistently detectable plasma viral load (virologic non-responders) which occurs in 7–15% of the patients **[3]**, or conversely, CD4+ T-cell numbers do not rise despite plasma viral load suppression (immunologic non-responders) **[4]**. It is noted that 7%-20% of patients receiving

Long-term HAART are "immunologic non-responders," **[5]**, i.e. patients who fail to achieve a CD4+ T cell count above 200 cells/µl at 6, 12, 18, 24 months of HAART **[5]**.

Interleukin (IL) 12 was initially described as a cytotoxic lymphocyte maturation factor and a NK cell stimulatory factor **[6]**. It was identified by G. Trinchieri et al. **[7]**, as a heterodimer consisted of p35 and p40 subunits, which, when combined together form the bioactive IL-12p70 **[8]**. This cytokine increases T cell proliferation, elevates natural killer (NK) and cytotoxic T cell activity, and induces the production of interferon gamma (IFN-γ) **[6,9]**. In the murine model, administration of IL-12 up-regulates NK cell activity, elevates the serum IFN-γ level, and causes a shift toward a T-helper 1 (Thl) response to specific pathogens and antigens **[6]**. Many studies demonstrated that the macrophages, monocytoid lineage and myeloid dendritic cells are the primary and the physiological sources of IL-12 in response to a large variety of infectious agents **[9]**. It was reported that there is an impaired of IL-I2 production in HIV-infected patients, and addition of exogenous IL-12 in vitro can restore HIV-specific cell-mediated immune responses in HIV-positive persons **[10]**.

Therefore, this study was conducted to assess the potential relation of serum interleukin 12 levels in the suppression of CD4+ T-cell count in HIV patient in spite of low viral load after Highly Active Anti-Retroviral Therapy.

PATIENTS AND METHODS

This study was conducted between November 2014 and April 2016, at the Infectious Disease Hospital (IDH), Kuwait. In this study, the patients were diagnosed as seropositive HIV and two years prior to the study were started on HAART in the form of combination of three or more anti-HIV medications from at least two different classes [non-nucleoside reverse transcriptase inhibitors (NNRTIs), nucleoside reverse transcriptase inhibitors (NRTIs), or protease inhibitors (PIs)]. All patients received HAART regimen according to individual needs. Of 148 patients who were on HAART for more than two years, 118 were excluded due to any opportunistic infections, co-infection with viral hepatitis, advanced age, advanced stage of the disease, diabetic state, female sex and history of previous treatment interruption. Thirty patients were selected with low viral load after HAART and divided into two groups. The first group (group I) included 15 male patients with low CD4 count. The second group (group II) included 15 male patients with high CD4 count. All patients were subjected to full history taking, thorough clinical examination, body mass index (BMI) (kg/m²), complete blood count (CBC), liver function test (LFT), kidney profile (KP), fasting blood sugar, estimation of the levels of TNF-α, IFN-γ, IL-10, IL-12 (Beckman Coulter, France) using enzyme-linked immunosorbent assay (ELISA).

Statistical analysis:

The statistical package for social sciences (SPSS) version 8.0 software was used for analysis the data. Quantitative data were represented as mean ± standard deviation (SD) and the independent t-test was used to evaluate the significance of differences between mean values of the study variables. Quantitative data were represented as number & percentage (%) and the significance of differences between proportions was performed using the Chi-square test. Pearson correlation coefficient was used to measure the correlation between the studied parameters. P value is considered significant when it is less than 0.05.

RESULTS

Thirty seropositive HIV male patients with low viral counts after HAART were selected for this study; they were divided into two groups according to their immunological response to HAART. Group I consisted of patients with low CD4 counts, while Group II comprised of patients with high CD4 counts.

The age and BMI of patients in both groups were compared; no significant differences in their means between the groups were observed (as shown in table I). There was highly significant difference as regard virological suppression between the studied groups (as shown in table I). IL-12 levels were significantly higher in Group II than in Group I (mean IL-12 levels of 11.91 versus 6.9, $p<0.05$).

A positive correlation between IL-12 levels and CD4 counts ($r = 0.514$; $p<0.05$) were observed (Table II). Similarly, a positive correlation between IL-12 levels and IFN-γ levels were noted ($r=0.602$, $p<0.01$).

As far as serum cytokine levels are concerned, we found significantly higher levels of the pro-inflammatory cytokine TNF-α in Group I; the

TNF-α/IL-10 ratio is also higher in Group I as compared to Group II, which is suggestive of a stronger pro-inflammatory bias in Group I. Furthermore, there was a significant negative correlation between the TNF-α and the CD4 level (Table II). No significant correlations were observed between IL-12 levels and viral loads and also, no correlation between serum levels of IL-12 and serum levels of IL-10 and TNF-α. (Table II).

Table I: Comparison between age, BMI, Interleukin 10, Interleukin 12, IFN-γ, TNFα, CD4 count and viral load in studied groups.

	Group I (Target group)	**Group II (control group)**	**Significant Difference (p)**
	n=15	**n=15**	
Age	40.4±1.4	39.4 ±1.74	0.66 (**NS**)
BMI	23.5 ±0.39	24.02 ±0.35	0.29 (**NS**)
FBS	6.09±0.18	5.66±0.26	0.184 (**NS**)
CD4	238.3±26.17	691±43.93	<0.0001 (**S**)
Viral Load	309.1±67.1	697.3±128.06	0.014 (**S**)
IL-12	6.9±2.9	11.91±2.8	0.05 (**S**)
TNF-α	11.14±1.76	5.58±0.45	0.045 (**S**)
IFN-γ	9.73±1.31	13.27±1.17	0.02
IL-10	10.23±2.37	12.11±3.08	0.89 (**NS**)
IFN/IL-10	1.07±0.16	1.93±0.29	0.81
TNF-α /IL-10	1.34±0.23	0.79±0.09	0.042 (**S**)

Table II: Correlation between Interleukin 10, Interleukin 12, IFN-γ, TNFα, CD4 count and viral load in studied groups.

	IL-12		**TNF-α**	
	r	p	r	P
CD4 count	0.514	0.05 (**S**)	-0.423	0.021 (**S**)
Viral Load	0.398	0.087 (**NS**)	-0.279	0.143 (**NS**)
IL-12	1.000	0	-0.242	0.235 (**NS**)
IFN-γ	0.602	0.01 (**S**)	0.419	0.091 (**NS**)
IL-10	-0.286	0.151 (**NS**)	0.21	0.51 (**NS**)

DISCUSSION

The advent of highly active antiretroviral treatment (HAART) has transformed HIV infection from an inevitably fatal disease and a death sentence, to a chronic condition marked by reduced morbidity and mortality **[11]**. In this study, we have attempted to identify a possible link between reduced serum IL-12 levels and failure of immunological response to HAART and to demonstrate a possible connection between serum levels of IL-12 and CD4 counts in HIV patients.

We have investigated serum IL-12 levels in a total of 30 HIV patients who were selected from 148 HIV patients and classified according to their response to HAART into two groups: group I (immunological non-responders) and group II (immunological responders). The results of current study indicated that serum IL-12 levels were significantly elevated in group II when compared to those in group I.

An early immune dysfunction characterized by the gradual erosion of CD4+ T cell is one of the hallmarks of HIV infection since the CD4+ T cells are the primary target of virus infection. HIV infection causes a reduction in levels of CD4+ T cells by three main mechanisms: First, increased rates of apoptosis of infected cells; second, direct viral killing of infected cells; and third, killing of infected CD4+ T cells by CD8+ cytotoxic T lymphocytes that recognize infected cells **[7]**.

Several studies of antigen-specific immune responses concluded that both CD4+ and CD8+ T cell responses were markedly enhanced ex vivo

by the addition of IL-12 **[12]**. The enhancement of CD4+ T cell antigen-specific responses was boosted further in HIV-infected patients by the demonstration that IL-12 inhibited apoptosis in this cell lineage **[13]**.

Despite the virological suppression is better in Group I than in group II, the CD4 counts in Group II are higher than in group I (Table I). In addition, we found a clear positive correlation between serum IL-12 levels and CD4 count (Table II) which supports the notion that the high levels of IL-12 in group II has an important role in improving CD4 counts in HIV patients.

IL-12 is a central inducer of the Th1 response and cell-mediated immunity by inducing differentiation and proliferation of Th1-type cells and also, by stimulating the production of IFN-γ from T cells and NK cells **[8]**. IFN-γ is the primary cytokine that defines Th1 cells: Th1 cells secrete IFN-γ, which in turn differentiate CD4+ cells (Th0 cells) to Th1 cells, representing a positive feedback loop, while suppressing Th2 cell differentiation **[8]**. The current study showed that group I of HIV patients with low CD4 count had significantly lower concentration levels of IFN-γ ($p=0.02$) compared to group II of HIV patients with high CD4 count. A clear positive correlation was noted between IL12 levels and IFN-γ levels.

In this study, group II had slightly higher concentration levels of IL-10 compared to group I and no significant difference was noted between the two groups. Stylianou et al. **[14]** observed significantly higher circulating IL-10 levels in HIV-infected patients; the same study demonstrated a significant fall in concentration levels of IL-10 during HAART and observed that HAART had an effect on IL-10 levels. Also, the same study found that the HIV patients on HAART had slightly higher IL-10 levels ($p=0.008$) compared to HIV negative patient **[14]**. Jane et al., also demonstrated that the HIV patients on HAART had significantly low concentration levels of IL-10 ($p=0.001$) compared to treatment naïve HIV patients **[15]**.

In the current study, we could not find a correlation between IL-12 and IL-10 in the studied groups. Some studies have found that HIV and/or its proteins increases expression of IL-10, an anti-inflammatory cytokine, leading to myeloid dendritic cells suppression **[16,17]**. High expression of IL-10 has been implicated in the suppression of IL-12 during HIV infection **[18]**, though other studies have shown IL-12 levels to be independent of IL-10 **[19]**. Consistent with these latter studies, we did not find evidence to support a role of IL-10 in suppression of IL-12 in group I.

An interesting feature of this study is the finding of a significant difference in the levels of the pro-inflammatory cytokine TNFα between the two groups (Table I). This matches what has earlier been reported by Resino S et al. who found higher levels of TNF-α at lower HIV viral loads **[20]**. Another report by Hestdal P et al., describes higher levels of serum TNF-α in HIV patients with lower CD4 counts **[21]**. This is in line with our observation of elevated TNF-α levels in patients with decreased CD4 counts. A plausible explanation for high TNF-α levels in patients with low CD4 counts is that the increased TNF-α may have been produced by other cells such as CD8+ T cells the levels of which may have been increased in these patients. The ratio of TNF-α to IL-10 is higher in group I. In other words the Th1/Th2 cytokine ratio is higher in this group, again suggestive of CD8+ T cell predominance in those subjects.

Finally, we can concluded that there was significant reduction in IL-12 levels in immunologic non-responders HIV patients which is not linked to the immunomodulatory effect of IL10 or TNF-α , on the other hand IL12 showed a statistically significant positive correlation with IFN-γ. Therefore, one of the underlying mechanisms leading to a poor immune reconstitution despite good virological control following HAART may be driven primarily by reduction in the concentration levels of IL-12. Although, we found that IL12 production was correlated with IFN-γ, the mechanism by which the reduced production of IL-12 in immunologic non-responders HIV-1-infected patients remains poorly understood.

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

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Study of the Correlation between Serum Testosterone Level and Sarcopenia in Egyptian Male Patients with Liver Cirrhosis

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Key words: Cirrhosis, Sarcopenia, Testosterone

Background and study aim: Irrespective of etiology, liver cirrhosis together with its complications can affect other body organs and lead to a great morbidity and mortality. In Cirrhotic patients, Sarcopenia block normal life activities. Low serum testosterone has been reported in up to 90% of men with liver cirrhosis. This study aimed to assess the correlation between serum testosterone level and sarcopenia in Egyptian male patients with liver cirrhosis.

Patients and Methods: This prospective study included sixty cirrhotic males. Three groups were designed according to Child-Pugh classification. Twenty healthy males were included as control group. Patients and controls were subjected to complete blood picture, liver and kidney functions. Serum total & free testosterone was analyzed by specific enzyme-linked immunosorbent assay (ELISA) kit and Skeletal Muscle Index (L3 SMI) was assessed by CT scan.

Results: There was significant decrease in serum levels of free and total testosterone in cirrhotic patients than controls, with lowest levels in child C cirrhotic males (p value <0.001). 32 (53.3%) cirrhotic patients were sarcopenic. At cutoff point 14.1 nmol/L total testosterone level has Sensitivity 91%, Specificity 94% and Accuracy 93.0% to predict sarcopenia in cirrhotic males with AUC = 0.938. There was significant positive correlation between total testosterone level and the L3 SMI (r= 0.819, P<0.001). In addition, a positive correlation was detected between total testosterone and hemoglobin (r= 0.668, P<0.001), serum Na (r= 0.846, P<0.001) and Platelet count (r= 0.904, P<0.001), also negative correlation with MELD score (r= - 0.928, P<0.001).

Conclusion: Significant positive correlation between total testosterone level and Skeletal Muscle Index (L3 SMI) and low testosterone level is associated with sarcopenia in cirrhotic males.

INTRODUCTION

Cirrhosis is a progressive chronic liver disease characterized by diffuse fibrosis, severe interruption of the intrahepatic venous flow and portal hypertension **[1].**

Clinical features of hypogonadism like gynecomastia, loss of libido and infertility are common in cirrhotic male patients **[2].** In men with cirrhosis the more liver disease is severe the more testosterone levels decrease **[3].**

Sarcopenia, defined as muscle loss and dysfunction, is a common feature of all chronic inflammatory diseases and involves impairment of either the contractile, metabolic or endocrine functions of skeletal muscle **[4].**

Sarcopenia is an important diagnostic lineament of malnutrition, a clinical condition that is often challenging to objectively define in cirrhotic patients **[5].** Sarcopenia is common in cirrhotic patients and block normal life activities. Muscle weakness limits exercise capability, can profoundly impact simple activities of daily living and may also contribute to fatigue. These effects impact participation in the community, family and workforce. They also suffer from muscle cramps which have been identified as a major factor affecting quality of life **[6].**

Lumbar vertebrae-3 skeletal muscle index (L3 SMI) was expressed as cross-sectional muscle area/height². The cutoff for sarcopenia was based on the study of Prado et al., 2008 **[7].** It has been shown to be of higher accuracy and the most commonly employed method in studies investigating sarcopenia in cirrhosis **[8].**

Testosterone deficiency has been identified as an independent prognostic marker in liver cirrhosis. In addition, many clinical sequelae of advanced liver disease, as anemia, bone disease, gynecomastia and sarcopenia are also found in hypogonadism. Therefore, low testosterone levels may contribute to at least some of these manifestations **[9].** This study aimed to assess the correlation between serum testosterone level and sarcopenia in Egyptian male patients with liver cirrhosis.

PATIENTS AND METHODS

Sixty men with liver cirrhosis were selected from inpatient and outpatient clinic of Tropical Medicine Department, Menofia University Hospital and the Hepatology Department, Al haram Specialized Hospital in the period between June 2016 to December 2016. Their ages ranged from 33 to 57 years with a mean age of 46.5±6.53 years. Twenty healthy men as a control group of matched age were incorporated in the study.

Two groups were designed including all participants: Group I: Included 60 patients with liver cirrhosis. They were subdivided according to child-Pugh classification into: Group Ia: included 20 patients with Child-A liver cirrhosis. Group Ib: included 20 patients with Child-B. Group Ic: included 20 patients with Child-C. Group II: included 20 healthy subjects as a control group. Patients aged more than70 years, immobilized patients, patients with diabetes mellitus, renal impairment, hypogonadism, cancer prostate, previous or ongoing malignant disease including HCC were excluded. Also patients receiving medications that influence androgen levels, such as high dose opiates, glucocorticoids, or antiepileptic, etc. and obese & overweight patients were excluded from the study.

Approved by the local ethics committee of Tropical Medicine Department of Faculty of Medicine Menoufia University and Hepatology Department, Al haram Specialized Hospital; and informed consent was taken from each patient to provide a blood sample and to review the medical record for research purposes.

All patients and controls were subjected to proper and detailed history taking, general and local examination. Weight had two measures for assessment: objective scale weight (kg) and subjective estimation of dry weight. Estimated dry weight (kg) was calculated using scale weight minus ascites weight based upon severity (mild: 5%; moderate: 10%; severe: 15%). And 5% was subtracted for bilateral pedal edema. Body mass index (BMI) was measured using estimated dry weight divided by height² (kg/m²) **[5].**

Laboratory investigations: Complete blood count, fasting & 2hour post-prandial blood sugar, serum sodium, liver and kidney function tests were performed for all patients and controls. MELD score was calculated for all cirrhotic patients. Serological tests for viral markers (HBs Ag and HCV Ab by enzyme-linked immune sorbent assay and were confirmed by HCV RNA and HBV DNA by quantitative PCR. Serum free **[10]** & total testosterone **[11]** levels were done by ELISA.

Radiological evaluation was in the form of abdominal ultrasonography to evaluate liver, spleen, portal vein, the amount of ascites and both kidneys. CT scan was done for Para spinal skeletal muscle at the level of the 3rd lumbar vertebral body (L3 SMI) **[7].** The L3-L4 slice was selected for the quantitative analysis. Images were analyzed using a dedicated workstation (Leonardo Syngo; Siemens Medical System, Erlangen, Germany) that enabled specific tissue demarcation using thresholds of Hounsfield unit (HU) which was previously recorded. Skeletal muscle tissue was separated by using different density thresholds: using the density value from +35 HU to +150 HU was used to separate muscle tissue from fat and bone tissues **[12].** By using a special computer software, cross-sectional areas (cm²) = tissue pixels × the pixel surface area. The skeletal muscle area and intramuscular adipose tissue was normalized by divided on height^2 (cm²/m²). Sarcopenia was diagnosed if the L3 Skeletal Muscle Index (SMI) was ≤53 cm² /m² for men with BMI ≥25 and if SIM ≤43 cm² /m² for men with BMI <25) **[13].**

Statistical Analysis

Data was statistically analyzed using an IBM compatible personal computer with SPSS statistical package version 20 (SPSS Inc. Released 2011. IBM SPSS statistics for windows, version 20.0, Armnok, NY: IBM Corp.), and for all the analysis a p value <0.05 was considered statistically significant.

RESULTS

A total of 60 males with liver cirrhosis and 20 healthy males as controls were enrolled in this study. Child A (GIa) were 20 males. Their ages ranged between 40 and 57 years with mean 49.40 years and BMI ranged between 18.7 and 23.6 kg/m². Child B (GIb) included 20 males. Their ages ranged between 36 and 56 years with mean 45.70 years and BMI ranged between 17.50 and 23.20 kg/m². Child C (GIc) included 20 males. Their ages ranged between 33 and 52 years with mean 44.40 years and BMI ranged between 17.1 and 23.4 kg/m². Controls (GII) were 20 males. Their ages ranged between 38 and 57 years with mean 46.80 years and BMI ranged between 18.9 and 24.9 kg/m². Age (p value <0.075) given that the null hypothesis is true but statistical significance difference (p value <0.05) between the studied groups regarding BMI (using estimated dry weight) Table (1).

In cirrhotic patients, HCV was the etiology of cirrhosis in 93.3% of patients and the rest was caused by HBV & HCV co-infection. There was statistically significant difference between studied patients regarding history of hematemesis and/or melena, hepatic encephalopathy, edema of the lower limb, jaundice and muscle wasting, while there was no statistically significant difference as regard the presence of palmer erythema (p value <0.07) and spider naevi (p value <0.153). Gynecomastia, feminine hair distribution and abdominal wall hernia were statistically differed among studied patients Table (1).

Regarding the free and total testosterone levels in all studied groups, there was significant decrease in cirrhotic patients than controls (p value <0.001). The mean value of free and total testosterone levels in controls (GII) (3.54± 0.86 ng/mL & 18.68± 2.69 nmol/L respectively) is higher when compared with Child A (2.56± 0.66 ng/mL & 16.13± 2.08 nmol/L) and Child B (1.56± 0.47 ng/mL & 13.72±2.51 nmol/L) with the lowest levels in child-C cirrhotic patients (0.75± 0.15 ng/mL & 6.21± 2.38 nmol/L) (Table 2).

In studied groups the L3 skeletal muscle index (SMI) was significantly different (p value <0.001). It was higher in controls (44.6± 0.79) than in cirrhotic patients Child A, B and C (43.3± 0.66, 42.4± 0.89 and 40.9± 2.26 respectively). The presence of sarcopenia was significantly different among studied groups. It was not detected in controls, and significantly increased with increasing child grade from child-A (15%) to child-B (55%) and highest in child-C (90%) of cirrhotic males (Table 3).

Receiver operating curve to assess the best cut off point of total testosterone level to predict the presence of sarcopenia showed that at cutoff point 14.1 nmol/L, total testosterone level has sensitivity 91%, specificity 94% and accuracy 93.0% to predict the presence sarcopenia in cirrhotic males (Figure 1).

In cirrhotic males, serum total testosterone level showed high significant positive correlation with SMI (r= 0.819 and p value <0.001) (Figure 2).

There was a statistical high significant positive correlation between serum total testosterone level (p value <0.001) with Body mass index, hemoglobin concentration, ALT, Serum albumin, serum Na & Platelets. There was high significant negative correlation between total testosterone and MELD score, INR & bilirubin in cirrhotic patients (Table 4 and Figure 3). Significant positive correlation (p value <0.001) was found between L3 SMI and Serum Na & Platelets in cirrhotic males, and significant negative correlation (p value <0.001) with MELD score (Table 4).

Table (1): Demographic data & General examination

Studied variables	GIa (N=20)	GIb (N=20)	GIc (N=20)	GII (N=20)	Test	*P* value
Age ($\bar{X}$ ±SD)	49.40± 5.60	45.70± 6.87	44.40± 6.11	46.80±5.91	F (2.39)	0.075
BMI (kg/m2) ($\bar{X}$ ±SD)	20.27± 1.44	20.00± 2.11	19.36± 1.88	21.68±1.78	F (5.80)	0.001
Hepatic encephalopathy	0 (0%)	11 (55%)	19 (95%)		χ^2 (43.33)	0.001
Muscle wasting	0 (0%)	9 (45%)	16 (80%)		χ^2 (26.47)	0.002
Gynecomastia	0 (0%)	3 (15%)	7 (35%)		χ^2 (8.88)	0.012
Feminine hair distribution	0 (0%)	9 (45%)	15 (75%)		χ^2 (23.75)	0.007

($\bar{X}$ ±SD); mean ± standard deviation, F; ANOVA (analysis of variance) test of significance, **χ^2;** chi square test, BMI; Body Mass Index.

Table (2): Biochemical investigations of studied groups

Studied variables	GIa (N=20)	GIb (N=20)	GIc (N=20)	GII (N=20)	Test of significance	P value
	±SD$\bar{X}$	**±SD$\bar{X}$**	**±SD$\bar{X}$**	**±SD$\bar{X}$**		
INR	1.49± 0.13	1.80± 0.22	2.39± 0.30	0.94± 0.1	F (176.1)	<0.001
Serum albumin (g/dl)	3.65±0.15	2.98±0.24	2.28±0.39	4.33±0.21	F (222.51)	<0.001
Total bilirubin (mg/dl)	0.94± 0.17	2.87± 0.63	3.76± 0.39	0.71± 0.16	F (622.56)	<0.001
Serum Na (mmol/L)	138.7±1.64	129.20±3.30	123.10±3.02	139.40±1.60	F (156.52)	<0.001
MELD score	11.00± 1.65	14.20± 2.28	22.20± 2.85		F (123.82)	<0.001
Free testosterone (ng/dl)	2.56± 0.66	1.56± 0.47	0.75± 0.15	3.54± 0.86	F (82.95)	<0.001
Total testosterone (nmol/L)	16.13± 2.08	13.72±2.51	6.21± 2.38	18.68± 2.69	F (98.09)	<0.001

($\bar{X}$ ±SD); mean ± standard deviation, F; ANOVA (analysis of variance) test of significance, INR; international normalized ratio, MELD; Model for End-Stage Liver Disease.

Table (3): L3 Skeletal muscle index (SMI) & Sarcopenia in studied groups (No=80)

Studied variables	GIa (N=20)		GIb (N=20)		GIc (N=20)		GII (N=20)		Test of significance	P value
	±SD$\bar{X}$		**±SD$\bar{X}$**		**±SD$\bar{X}$**		**±SD$\bar{X}$**			
SMI (cm^2/m^2) range	43.3± 0.66 41.8-43.9		42.4± 0.89 40.6- 43.6		40.9± 2.26 37.8- 44.6		44.6± 0.79 43.5-46.2		F (27.31)	<0.001
Sarcopenia	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	χ^2 (41.25)	0.001
	3	15	11	55	18	90	0	0		

$\bar{X}$ ±SD; mean ± standard deviation, SMI; Skeletal muscle index, F; ANOVA (analysis of variance) test of significance, χ2; chi square test

Cutoff point of total testosterone	AUC	Sensitivity	Specificity	Accuracy	Positive predictive value (PPV)	Negative Predictive value (NPV)
14.1 nmol/L	0.938	91%	94%	93.0%	91.0%	94.0%

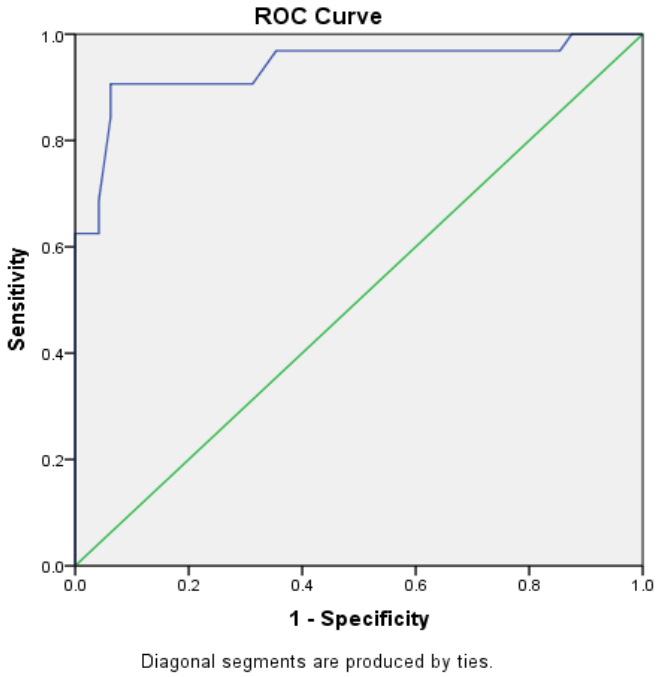

Fig. (1) : ROC curve of total testosterone level to predict sarcopenia in cirrhotic males

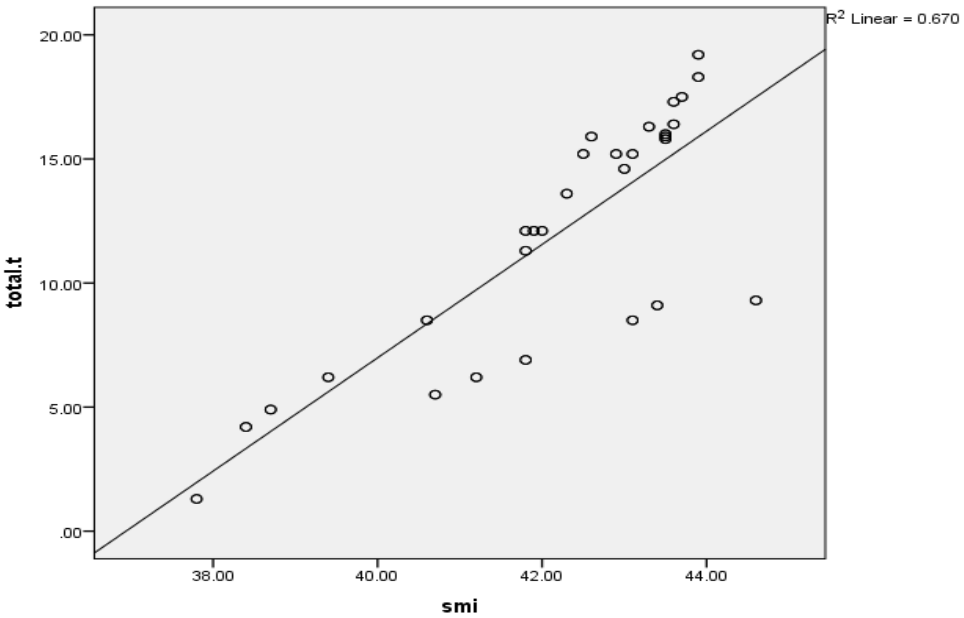

Fig. (2) : Correlation between total testosterone level and SMI in cirrhotic males

Table (4): Correlation between total testosterone level & SMI and other studied parameters among cirrhotic males

Studied variables	Total testosterone level	
	(r)	p value
BMI	0.536	<0.001
Hb	0.668	<0.001
MELD score	-0.928	<0.001
INR	-0.853	<0.001
ALT	0.848	<0.001
Serum albumin	0.848	<0.001
Total bilirubin	-0.795	<0.001
Serum Na	0.846	<0.001
Platelet count	0.904	<0.001
	Skeletal muscle index (SMI)	
MELD score	-0.698	<0.001
Serum Na	0.630	<0.001
Platelet count	0.705	<0.001

BMI; Body Mass Index, Hb; hemoglobin concentration, MELD; The Model for End-Stage Liver Disease, INR; international normalized ratio, ALT; alanine aminotransferase, Na; serum sodium.

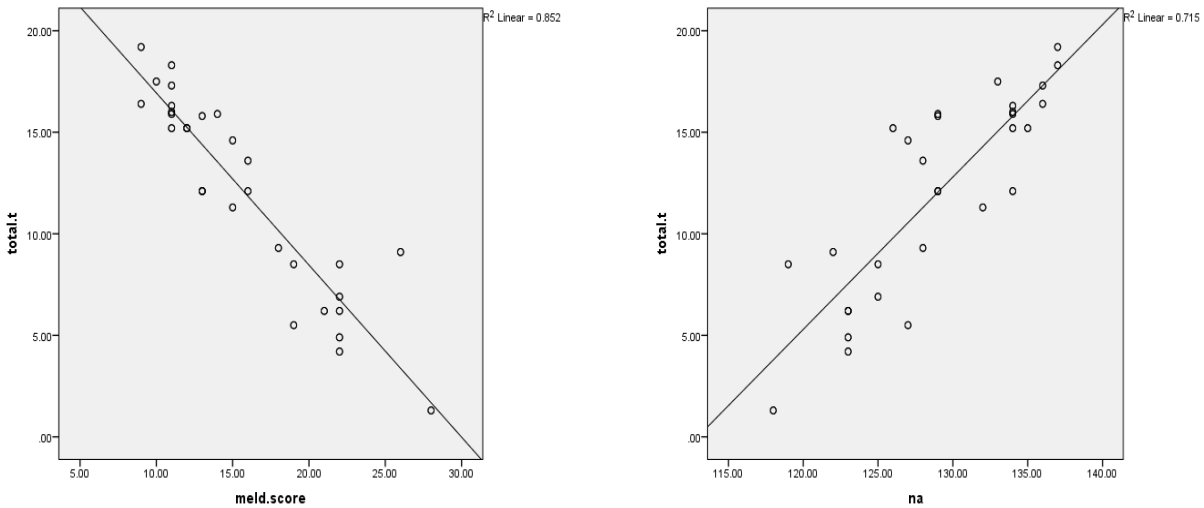

Fig. (3) (a&b): Correlation between total testosterone level and MELD score & serum sodium in cirrhotic males

DISCUSSION

Cirrhosis is considered as an increasing cause of morbidity and mortality in developed countries **[14].** Cirrhotic males have clinical features of hypogonadism including gynecomastia, loss of libido and infertility while females may experience amenorrhea or oligomenorrhea **[15].**

A decreased testosterone level has been detected in up to 90% of males with liver cirrhosis **[16].** It is blamed to cause many of the clinical features of end stage liver disease in males, including altered body hair distribution, fatigue and impaired sexual function **[17].**

In this study there was significant difference (p value <0.001) between cirrhotic groups regarding feminine hair distribution. This agreed with Yee and Lidofsky **[18]** and also with De Bruyn and Graviss **[19]** who reported that examination of cirrhotic patients may show diminished or feminine distribution of body hair.

Our study revealed that there was statistical significant increase (p value <0.001) in both free and total testosterone levels in controls when compared with cirrhotic males. In addition there was significant decrease in free and total testosterone levels with increasing Child Pugh scores. These results agreed with Yurci et al. **[20]** who reported that in cirrhotic patients, low plasma testosterone levels was correlated with Child Pugh scores and express the severity of liver damage. Southren et al. **[21]** as well proved decreased plasma level and production rate of testosterone in cirrhotic males and that the mechanism is probably resulting from suppression of hypothalamic/pituitary function rather than testicular atrophy.

Sarcopenia is one of the commonest complications of cirrhosis and can contribute significantly to morbidity and mortality in patients with liver cirrhosis **[22]**. It is defined as a muscle mass two standard deviations below the healthy young adult mean. The L3 Skeletal Muscle Index (SMI) was expressed as cross-sectional muscle area/ height² [**23].** We measured BMI by using estimated dry weight divided by height² (kg/m2) **[5].** The calculated BMI for all cirrhotic males was <25, at the same time we selected the controls with BMI <25 so we used L3 SMI≤43 cm2 /m2 as a cut off for detection of sarcopenia. This cutoff was also used by krell et al. **[24]** based on a CT-based study in patients with solid tumors. In our study there was high statistical significant difference (p value <0.001) among studied groups regarding SMI with the lowest level in child-C cirrhotic patients.

Sarcopenia was significantly different among studied groups in our study. It was not detected in control group, and significantly increased with increasing child grade from A to B and highest in child-C cirrhotic patients. The present study revealed that, sarcopenia was detected in 53.3% of cirrhotic patients (32 out of 60 patients). Cross-sectional imaging studies revealed that among patients with cirrhosis sarcopenia can be detected in 30%-70% **[25].**

Many factors were reported to contribute to development of sarcopenia in cirrhosis, including elevated inflammatory mediators, malnutrition, reduced insulin-like growth factor-1 **[26]** and breakdown of muscle protein for energy use because of low hepatic glycogen synthesis and storage **[27].**

In this research we found that at cutoff point 14.1 nmol/L, total testosterone has sensitivity 91%, specificity 94% and accuracy 93% to predict sarcopenia in men with liver cirrhosis. Tandon et al. **[28]** has reported that male sarcopenic patients had lower total testosterone levels than non-sarcopenic patients (15±1 nmol/L versus 22±1.2, P= 0.005) & low testosterone levels (OR 0.95, 95% CI 0.91-0.99, P= 0.02) were associated with sarcopenia by multivariate regression analysis.

The current study demonstrated that there was significant positive correlation between serum total testosterone and SMI (r =0.819, P<0.001) in agreement with Sinclair et al. **[3]** who reported that muscle mass was positively associated with serum total testosterone levels (tau 0.13, P = 0.02). Also we detected a significant positive correlation between serum total testosterone and hemoglobin concentration, serum albumin & serum sodium in cirrhotic patients. These results were in agreement with those reported by Sinclair et al. **[29]** who detected a positive association between total testosterone and hemoglobin concentration (P<0.001), serum sodium and albumin. Exogenous testosterone administration increases erythropoiesis, and predispose to polycythaemia thus, this relationship could plausibly be a causal one **[30].**

We found significant positive correlation between serum total testosterone and platelets (r =0.904, P<0.001). However Sinclair et al. **[29]** demonstrated non-significant correlations between total testosterone levels and platelet count. The

present study revealed that, there was high significant negative correlation between total testosterone and MELD score **(**r -0.928, P<0.001**)**, INR **(**r -0.853, P<0.001**)** & Bilirubin **(**r -0.795, P<0.001**)**. This result agreed with Sinclair et al. **[3]** and Sinclair et al. **[29].**

We found that SMI positively correlated with serum Na **(**r 0.630, P<0.001**)** in agreement with Sinclair et al. **[3]** who found a positive correlation between muscle mass & serum sodium (tau 0.18, P= 0.002). Also we found a positive association between SMI and Serum albumin **(**r 0.681, P<0.001), and a significant negative correlation between SMI and MELD score **(**r -0.698, P<0.001**)**. This result agreed with Sinclair et al., **[3]** who reported that (SMI) muscle mass negatively correlated with MELD score (tau 0.16, P= 0.007**).** On the other hand Montano loza et al **[8]** reported a poor correlation between sarcopenia and serum sodium (r= 0.11, P= 0.2), serum albumin (r 0.02, P=0.9) and MELD score (r= 0.04, P= 0.7). This disagreement may be due to difference in L3 SMI cutoff used in diagnosis of sarcopenia, as they depend on L3 SMI, ≤52.4 cm2/m2 for men whatever their body mass index.

CONCLUSION

Our data demonstrated that serum levels of free and total testosterone were significantly lower in cirrhotic males than controls, with the lowest levels in child C cirrhotic patients. Additionally, serum testosterone level positively correlated with The L3 Skeletal Muscle Index (SMI) and the presence of sarcopenia in male patients with liver cirrhosis, with negative correlation with severity of liver cirrhosis as assessed by MELD score.

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

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Circulating Micro RNA- 21 and - 92a as Biomarkers of Colorectal Cancer

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Key words:
Colorectal cancer (CRC); miRNA-21; miRNA-92a; biomarkers

Background and study aim: Colorectal cancer (CRC) is a major cause of cancer-related deaths in both men and women. Colonoscopy is the most reliable tool for CRC diagnosis, but its complexity and costs hamper its wide application. There is a pressing need for new non-invasive biomarkers to improve early diagnosis of CRC. Aim was to assess serum micro RNA (miR) -21and miR-92a for diagnosis of CRC.

Patients and Methods: This comparative cross sectional study was carried out on 50 subjects. The cases group comprised 35 consecutive treatment naive patients with sporadic CRC proved by colonoscopy and histopathology of the biopsied specimens as well as abdominal CT which all together helped in tumor staging applying TNM, Duke's and MAC Coller stages. Serum carcinoembryonic antigen (CEA) and cancer antigen (CA) 19-9 were also assessed. Fifteen matched healthy subjects (with normal colonoscopy) served as the control group.

Results: Serum levels of miR-21 and miR-92a were significantly higher (P<0.001) in cases compared to the control (5.53 ± 0.17≠4.82 ± 0.20 and 7.01 ± 0.234 ≠ 6.56 ± 0.20 log RU, respectively). Serum miR-21 and miR-92a levels revealed a significant positive relation with tumor size and TNM and MAC Coller stages (P<0.05). No significant relationship was detected between either serum miR-21 or miR-92a levels with age or sex. Applying ROC curve, at a cutoff value of ≥5.25 log RU, serum miR-21 was 94.3% sensitive and 93.3% specific for detection of CRC with an AUC= 0.99 and serum miR-92a level at a cut off value ≥6.75 log RU, was 91.4% sensitive and 80% specific for detection of CRC with AUC= 0.91. When both markers were combined, the sensitivity and specificity were 97.1% and 93.3% respectively.

Conclusion: Serum miR-21 and miR-92a levels represent a sensitive and specific tool for CRC diagnosis, with higher accuracy of miR-21.

INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer diagnosed in both men and women **[1]**. The lifetime risk of developing CRC is about 1 in 21 (4.7%) for men and 1 in 23 (4.4%) for women. Many factors increase the risk including: obesity, diet high in red meat (such as beef, pork, lamb, or liver) and processed meat, smoking, heavy alcohol use, history of inflammatory bowel disease, family history of CRC or adenomatous polyps and diabetes mellitus **[1]**.

The CRC mortality rates can be decreased by early diagnosis through screening. However, the present CRC screening techniques **[**colonoscopy, faecal occult blood test (FOBT), and carcinoembryonic antigen (CEA) test**]** are limited by their difficulties and costs beside uncertain or delayed results **[2]**.

Although colonoscopic screening for CRC is the most reliable tool; its difficulties and costs have hampered its wide application. On the other hand, FOBT has the limitation

of low sensitivity and dietary restrictions **[3]**. Thus, there is a pressing need for new non-invasive biomarkers to improve the early detection of CRC **[4].**

The discovery of micro RNAs (miRNAs), that play important roles in oncogenesis, has opened new opportunities of non-invasive tests for the early diagnosis of cancers **[5]**.

MiRNAs are a family of small, noncoding RNAs (19-22 nucleotides) which post-transcriptionally regulate gene expression. In general, miRNAs are transcribed as a group called the pri-miRNA complex, which is cleaved in the nucleus to form the pre-miRNA which is then translocated to the cytoplasm where they undergo final maturation into a functional miRNA **[6]**. Studies have shown that profiles of miRNA expression differ between normal- and tumour- tissues and vary among different tumour types **[7]**. Aberrant miRNA expression profiles have been identified and emerged as potential screening biomarkers for CRC **[8,9]**.

Although most previous studies on miRNA expression have been performed on tissue specimens, some studies have shown diagnostic and prognostic potential for circulating miRNAs because tumor-derived miRNAs can be present in blood and appear to be stably protected from endogenous ribonuclease activity in the circulation **[10]**.

MiR-21 is an oncogenic miRNA that modulates the expression of multiple cancer-related target genes such as PTEN, TPM1, and PDCD and has been shown to be overexpressed in various human tumors **[11]**. In addition, miR-21 expression is upregulated in CRC tissues, is elevated during tumor progression, and is also associated with poor survival and response to chemotherapy **[12]**. Significantly elevated plasma miR-21 expression in CRC was concluded in many studies **[11,13]**. On the other hand, MiR-92a is part of the miR-17–92 gene cluster located at chromosome 13q13. As a known oncomir, the miR-17–92 cluster can promote cell proliferation, suppress apoptosis of cancer cells, induce tumor angiogenesis and accelerate tumor progression. Elevated expression of miR-92a has been observed in CRC, lung and thyroid cancers suggesting an important role in tumorigenesis. Many studies reported that circulating miR-92 is a potential biomarker for CRC diagnosis **[13,14]**. The aim of the present study was to assess miR-21 and miR -92a expression levels as a stable blood-based biomarker for detection of CRC.

PATIENTS AND METHODS

This comparative cross sectional study was carried out on 50 subjects. The cases group comprised 35 consecutive patients with CRC (19 males and 16 females) who were attending the Departments of Hepatology, Gastroenterology and Infectious Diseases and General Surgery at Benha University Hospitals, within the period between January 2015 and October 2016. All had sporadic CRC, proved by colonoscopy and histopathology. Fifteen age and sex matched apparently healthy subjects who had normal routine laboratory investigations and colonoscopy served as healthy control. The indication for colonoscopy was unexplained abdominal pain and/or altered bowel habits. Patients with familial adenomatous polyposis or hereditary non-polyposis CRC, those who received chemotherapy or radiotherapy and pregnant female patients were excluded from the study. The study protocol was approved by the Ethics Committee of Benha Faculty of Medicine, Benha University. An informed written consent was obtained from the participants.

All the studied 35 cases were subjected to the following :

Full history taking focusing on: family history of CRC, recent- onset constipation, bleeding per rectum, significant weight loss, and/or anemia of unexplained aetiology. Thorough clinical examination including general examination focusing on: cachexia, pallor and lymphadenopathy. Local abdominal examination focusing on: palpable abdominal masses, ascites, LN and PR examination.

Laboratory investigations, including: stool analysis, complete blood count (CBC), erythrocyte sedimentation rate (ESR), random blood sugar (RBS), liver profile tests, including: alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), serum albumin, serum bilirubin (total and direct) and prothrombin time (PT), serum creatinine and blood urea and Serum CEA and CA19-9.

Detection of miRNA- 21 expression level using real-time PCR technique: Extraction of total RNA including microRNA-21 from plasma samples using microRNA extraction kit according to the

manufacturer's instructions **[15]**. Relative quantitation of microRNA-21 level using real-time quantitative PCR (RT-PCR) according to the manufacturer's instructions **[16]**.

Detection of miRNA- 92a expression level using real-time PCR technique: Extraction of total RNA including microRNA-92a from plasma samples using miRNA extraction kit according to **[15]**. Relative quantitation of microRNA-92a level using real-time quantitative PCR (RT-PCR) according to Siege et al. **[17]**.

Pelvi-abdominal ultrasonography and computed tomography (CT scan) were done. Complete colonoscopy was done under sedation with patient preparation through Low-volume Polyethylene glycol-based lavage solution with ascorbic acid (Low-volume PEG-ELS with ascorbic acid) administered as split-dose regimen. Multiple biopsies were taken from any suspected lesion, and were sent for histopathological examination. TNM, Duke's and MAC Coller staging systems were applied.

Statistical analysis [18] :

The collected data were tabulated and analyzed using SPSS version 16 soft ware (SpssInc, Chicago, ILL Company). Categorical data were presented as number and percentages while quantitative data were expressed as mean ± standard deviation, median and range. Chi square test (X2) or Fisher's exact test (FET) were used to analyze categorical variables. Quantitative data were tested for normality using Shapiro-Wilks test, assuming normality at P>0.05. Student "t" test was used for normally distributed variables, while Man Whitney U (MWU) test, Kruskal Wallis test and Spearman's correlation coefficient (rho) were used for not normally distributed data. ROC curve was used to determine cutoff values of miRNA-21 and miR-92a with optimum sensitivity and specificity in prediction of patients with CRC. P value ≤ 0.05 was considered significant.

RESULTS

The studied cases included 19 males (56%) and 16 females (44%) with a mean age of 50.6 ± 15.1ys. Age categories among the studied cases showed 26% below 40 years and 63% above 50 years.

The main presenting symptom in the studied cases was bleeding per rectum (15/35 = 42.9%) followed by recent onset constipation (13/35 = 37.1%) and significant weight loss (7/35 = 20%). Positive family history was found in (6/35=17%) of the studied cases.

Colonoscopic examination of the studied cases revealed that the main lesion site of CRC was colon (24/35 = 68.6%), followed by rectum (9/35 = 25.7%) and lastly rectosigmoid (2/35 = 5.7%). Small lesions (<2.5 cm) were seen in 4 cases (11.4%), medium sized ones (2–5 cm) were 37.1% (13 cases), while large lesions were seen in 18 cases (51.4%). Mass was the main lesion seen (94.2%) with only one case showed ulcer and another one showed a stricture. The main histo-pathological CRC types in the studied cases were adenocarcinoma (24/35 = 68.6%) followed by mucinous adenocarcinoma (9/35 = 25.7%) and signet ring cell adenocarcinoma (2/35 = 5.7%).

The majority of the studied cases were Dukes B (88.6%). According to TNM staging, cases with TIIB were (54.3%) followed by TIIA (34.3%). Three cases only were Tis (8.5%). None of the studied cases had nodal involvement or metastases (all were N0M0).

Table (1): Circulating miR-21 and miR-92a levels in the studied groups**.**

Variable	Cases (N°=35)		Controls (N°=15)		Z of MWU test	P
	Mean ± SD	Range	Mean ± SD	Range		
MiR-21 level	5.53 ± 0.17	4.93 - 5.67	4.82 ± 0.20	4.62 - 5.41	*5.55*	**<0.001****
MiR-92a level	7.01±0.234	6.46 – 7.33	6.56 ± 0.20	6.05 – 6.9	4.56	**<0.001****

**Highly signifiant P value

Table (2): Mean values of serum miR-21& miR-92a in relation to colonoscopy findings**.**

Lesion		N° (T= 35)	Serum miR-21 (log RU)		KWT	P	Sig pairs	microRNA-92		KWT	P	Sig pairs
			Mean± SD	Range				Mean± SD	Range			
Site	Colon	24	5.51 ± 0.19	4.93 - 5.67	0.28	0.87	-----	6.98±0.25	6.94-7.32	4.02	0.13	-----
	Rectum	9	5.56 ± 0.09	5.41 - 5.66				7.14±0.13	6.94-7.32			
	Recto-sigmoid	2	5.57± 0.05	5.54 - 5.61				6.82±0.02	6.8-6.84			
Size	Small	4	5.22 ± 0.31	4.93 - 5.54	14.5	**0.001****	Small ≠ medium	6.75**±02**	6.46-6.94	11.9	**0.003***	.Small ≠ medium
	Medium	13	5.51 ± 0.09	5.41 - 5.65			Small ≠ large	6.93**±024**	6.48-7.28			.Medium ≠ large
	Large	18	5.61 ± 0.07	5.44 - 5.67				7.13±015	6.8-7.33			
Morphology	Mass	33	5.54 ± 0.14	4.93 - 5.67	2.93	0.23	-------	7.02±023	6.46-7.33	2.19	0.33	--------
	Ulcer	1	5.63 ± ----	5.64 - 5.64				6.79±--	6.8-6.8			
	Stricture	1	4.98 ± ----	4.98 - 4.98				6.94±--	6.94-6.94			
Pathological Grade	Grade I	3	5.27±0.296	4.93-5.45	8.06	**0.018 ***	I≠III	5.21± 0.286	4.83-5.45	8.02	**0.017 ***	I≠III
	Grade II	27	5.51±0.176	4.98-5.65				5.41±0.186	4.78-5.55			
	Grade III	5	5.64±0.028	5.61-5.67				5.60±0.029	5.51-4.67			

*Significant P vaule
**Highly signifcicant P value
RU:relative uncertainty RU

A statistically significant relationship was found between serum- miR-21& -miR-92a and the tumor size (P = 0.001 & 0.003 respectively) as well as the tumor pathological grade (P = 0.018 & 0.017 respectively), while tumour site and morphology showed non-significant relationships with serum miR-21 and miR-92a expression.

Table (3): Correlation between serum miR-21& miR-92a and assessed variables in the cases group.

Variable	miR-21		miR-92a	
	rho	P	rho	P
Age	0.231	0.18 (NS)	0.076	0.66 (NS)
Hb%	-0.138	0.43 (NS)	0.115	0.51 (NS)
RBCs	0.083	0.063 (NS)	0.232	0.18 (NS)
WBCs	-0.041	0.81 (NS)	-0.149	0.39 (NS)
PLTs	0.477	**0.008 (S)**	-0.177	0.31 (NS)
RBS	0.062	0.72 (NS)	0.234	0.17 (NS)
ALT	0.058	0.74 (NS)	0.016	0.92 (NS)
AST	0.055	0.75 (NS)	0.162	0.35 (NS)
ALP	-0.158	0.36 (NS)	0.199	0.25 (NS)
S.albumin	-0.1	0.57 (NS)	-0.161	0.35 (NS)
T. Bilirubin	0.126	0.47 (NS)	0.292	0.09 (NS)
Creat.	0.246	0.15 (NS)	0.249	0.15 (NS)
BUN	0.023	0.89 (NS)	0.052	0.77 (NS)
CEA	0.131	0.45 (NS)	0.022	0.9 (NS)
CA19-9	0.161	0.35 (NS)	0.034	0.84 (NS)

There was a statistically significant positive correlation between serum miR- 21and platelets count (P=0.008), while there were non- significant correlations between serum miR-21 and miR-92a and the other assessed variables.

Table (4) : Sensitivity and specifity of miRNA-21 and microRNA-92a for CRC diagnosis.

Variable	Sens %	Spec %	PPV%	NPV%	AUC	95%CI	P
miR -21 ≥ 5.25 *log RU*	94.3%	93.3%	97%	87.5%	0.99	0.97-1.0	**<0.001****
miR -92a ≥ 6.75 *log RU*	91.4%	80%	91.4%	80%	0.91	0.83-0.99	**<0.001****
Combined miR -21 & miR -92a	97.1%	93.3%	97.1%	93.3%			

**Highly signifcicant P value

At a cut off value ≥5.25 log RU, serum miR-21 was 94.3% sensitive and 93.3% specific for detection of CRC, with AUC = 0.99.At a cut off value ≥6.75 log RU, serum miR-92a was 91.4% sensitive and 80% specific for detection of CRC with AUC = 0.91. When both markers were combined, the sensitivity increased to 97.1% and specifity was 93.3%.

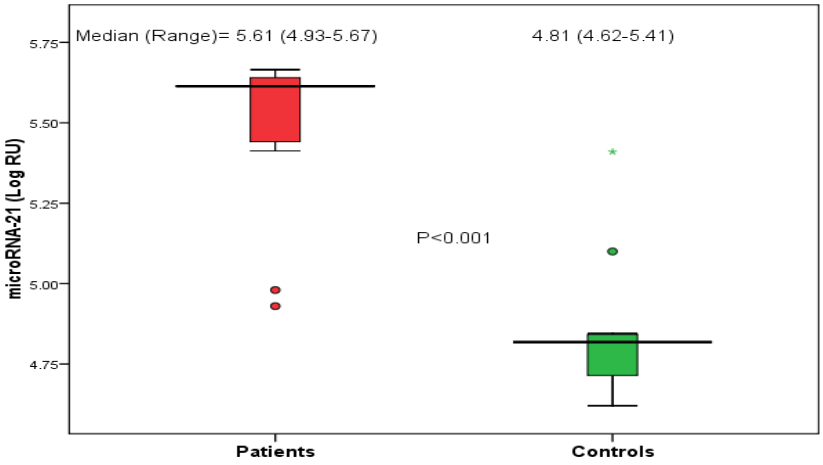

Fig. (1): Box plot showing the median and range of serum miR-21 among cases and control group

Serum miR-21 expression was significantly higher in the cases group compared to the control **(**5.53 ± 0.17≠4.82 ± 0.20 log RU**)** with (P<0.001)**.**

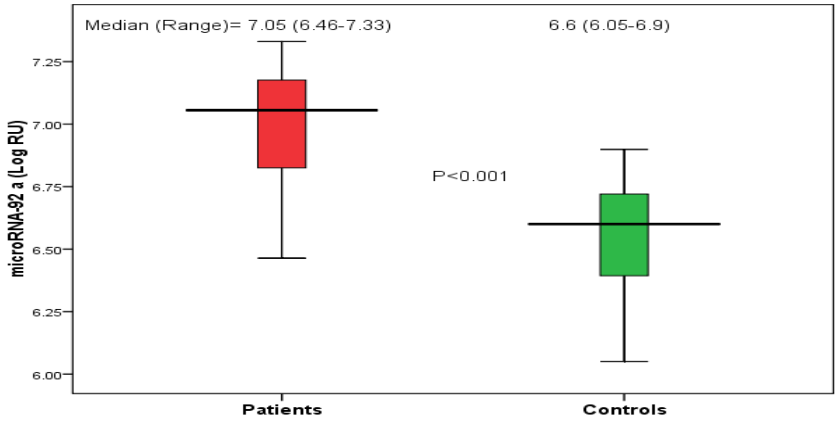

Fig. (2): Box plot showing the median and range of serum miR-92a in cases and control groups

Serum miR-92a expression was significantly higher in the cases group compared to the control (7.01 ± 0.234 ≠ 6.56 ± 0.20 log RU) with (P<0.001).

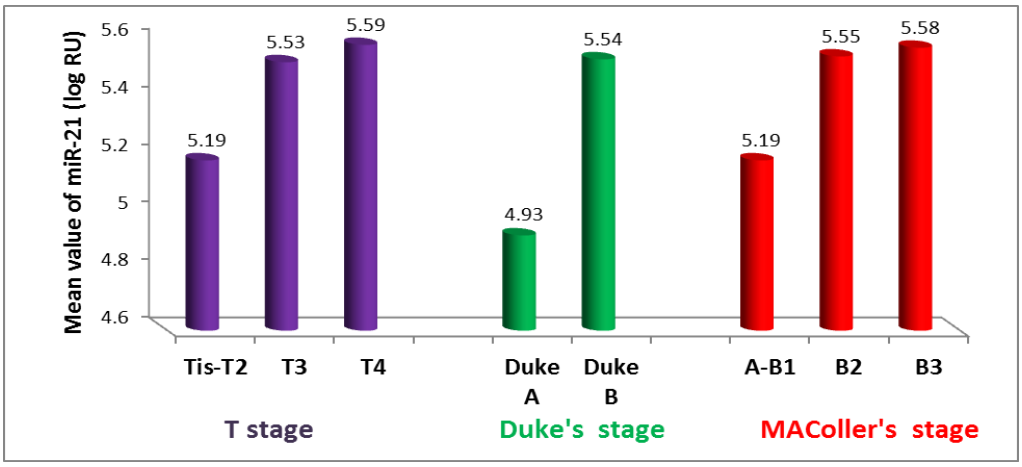

Fig. (3): Mean values of serum miR-21 in different CRC stages

A statistically significant positive relationship was found between T satge (P = 0.01) as well as MAC Coller stage (P = 0.008) and serum miR-21 expression, while a statistically non-significant relationship was found between Duke's Stage and serum miR- 21 expression.

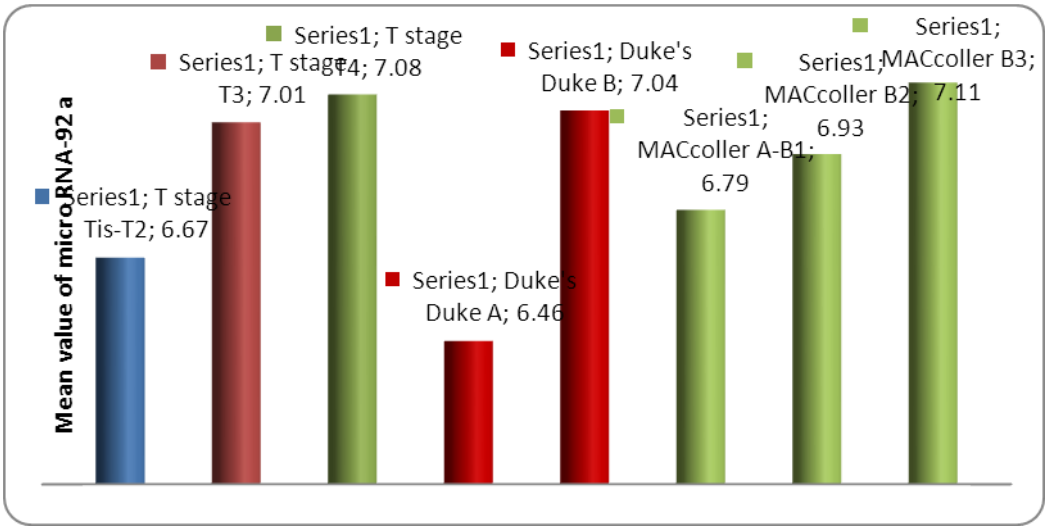

Fig. (4): Mean values of serum miR-92a in different CRC stages

A statistically significant positive relationship was found between T satge (P = 0.03) as well as MAC Coller stage (P = 0.031) and serum miR-92a expression, while a statistically non-significant relation was found between Duke's Stage and serum miR- 92a expression.

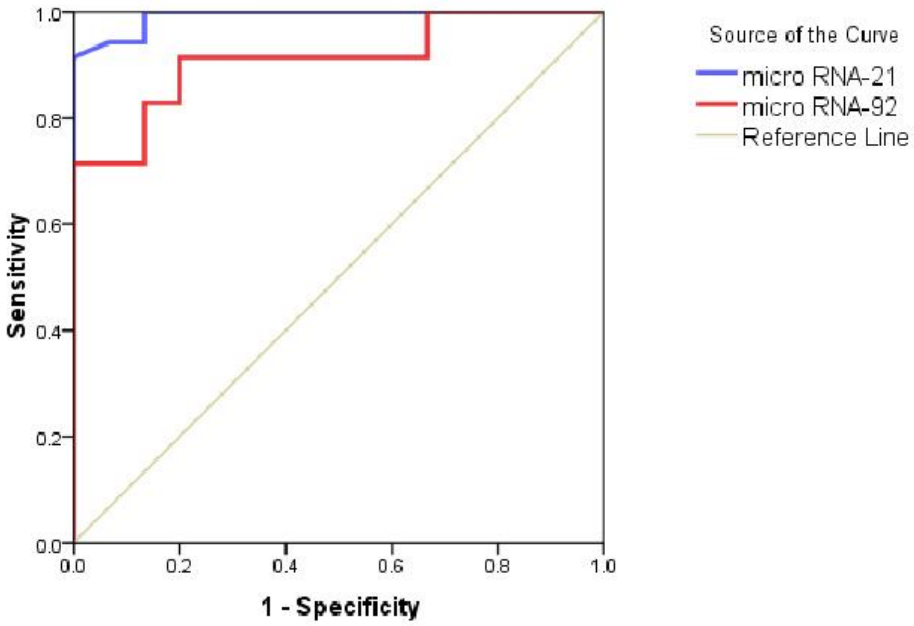

Fig. (5): ROC curve for the performance of miR-21 and miR-92 in CRC detection.

DISCUSSION

CRC is the third most prevalent cancer worldwide and is a leading cause of cancer-related mortality for both men and women **[19]**. In Egypt, CRC is one of the most common malignant neoplasms. It ranks the sixth most common cancer in both males and females **[20,21]**. The median age of CRC cases in Egypt is 48 and 51.5y_s for males and females, respectively **[22]**.

In the present study, the age of the studied cases ranged between 19-74ys, with a mean of 50.6 ± 15.1ys. More than 60% were >50ys and 26% (more than 1/4 of patients) were ≤40ys old. This comes in agreement with many Egyptian studies Gado et al. **[22]** found that the mean age of their assessed patients was 51ys and 44.8ys respectively and El Attar **[23]** and Sakr et al. **[21]** told that the mean age of their studied patients was 48ys and 51.2 ys respectively.

Males represented 56% of the studied cases in the present study. Rim et al. **[24]** and Murphy et al. **[25]** reported that men have more incidence of CRC than women, while El-Bolkainy et al. **[26]**, Abotchie et al. **[27]** and Sakr et al. **[21]** told that CRC affects men and women almost equally

The percentage of the studied cases with CRC with a positive family history was 17%. This comes in agreement with chan et al. **[28]** and Haggar & Boushey **[29]** who told that the percentage of patients with CRC with a positive family history was up to 20%. Fatemi et al. **[30]** reported a higher figure (31.3%) in their studied Iranian patients. These data make adherence to CRC screening programs is mandatory in subjects with family history of CRC. Lieberman and his colleagues **[31]** told that screening should be offered to individuals with a family history of CRC earlier than for the average-risk population.

As regards the tumor site, colonic lesions (68.6%) were encountered more than rectal (25.7%) and rectosigmoid (5.7%) ones. Yi et al. **[32]** reported similar finding of a lower incidence of rectal lesions (25.5-30.5%) compared to colonic lesions. On the other hand, Kenawi et al. **[33]** and Soliman et al. **[34]** told that about half of their studied cases were rectal lesions, this disagreement may be attributed to the large number of their studied cases (400 cases) compared to the small number in our study.

Upon histopathological examination, most of the studied cases in the present study were adeno-carcinoma (68.6%). Mucinous and signet ring adenocarcinomas constituted around 25.7 and 5.7% respectively. This comes in agreement with Hamilton et al., (2010) [49], Veruttipong et al. **[35]**, Said et al. **[36]** and Sakr et al. **[21]**.

Our results exhibited significantly higher mean serum miR-21 and miR-92a levels (P<0.001) in the studied cases compared to the control group

(5.53 ± 0.17 and 7.01±0.234 ≠ 4.82± 0.20 and 6.56 ± 0.20 log RU, respectively). This was in agreement with, Wang et al. **[37]** who found that miR-21& miRNA-92a levels were significantly higher in CRC cases and added that miR92a could distinguish CRC and advanced adenoma from normal controls, with a sensitivity of >62% and specificity of >84%. No statistically significant association was found between serum miR-21 or miR-92a level and either age or sex. This comes in agreement with Guang-Hui and colleagues **[38]**.

The mean value of serum miR-21 level showed a statistically significant positive relationship with the tumor size (P<0.001) and the mean values in small (<2.5 cm), medium and large (>5cm) CRC lesions were 5.22, 5.51 and 5.61 log RU, respectively. Also serum miR-21 level showed a statistically significant positive relationship with the pathological grade of CRC (P<0.05). The mean value of grade I was (5.27 log RU), grade II (5.51 log RU) and grade III (5.64 log RU). Higher mean values of serum miR-21 were found in higher CRC pathological grades. This comes in agreement with Toiyama et al. **[39]**. In the present study, the mean value of serum miR-21 level showed a statistically significant difference in different TNM stages (P>0.05) with a statistically significant positive correlation. The mean values in Tis-T2, T3 and T4 were 5.19, 5.53 and 5.59 log RU, respectively. This comes in agreement with Schetter et al. **[40]** who found that higher expression levels of miR-21 were associated with more advanced clinical stages of CRC. The mean value of serum miR-21 level showed a statistically significant difference between different MAC Coller stages (P>0.05) in the present study with a statistically significant positive correlation. The mean values in A-B1, B2 and B3 were 5.19, 5.55 and 5.58 log RU, respectively.

The mean value of serum miR-92a level showed a statistically significant positive correlation with tumor size (P < 0.003). The mean values in small, medium and large CRC lesions were 6.75, 6.93 and 7.13 log RU, respectively This comes in agreement with Zhou et al. **[41]** who reported that over expression of miR-92a is correlated with TNM stages and poor prognosis in CRC. Nami and his colleagues **[42]** told that a significant increase in miR-92a expression was more frequently observed in CRCs than in colorectal adenomas, and suggested that miR-92a could be a potential marker for discrimination between cancers and adenomas and a potential promoter for the phenotypic changes from adenoma into carcinoma. They added that miR-92a expression was related to advanced clinical stages and to the depth of invasion.

The mean value of serum miR-92a level showed a statistically significant positive correlation and different TNM stages (P>0.03). The mean values in Tis-T2, T3 and T4 were 6.67, 7.01 and 7.08 log RU, respectively .This comes in agreement Zhang et al. **[43]** who reported that expression of miR-92a was associated with more advanced tumor–lymph node–metastasis (TNM) stage (P=0.07).

When ROC curves were applied; serum miR-21 at a cutoff value of ≥5.25 Log RU was 94.3% sensitive and 93.3% specific for diagnosis of CRC with an AUROC = 0.99. Wang et al. **[37]** reported that serum miR-21 showed a sensitivity and specificity of 93% and 91%, respectively. While, Ahmed et al. **[44]** and Schetter et al. **[40]** reported that serum miR-21 could detect CRC with 90% sensitivity and specificity. On the other hand, Guang-Hui et al. **[38]** reported that the sensitivity of serum miR-21 for detection CRC was only 65% and the specificity was 85%.

In the present study, serum miR-21 level ≥5.61 Log RU, could significantly discriminate cases of CRC lesions larger than 5 cm with 83.3% sensitivity and 82.4% specificity, with AUROC =0.87 as well as cases with high grade adeno-carcinoma with 100% sensitivity and 63.3% specificity and AUROC = 0.85. At a level ≥ 5.43 log RU, miR-21 could significantly detect cases of T stage >2 with 90% sensitivity and 100% specificity, with AUROC = 0.95, and at a level ≥5.6 log RU, it could significantly detect cases of MaCollerB3 with 68.4% sensitivity and 68.8% specificity, with AUROC = 0.71.

Serum miR-92a at a cut off value of ≥ 6.75 Log RU, was 91.4% sensitive and 80% specific for diagnosis of CRC with an AUROC = 0.91. Other studies reported plasma miR-92a to have a sensitivity and specificity of 89% and 70% respectively at a cut off value of ≥ 6.75 Log RU in distinguishing CRC patients from healthy controls **[43,44]**. Huang et al. **[14]** also reported similar results (sensitivity: 84, specificity: 71.4). In addition, many studies have found miR-92a expression to be associated with other diseases such as hepatocellular carcinoma, breast cancer, and even cardiovascular diseases, pointing to the low specificity of miR-92a. MiR-92a which has been studied extensively in plasma has also been

observed to have higher expression levels in the stool of CRC patients **[13]**. However, the sensitivity and specificity of the miRNA 92a for detecting CRC was significantly lower in stool than in plasma**[13].**

The present study found that serum miR-92a at a cut off value ≥7.05 log RU, could significantly detect cases of CRC lesions larger than 5 cm with 72.2% sensitivity and 70.6% specificity, with AUROC = 0.82. This was in agreement with Guang et al. **[38]** who reported that, at a cut off ≥7.0, AUROC value of 0.722 (95% CI = 0.633–0.811) with a sensitivity of 70% and specificity of 70%. At level ≥7.1 log RU, miR-92a could significantly detect cases of MaC CollerB3 with 63.2% sensitivity and 81.8% specificity, with AUROC= 0.76 .This was in agreement with Guang et al. **[38]** who told that serum levels of miR -92a were potential biomarkers for CRC, and at the cut-off value of ≥7.1, the sensitivity was 65.5% and the specificity was 82.5% with an AUROC = 0.786.

At level ≥6.96 log RU, miR-92a could significantly detect cases of T stage >2 with 77.4% sensitivity and 100% specificity, with AUROC= 0.9 This was in agreement with Guang et al. **[38]**.

When serum miR-21 (≥5.25 log RU) and miR-92a (≥6.75 log RU) were combined together, the sensitivity for detection of CRC was increased to 97.1% and the specificity was 93.3%.

In conclusion, serum miR-21 and miR-92a expression levels represent a sensitive and specific tool for detection of CRC with higher accuracy of miR-21.

Funding: None.

Conflicts of interest: None.

Ethical approval: Approved

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Response to Hepatitis B Vaccine in Egyptian Chronic Hepatitis C Patients

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Key words:
Hepatitis B; HBV-vaccine; hepatitis C; Schistosomiasis; vaccination response

Background and study aim: Egypt unfortunately has the highest worldwide prevalence of chronic hepatitis C (CHC). Patients with CHC are advised to be vaccinated against hepatitis B virus (HBV) infection. Response to hepatitis B vaccination and risk factors for a weak response are not clearly defined.. The aim of this study is to assess the response to hepatitis B vaccination in CHC patients and identify predictors of a weak response.

Patients and Methods: This prospective study included 112 consecutive adult, treatment- naive, CHC patients (cases group) and 54 non-hepatitis C virus (HCV) subjects (control group). Demographic and laboratory variables including HCV-viral load, schistosomal antibody (Ab) titre, and histopathological examination of liver biopsy were assessed. Three intramuscular 20 µg doses (given at 0, 1 & 6 months) of HBV-vaccine (Euvax-B, Korea) were administered, and hepatitis B surface antibody (HBsAb) titres were evaluated 6 – 8 weeks after the 3rd dose.

Results: Out of 112 CHC patients, five (4.5%) had HBsAb titres of <10 mIU/mL, 20 (17.9%) had <100, and 50 (44.6%) had titres of >1000. In comparison, out of 54 controls, one (1.9%) had a titre of <10 mIU/mL, 2 (3.8%) had <100, and 41 (75.9%) had >1000 (P= 0.001). CHC patients had highly significant lower mean Ab titres than controls (P<0.001). In a univariate regression analysis, HBsAb titre was negatively associated with age (P<0.001), ALT (P=0.03), AST (P=0.03), FIB-4 score (P=0.008) and schistosomal Ab titre (P= 0.007) and positively associated with platelet count (P=0.01). There was no association with gender, BMI, viral load or other variables (including METAVIR grade or stage). A multivariate regression analysis in CHC patients showed that age (P= 0.02) and schistosomal Ab titre (P= 0.04) were independent predictors of HBsAb titre response.

Conclusions: CHC patients, particularly of older age or with schistosomiasis, have a significantly weakened response to the HBV-vaccine.

INTRODUCTION

The most common viral diseases causing chronic liver diseases (CLD) worldwide are hepatitis B virus (HBV) and hepatitis C virus (HCV) infections **[1]**. The global prevalence of hepatitis B surface antigen (HbsAg) positivity varies greatly and studies in the Middle East have shown that Egypt lies in the zone of intermediate prevalence, with HBsAg seroprevalence ranging between 3% to 11%, and genotype D is the most prevalent **[2,3]**. On the other hand, Egypt is cursed with the highest worldwide prevalence of chronic hepatitis C (CHC), with an overall anti-HCV Ab prevalence of 14.7% and it is estimated that 9.8% of Egyptians are chronically infected with HCV. The main genotype in Egypt is genotype 4 (G4), which is responsible for >90% of infections, while the remaining infections are attributable to genotype-1 **[4]**. In patients with dual chronic HCV and HBV infections, disease outcomes, including the development of liver cirrhosis and HCC, are generally more severe than those in patients with monoinfection. In addition, the incidence of HCC in co-infected patients is higher than the incidence in monoinfected patients **[5,6]**.

Both acute and chronic coinfection with HBV in CHC patients is preventable by HBV vaccination **[7,8]**. However, responses to the HBV vaccine are variable among different patients. Response of CHC patients to the HBV vaccine, factors affecting this response, suitable doses and the interval between doses are active areas of research especially in Egypt where HCV-G4 predominates and schistosomiasis is present.

Aim of the study:
To assess the response to HBV- vaccination in CHC patients and identify predictors of a weak response.

PATIENTS AND METHODS

This study was designed as a prospective clinical cohort study to assess the immunogenicity of the HBV vaccine in patients with CHC in comparison to healthy control subjects. The study protocol was approved by the Ethics Committee of Benha Faculty of Medicine. All patients gave written informed consent before enrollment in the study.

One hundred and twelve adult treatment-naive patients with CHC (cases group) and 54 non-HCV subjects (control group) who gave informed consent were included in the study. The inclusion criteria were: age older than 18 years, and CHC (in the cases group) that was diagnosed by both HCV- Ab (by 4th generation ELISA test) and HCV- RNA- PCR positivity for ≥6 months before inclusion in the study. We excluded patients (or controls) who were positive for HBs Ag or HBc Ab (total); underwent previous HBV-vaccination; were pregnant, diabetic; underwent haemodialysis, organ transplantation, or immunosuppressive therapy; or who demonstrated malignancy and/or decompensated cirrhosis with ascites and/or HCC. Non-HCV healthy controls were recruited from subjects who came for vaccination for pre-employment and pre-marital purposes or contacting HbsAg- positive patients.

Demographic data for patients and controls including age, gender and body mass index (BMI) were collected. Laboratory data, including haemoglobin level, white blood cell count, platelet count, liver function profile (ALT, AST, total bilirubin, prothrombin concentration and serum albumin), HCV-viral load and schistosomal Ab titre, were collected for all cases. FIB-4 score was calculated for all cases according to the standard formula **[9,10]**. Abdominal ultrasonography was performed to exclude the presence of ascites and/or hepatic focal lesions, and histopathological data comprising METAVIR necroinflammatory grade (A0-3) and fibrosis stage (F0-4) were reported for those cases who underwent liver biopsy before receiving antiviral treatment within the national project of the Egyptian Ministry of Health.

The Euvax B vaccine (Euvax B, LG Life Sciences, Korea) used in this study is a liquid vaccine consisting of highly purified, non- infectious HBsAg particles absorbed onto aluminium salts as an adjuvant and preserved with thimerosal. It is a recombinant DNA hepatitis B-vaccine derived from HBsAg produced by recombinant DNA technology in yeast cells (Saccharomyces cerevisiae).

Vaccination of both cases and controls was accomplished by administering 3 doses of the Euvax B vaccine, each dose containing 20 µg of the active ingredient, purified HbsAg in a 1-mL volume; the vaccine was intramuscularly injected into the deltoid muscle at 0, 1, and 6 months. The response to the vaccine was measured by quantitatively assessing HBsAb titres (by ELISA test, according to the manufacturer's instructions), 6 - 8 weeks after the 3rd vaccination dose. Non-responders were defined as patients who had an HBs-Ab titre of less than 10 mIU/mL, poor responders were patients with an HBs-Ab titre between 10 and 100 mIU/mL, and good (robust) responders were those who had HBs-Ab titre of more than 100 mIU/mL.

Statistical Methods :
SPSS (version 21) was used for statistical analysis. Comparison of patients and control groups was performed by using a two tailed "**t**" test for continuous variables and a Chi square test for categorical or dichotomous variables. Non-parametric tests were used when indicated. Univariate regression analysis was performed to assess the association between continuous variables and HBs-Ab titre within CHC patients. Independent samples two-tailed "**t**" test was performad to assess the association between categorical or dichotomous variables and HBs-Ab titre. Significant variables associated with HBs-Ab titre in all univariate analyses were included in a multivariate regression analysis to identify independent predictors of the response. Pearson correlation test was performed to test the correlation between age and HBs-Ab titre. For all tests, 0.05 was set as the level of significance.

RESULTS

This study included 112 CHC patients and 54 healthy controls. Out of the 112 patients, 36 (32.1%) were males. Tables (1) and (2) show descriptive demographic, laboratory and histo-pathological data for CHC patients. Only 79 patients had schistosomal- Ab data, and 60 had histopathological data of liver biopsy. Table (3) shows the comparison between cases and control groups in terms of age, gender distribution and BMI. Regarding the response to hepatitis B vaccine, we found that CHC patients had significantly lower HBs-Ab titres than healthy controls and significantly more number of non responders (Table 4 and Figure 1).

Univariate regression analysis showed that there were significant associations between HBs-Ab titre levels and age (P<0.001), AST (P= 0.03), ALT (P= 0.03), FIB4 score (P= 0.008), and platelet count (P= 0.01). Age showed a significantly negative linear correlation with HBs Ab response (Figure 2). There was no statistically significant difference between males and females regarding HBs-Ab titres while there was a statistically significant negative association between the presence of schistosomal Ab and HBs-Ab titre (P= 0.007).

Variables that showed a significant association with HBs-Ab titre in univariate analysis were included in multivariate regression analysis to identify independent predictors of HBs-Ab titre response. The only independent predictors for HBs-Ab response were age and the presence of schistosomal Ab (Table 5).

Table (1): Description of demographic and laboratory data of CHC patients**.**

Parameter	Mean ± SD	Range
Age (Years)	44.2 ± 10.2	**40** (19-59)
Weight (Kg)	82.1 ± 14.4	**81** (45-126)
Height (Meter)	1.62 ± 0.08	**0.35** (1.47-1.82)
BMI	31.5 ± 6.1	**34** (15.2-49.2)
Hb (gm/dL)	13.1 ± 1.5	**8.2** (9.8-18)
WBC (/mm3)	6379 ± 1855	**10300** (2700-13000)
Platelet (×1000)	198.9 ± 62.6	**303** (72-375)
ALT (U/L)	50.9 ± 33	**160** (10-170)
AST (U/L)	47.1 ± 30.2	**193** (12-205)
Bilirubin (mg/dL)	0.87 ± 0.28	**1.35** (0.35-1.7)
Albumin (gm/dL)	4.1 ± 0.39	**2.3** (2.4-4.7)
Proth. Concent. %	89.1 ± 8.1	**40** (60-100)
FIB-4	1.71 ± 1.25	**8.5** (0.34-8.84)

Table (2): Description of schistosomal Ab and histopathology of liver biopsy of CHC patients**.**

Parameter	Number (%)
Schistosomal Ab (79 patients)	
- Positive	**37 (53.2%)**
- Negative	**42 (46.8%)**
METAVIR Necroinflammatory Grade (A) (60 patients)	
A1	**34 (56.7%)**
A2	**24 (40%)**
A3	**2 (3.3%)**
METAVIR fibrosis stage (F)	
F1	**7 (11.7%)**
F2	**28 (46.7%)**
F3	**23 (38.3%)**
F4	**2 (3.3%)**

Table (3): Comparison of demographic variables between cases and controls.

Variables	Cases (N = 112)	Control (N = 54)	P
Gender; **Male**	36 (32.1%)	19 (36.5%)	0.3
Age (years)	44.3±10.3	35.9±7.9	0.05
BMI (kg/m^2)	31.1±7.6	30.8±6.1	0.8

Table (4): Comparison of HBV vaccine responses (HBs Ab titres) between cases and controls.

Variables	Cases (N = 112)	Control (N = 54)	P
HBs-Ab titre (mIU/ml)	675.3 ± 446.5	931.9 ± 337.1	<0.001
Vaccine response			0.001
Non Responders (titer <10 mIU/mL)	5 (4.5%)	1 (1.9%)	
Poor Responders (10-100 mIU/mL)	15 (13.4%)	1 (1.9%)	
Good Responders (>100 mIU/mL)	92 (82.1%)	52 (96.2%)	

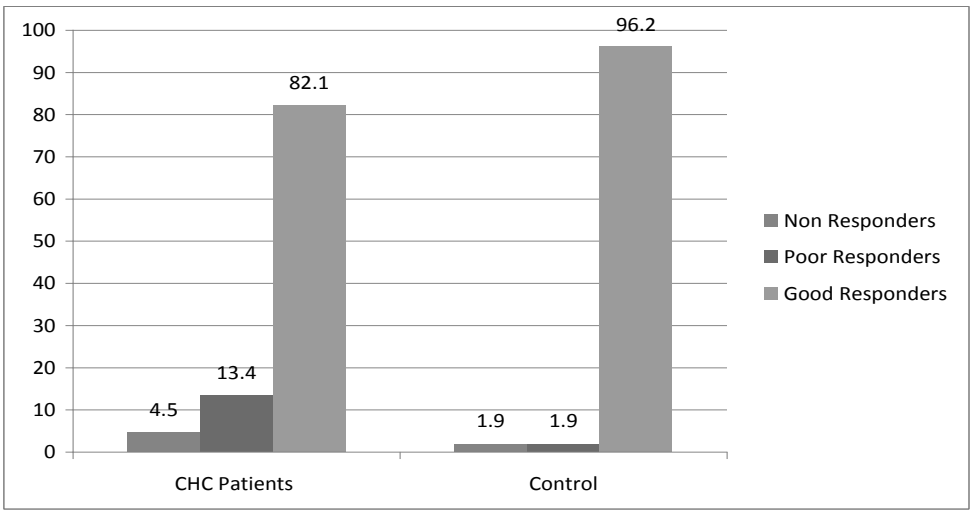

Figure (1): Percentages of non-, poor-, and good- responders among cases and controls.

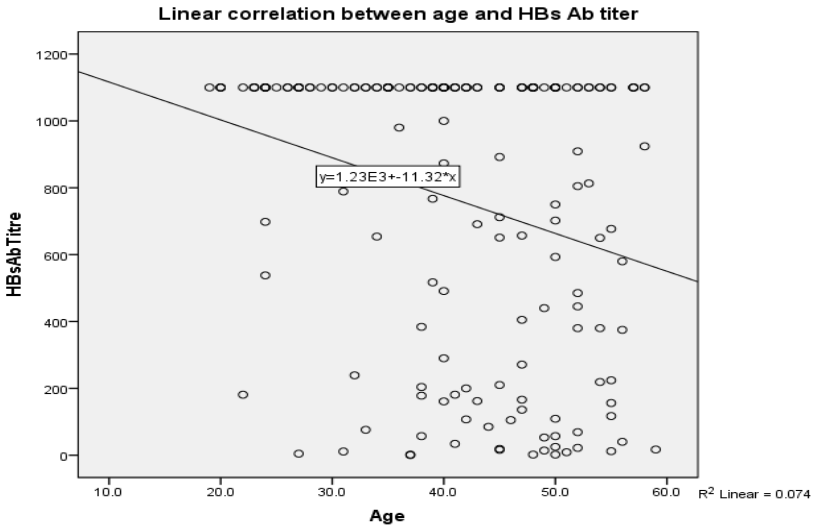

Figure (2): Linear correlation between age and HBs Ab titre..

Table (5): Multivariate regression analysis for independent predictors of the response to HBV vaccination (HBs Ab titre).

Variables	B	95% CI	P
Age (years)	- 15.9	- 29.4 – - 2.6	0.02
Bilharzial Ab	- 227.2	- 438.7 – - 15.7	0.04
BMI (kg/m^2)	31.1±7.6	30.8±6.1	0.8
Platelet count	2.2	- 0.9 – 5.4	0.2
ALT (U/mL)	4.8	- 3.6 – 13.2	0.3
AST (U/mL)	- 8.8	-24.1 – 6.4	0.3
FIB-4 score	155.4	- 163.1 – 473.9	0.3

DISCUSSION

Increased occurrence of fulminant liver failure, rapid progression to cirrhosis and HCC in patients co-infected with HBV and HCV have been documented in many epidemiological studies **[11,12]**. Fortunately, HBV infection is a preventable disease due to the development of HBV-vaccine. However, the vaccination response in patients with CLD is variable.

In the present study, five out of 112 patients with CHC (4.5%) did not respond to HBV- vaccine in comparison to one out of 54 (1.9%) healthy controls. A good (robust) response (HBs Ab >100 mIU/ mL) was achieved in 87/112 (77.6%) cases compared to 51/54 (94.4%) controls. This result is in agreement with a previous study by Minakari et al. **[13]** who found that 4/32 patients with CHC (12.5%) did not respond to HBV vaccination in comparison to 0% of healthy controls. This finding is explained by the evidence that HCV infects immune cells, such as macrophages, B cells, and T cells, with many reports suggesting that the HCV- core, the first protein expressed during the early phase of viral infection, moderates immunomodulatory functions to suppress host immune responses. This altered function of immune cells caused by HCV infection may explain the ineffective immune response to HCV **[14,15]** and may subsequently affect the response to vaccination.

Surprisingly, in Minakari's study, there was an unexplained low but robust response in healthy controls (17/32, 53.1%) compared to the response in 21/32 (65.6%) cases of CHC with genotypes 1 and 3 (in contrast to genotype 4 in >90% of Egyptian cases). Wiedmann et al. **[16]** reported higher failed response rates, observing non-response in 18/59 (31%) of their studied CHC cases compared to 5/58 (9%) healthy controls ($P<0.05$) and low response (anti-HBs 10-99 mIU/mL) in 19% of cases and 17% of controls. The authors were to explain the cause of such a high rate of response failure. On the other hand, Keeffe and Krause **[17]** found that 100% of their studied patients with CHC responded to HBV vaccination. The difference in the seroconversion incidence may be attributed to the difference in the distribution of fibrosis stages or liver disease advancement within each group, as patients with more advanced CLD demonstrate lower response to HBV vaccine **[18,19]**. This phenomenon was also observed in our study, as there was an association between a higher FIB-4 score (reflecting advancement of liver fibrosis) and mean HBs- Ab response level ($P=0.008$).

In a univariate regression analysis, we found that parameters that indicate more fibrosis, such as increased AST levels, decreased platelet counts and increased FIB4 scores, were associated with decreased HBs-Ab titres.

In this study, we found that age was an independent predictor of HBs-Ab response. As age increased, the vaccine response decreased, confirming the concept that older patients demonstrate lower responses to HBV vaccination, as reported by Denis et al. **[20]** who found that only 32 out of 70 patients over 60 years (45.7%) responded to the HBV vaccine, and by de Rave et al. **[21]** who reported a 60% response rate in patients ≥60 years old; this is also in agreement with Al-Zahaby and colleagues **[22]** who recommended a double dose vaccination schedule for such patients.

Schistosomiasis represnts a historical and current health problem in Egypt with estimated prevalence of 3 - 10% in the general population **[23].** In their study of 3,596 Egyptian patients, Abdel-Rahman et al. **[24]** reported that 27.3%

had both HCV-RNA and schistosomal Ab in their sera. Patients demonstrating schistosomiasis/ HCV coinfection have increased HCV morbidity and chronicity **[25]**. Defects in immune response with altered IFN-γ and IL-5 serum levels (related to cell-mediated immunity) and IgE levels (humoral immunity) have been reported, with a relative shift from cellular to humoral immunity, which might play a role in the persistence and severity of both diseases and lower HCV clearance rates **[26,27]**.

The novel finding of our study is the association between the presence of schisosomal Ab and low HBs-Ab titres after vaccination, indicating that schistosomiasis alters the immunologic response to HBV vaccine.

The presence of schistosomal Ab and older age were found to be independent predictors of the response to HBV vaccine in our studied CHC patients.

CONCLUSIONS

Patients with CHC, especially older patients and patients who are schistosomal Ab positive, demonstrate a lower response to HBV vaccination.

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

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