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CONTENTS

Expression of Vascular Endothelial Growth Factor in the Gastric Mucosa of Patients with Portal Hypertensive Gastropathy

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Key words:
VEGF, Gastric mucosa, Portal Hypertensive Gastropathy

Background and study aim: Portal hypertensive gastropathy (PHG) is a common finding in patients with cirrhosis and portal hypertension that occurs in between 7% and 98% of cases. High levels of vascular endothelial growth factor (VEGF) in mesentery suggested their contribution in portal hypertension secondary to liver cirrhosis. VEGF participates in regulation of angiogenesis in gastric wall in portal hypertension. The aim was to evaluate the serum concentration of VEGF and gastric mucosal expression of VEGF and its possible association with PHG.

Patients and Methods: Serum levels and gastric mucosal expression of VEGF were measured in fifty seven patients with liver cirrhosis and portal hypertensive gastropathy as well as eleven patients with liver cirrhosis without portal hypertensive gastropathy and another twenty one persons served as control group. They were clinically assessed and laboratory investigations were done including liver biochemical profile, and viral markers. Severity of liver disease was assessed by Child-Pugh, model for end stage liver disease (MELD) and updated (uMELD) scores. The presence of PHG was diagnosed by esophago-gastroduodenoscopy.

Results: Serum VEGF increased in patients with liver cirrhosis compared to healthy control. But there was no significant difference between patients with PHG and patients without PHG as regard to serum VEGF.VEGF expression in the gastric mucosa was highly significant in patients with PHG than patients without PHG and control group. Serum VEGF has no correlation with severity of liver disease or PHG grade.

Conclusion: VEGF was highly expressed in gastric mucosa rather than elevation of serum VEGF in patients with PHG.

INTRODUCTION

Portal hypertensive gastropathy (PHG) is a common finding in patients with cirrhosis and portal hypertension **[1]** and it is endoscopically characterized by a mosaic-like or snake skin pattern of the gastric mucosa, mainly in the body and fundus of the stomach and more rarely in the gastric antrum. These gastric mucosal lesions represent another frequent cause of upper gastrointestinal bleeding, even though esophagogastric varices remain the major source of bleeding in patients with portal hypertension **[2]**. PHG observed during endoscopy in patients with cirrhosis are very common, occurring in between 7% and 98% of cases according to different series **[3]**.

Vascular endothelial growth factor (VEGF) is a secreted, 46 kDa dimeric protein, active as direct and specific mitogen for vascular endothelial cells thus a well-known mediator of angio-genesis in physiological and pathological conditions **[4]**. High levels of VEGF in mesentery suggested its contribution in portal hypertension secondary to liver cirrhosis.[5] High serum VEGF in late stage may reflect its prognostic value in liver cirrhosis **[5]**. VEGF participates in regulation of angiogenesis in gastric wall in portal hypertension **[6]**. Very few studies have been recently published about this issue. So, the aim of this study was to evaluate the serum concentration of VEGF and gastric mucosal expression of VEGF and their possible association with PHG in patients with cirrhosis.

PATIENTS AND METHODS

Patients :

The current study was carried out on 68 patients with liver cirrhosis divided into two groups according to presence or absence of PHG attended or admitted to Hepatology, Gastroenterology and Infectious Diseases Department, Benha University Hospital, within the period between October 2014 and March2015, after approval by the scientific committee of Benha Faculty of Medicine. Another twenty-one persons served as control group.

Patients with cirrhosis were diagnosed by clinical manifestations, laboratory investigations and ultrasonography. Patients were classified according to presence or absence of portal hypertensive gastropathy which was diagnosed by upper gastrointestinal endoscopy.

Patients with congestive heart failure, renal failure, lung disease, malignancy, hepatic encephalopathy, gastrointestinal bleeding was excluded from this work at the time of study .

Methods :

All patients were subjected to full history taking thorough clinical examination and routine laboratory tests including liver biochemical profile as serum bilirubin (total, direct), serum albumin, prothrombin time, international normalized ratio and serum creatinine, viral markers: HCVAb (Hepatitis C virus antibody) and HBsAg(Hepatitis B virus surface antigen).Each patient was assigned a score and a grade reflecting the severity of liver affection according to:

- The numerical system of Child Turcotte Pugh (CTP) **[7].**
- MELD score (Model for End Stage Liver Disease) **[8].**
- uMELD score (Updated Model for End Stage Liver Disease).**[9].**

VEGF was measured in serum of all subjects: using Human Vascular Endothelial Growth Factor ELISA Kit. Expression of VEGF in gastric mucosa was measured in all cases **[10,11]**.

Pelvi-abdominal Ultrasonography was done using (LOGIC LG) with a convex probe (3.75 MHZ} for evaluation of liver (size, echopattern and portal vein, presence of focal lesion), evaluation of spleen (size and echopattern) and the presence of ascites.

Esophagogastroduodendoscopy was done using disinfected upper gastrointestinal video scope (OLYMPUS model) after good preparation of the patient. Complete evaluation of the esophagus, stomach and the duodenum down to the second part of the duodenum. Esophageal varices were classified as small or large **[12]**.

- small esophageal varices were defined as: Varices that flatten with insufflation or minimally protrude into the esophageal lumen.
- While large esophageal varices were defined as: Varices that protrude into the esophageal lumen and touch each other (presence of confluence), or that fill at least 50% of the esophageal lumen.

The grading (I-IV) classification:

- grades I and II were reclassified as small and
- grades III and IV were reclassified as large for this study.

PHG were reported according to Modified grading system proposed by the Baveno III meeting (Baveno, Italy (2000) on portal hypertension **[13]** :

- PHG is mild when a pink mosaic-like mucosal pattern with no red signs or black–brown spots is present.
- PHG is severe when the mosaic-like mucosal pattern is red and superimposed by any red sign (red point lesions and/or cherry-red spots) or black–brown spots.

Gastric mucosal biopsies were taken for studying the expression of VEGF.

Statistical Analysis :

Statistical package (SPSS, version 10.0) was used for data management. Descriptive statistics was presented as mean±standard deviations for continuous variables, number and percentage for categorical variables (frequency distribution). Unpaired Student t-test (two sided) was used to test the significance of difference between the mean value of studied groups and chi -square test was used for comparison of categorical variables. Pearson correlation test was used to identify the correlation between VEGF and different clinicopathological variables. The significance level was set at $p<0.05$.

RESULTS

Characteristics of the studied patients :

Sixty eight patients with liver cirrhosis were included in this study. The cases were divided into two groups according to presence or absence of PHG, cases with PHG were fifty seven while the other eleven cases had no PHG. The mean age was 53.5±9.1 in patients with PHG compared

to 55.9±10.6 in patients without PHG. There was no statistically significant difference between groups as regards to the age and gender but PHG tends to be more common in males than females (males were 35 cases and females were 22 cases).

HCV infection was found in 98.2% of patients. Most of patients presented at Child B grade, and had a higher MELD and uMELD scores. By ultrasonography; spleen size and Portal vein diameter were higher in cirrhotics with PHG than in cirrhotic without PHG cases. Otherwise, other ultasonographic parameters did not distinguish between the two groups (Table 1).

Regarding endoscopy, fifty seven cases had endoscopically based portal hypertensive gastropathy (83.8%) and eleven had no portal hypertensive gastropathy (16.2%). large varices were detected in (61.4%) of patients with PHG and small varices in (31.6 %) of patients (Table 2).

Serum VEGF in the studied groups :

Concerning serum VEGF value, it was significantly increased in cirrhotic patients with PHG as it ranges between (34-234.1) pg/ml with mean (65.3) pg/ml and also in cirrhotic patients without PHG as it ranges between (38.8-99.5) pg/ml with mean (62.7) pg/ml compared to control group as it ranges between (24.4-37.5) pg/ml with mean (28.3) pg/ml , but there was no statistically significant difference between cirrhotic patients with PHG and cirrhotic patients without PHG (Table 3).

Expression of VEGF in gastric mucosa in studied groups :

VEGF expression in gastric mucosa was highly significantly expressed in patients with liver cirrhosis with PHG than cirrhotic patients without PHG and control group (Table 4 and Figure 1,2).

VEGF and severity of liver disease :

There was no significant correlation between VEGF and severity of liver disease (Child, MELD and uMELD), varices grade, number of varices or PHG grade (Table 5).

Table (1) : Demographic features of the studied patient groups

Characteristics	Patients with PHG (n = 57)	Patients without PHG (n = 11)	P. value
Age (years)			
Range	32- 72	35- 70	0.455
Mean ± SD	53.5 ± 9.1	55.9 ± 10.6	
Gender			
Male	35 (61.4%)	5 (45.5%)	0.256
Female	22 (38.6%)	6 (54.5%)	
Occupation			
Farmer	10 (17.5%)	1 (9.1%)	0.34
Non- farmer	26 (65%)	16 (80%)	
Etiology			
Smoking	19 (33.3%)	4(36.4%)	0.55
Shistosomiasis	20 (35.1%)	0 (0%)	0.015*
HCV	56 (98.2%)	11 (100%)	0.83
HBV	1 (1.8%)	1 (9.1%)	0.18
Child- Pugh score			
Child A	12 (21.05%)	3 (27.72%)	0.64
Child B	33 (57.89%)	4 (36.36%)	0.66
Child C	12 (21.05%)	4 (36.36%)	0.55
MELD Score			
Mean ± SD	21 ± 8.2	14.2 ± 4.6	0.02*
uMELD Score			
Mean ± SD	4.4 ± 0.79	3.2 ± 0.4	0.000*
Ultrasound			
Spleen size	13.8 ± 4.2	10.3±8.1	0.02*
PV (cm))	1.9 ± 0.6	1.4 ± 0.2	0.01*
Ascites	31(56.4%)	8(72.7%)	0.14

PHG, portal hypertensive gastropathy; SD, Standard deviation; HCV, hepatitis c virus; HBV, hepatitis B virus; MELD, Model for end stage liver disease; uMELD , updated MELD; PV, portal vein; *, Significant.

Table (2) : Endoscopic features of the studied patients

Variables	Group I PHG N=57		Group II Non PHG N=11		P-value
	N	%	N	%	
Varices	53	93	9	81.8	0.24
Small varices	18	31.6	5	45.5	0.24
Large varices	35	61.4	4	36.4	0.26
Gastric varices	9	16.1	1	9.1	0.48
PHG grade					
Mild	26	45.6	---	---	---
Severe	31	54.4	---	---	---

PHG, portal hypertensive gastropathy

Table (3) : Values of VEGF among the studied groups

Variable	Group I PHG N=57		Group II Non PHG N=11		Group III Control N=21		P-value
	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	
VEGF (pg/ml)	34- 234.1	65.3± 31.1	38.8- 99.5	62.7± 17.9	24.4- 37.5	28.3± 3.9	0.000*

VEGF, vascular endothelial growth factor; PHG, portal hypertensive gastropathy; *, Significant; SD, Standard deviation.

Table (4) : Expression of VEGF by folds in gastric mucosa among the studied groups

Variable	Group I PHG	Group II Non PHG	Group III Control	P - value
VEGF (folds)	31.12	6.06	1.0	I,II :0.002* I,III:0.000* II,III:0.009*

VEGF, vascular endothelial growth factor; PHG, portal hypertensive gastropathy; *, Significant.

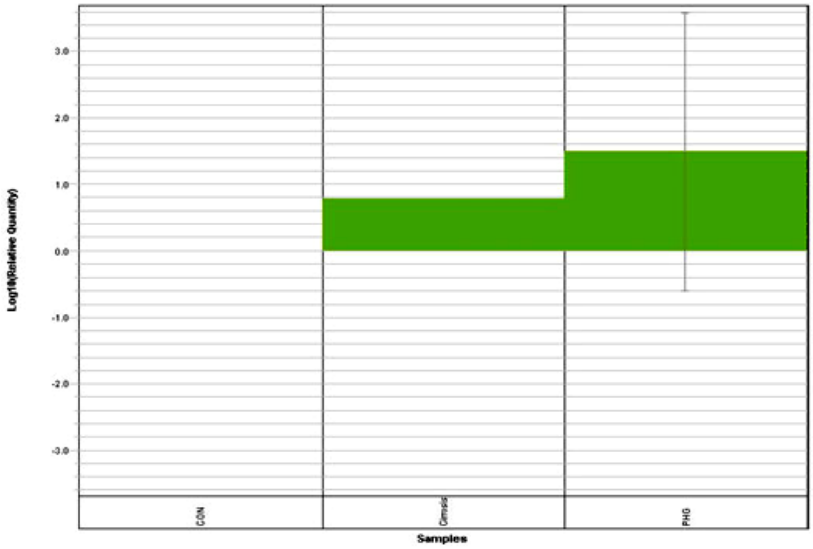

Figure (1) : Expression levels of VEGF mRNA for Both PHG and non PHG Sample. Expression levels of VEGF mRNA in both PHG and non PHG samples are indicated by green bars. This color also indicates the samples in RQ. Because control samples are used as calibrators, the expression levels are set to one. But because the expression levels were blotted as log¬10 values (and the log 10 of 1 is 0), the expression level of the control samples appear as 0 in the graph. Because the relative quantities of the VEGF mRNA are normalized against the relative quantities of the GAPDH (endogenous control), the expression level of the endogenous control is 0; there are no bars for GAPDH.

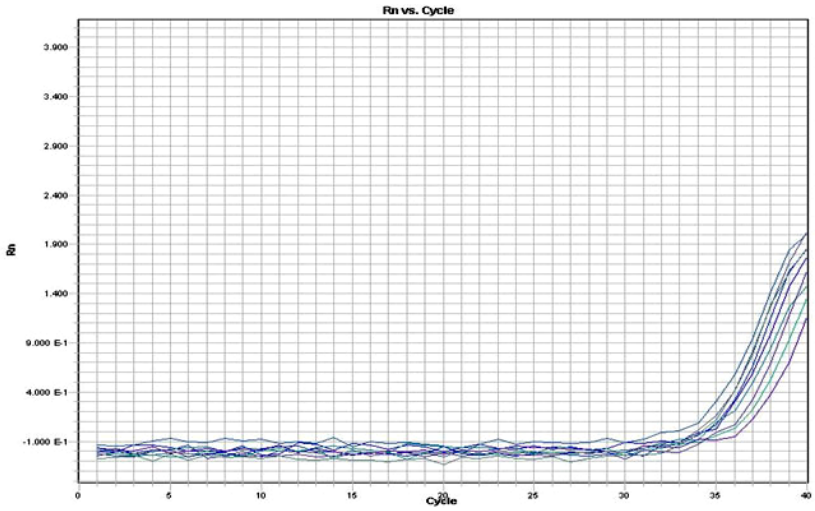

Figure (2) : Amplification plot curves for all detectors in the studied groups (Curves by ABI 7900 Real Time)

Table (5) : Correlation between VEGF and different parameters in PHG cases

VEGF	Pearson correlation	P-value
Child Score	-0.18	0.34
MELD	-0.14	0.5
uMELD	-0.13	0.53
Varices grade	-0.04	0.69
Number of varices	-0.1	0.42
PHG garde	-0.03	0.78

VEGF, vascular endothelial growth factor; PHG, portal hypertensive gastropathy; MELD, Model for end stage liver disease; uMELD , updated MELD.

DISCUSSION

Portal hypertensive gastropathy (PHG) is a unique endoscopic finding in cirrhosis and portal hypertension is the main cause for the development of PHG **[1]**.

PHG is clinically important because it may cause acute (and even) massive or insidious, blood loss. The diagnosis of PHG is (only) made endoscopically; which is often characterized by an abnormality of the gastric mucosa described as a mosaic-like pattern resembling 'snake-skin', with or without red spots **[14]**.

In the present study, PHG was observed in (83.8%) of patients, PHG was mild in (45.6%) of patients and severe in (54.4%) of patients, these results were near to the results of Kim et al**.** who observed PHG in (90%) of patients, PHG was mild in (25.4%) and severe in (64.7%) of patients **[15]**.

In this study, endoscopy revealed that PHG cases were associated with varices in (93%) of patients, (35) of them (61.4%) were with large varices and (18) of them (31.6%) of patients were with small varices; these results were in agreement with the results of Kim et al**.** who documented that PHG was associated with esophageal varices grade and the prevalence of PHG was higher in patients with large esophageal varices than in those with small sized varices **[15]**. This may results from a more severe portal hypertension in patients with both severe PHG and large esophageal varices. Moreover, in our study, endoscopy revealed that there was no significant difference between the two groups as regard to presence of gastric varices, these results agreed with the results of Kim et al. who stated that there was no correlation between gastric varices and PHG **[15].**

Concerning VEGF serum values, our results showed that it was significantly increased in cirrhotic patients with PHG as it ranges between (34-234.1) pg/ml with mean (65.3) pg/ml and also in cirrhotic patients without PHG as it ranges between (38.8-99.5) pg/ml with mean (62.7) pg/ml compared to control group which ranges between (24.4-37.5) pg/ml with mean (28.3)pg/ml, these results agreed with the results of Jaroszewicz et al. who observed also that VEGF value was significantly increased in liver cirrhosis with mean (153.1) pg/ml compared to healthy individuals (46.8) pg/ml **[16],** our results also agreed with Abdelmoaty et al. who documented that VEGF value was significantly increased in liver cirrhosis with mean(106.1) pg/ml compared to healthy individuals (41.5) pg/ml **[5].**These results indicate possible association between VEGF signaling pathway and enhanced angiogenesis during liver cirrhosis **[16].**

As regard to serum VEGF level, the results showed that there is no significant difference between patients with PHG and patients without PHG, this may be due to the fact that the level of portal VEGF is significantly higher than that of systemic VEGF **[17]**, while in the present study we measured the level of VEGF in systemic circulation only and not in portal circulation as recorded by Snowdon et al**. [18]**.

Expression of VEGF in gastric mucosa was highly significant in patients with liver cirrhosis without PHG (6.06 folds to control) and cirrhotic patients with PHG (31.12 folds to control) than control group, these results may be explained by Abdelmoaty et al who stated that VEGF might be involved in cirrhosis associated angiogenesis **[5].**

The previous results agreed with the results of Pan et al. who showed a significantly elevated expression of VEGF in the gastric walls during the development of portal hypertension, the expression was mainly located in the basal layer of the gastric mucosa, suggesting that VEGF plays a certain role in the vascular changes of the gastric wall in portal hypertensive gastropathy **[6].** These results agreed also with the results of Colle et al. who observed in patients and in animal models that there is an increased expression of VEGF in portal hypertensive gastric mucosa and can be involved in the development of portal hypertensive gastropathy **[19]**. Previous studies found in vivo increased angiogenesis in the mesenteric microvasculature of an experimental model of portal hypertension rats with cirrhosis, also showed increased expression of VEGF in the mesentery of these rats, which was significantly higher compared with the control groups **[5]**. Gjeorgjievski and Cappel proposed that gastric mucosal hypoxia and elevation of VEGF might accelerate mucosal angiogenesis and increase blood flow **[20]**.

Concerning the VEGF and severity of liver disease assessed by Child-Pugh score, MELD score and uMELD score, our results showed that there is no significant correlation between VEGF and these scores which was in agreement with Assy et al. who documented that circulating VEGF level in patients with liver cirrhosis could not serve as an indicator of the progression of

chronic liver disease but rather, they may reflect increased portal hypertension or decreased hepatic regenerative activity or the combination of both **[21]**.

Concerning the VEGF and varices grade, our results showed that there is no significant correlation between them, which is similar to the results of Makhlouf et al. **[22]**.

CONCLUSION

In conclusion, the serum VEGF increase in patients with liver cirrhosis compared to healthy control. According to the expression VEGF in gastric mucosa, it was highly significant in patients with PHG than patients without PHG and control group. The serum VEGF didn't increase in patients with advanced stages of liver cirrhosis, which is reflected by Child-Pugh score, MELD score and uMELD score or with PHG grade as well.

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Conflicts of interest: None

Ethical Approval: A written informed consent was taken from all included patients, and the study was approved by the Ethical Committee of our institution.

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Prognostic Role of Serum Alpha-Fetoprotein in Hepatocellular Carcinoma Patients with Radiofrequency Ablation

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Key words:
Alpha-fetoprotein, Hepatocellular carcinoma, Prognosis, Overall survival, Recurrence, Disease-free

Background and study aim: Prognostic value of serum alpha-fetoprotein (AFP) in hepatocellular carcinoma (HCC) is still debatable. We aimed to study this role in HCC patients who underwent radio-frequency ablation (RFA).

Materials and Methods: Records from HCC patients were retrospectively analyzed between January 2012 and December 2016. A minimum data set for each patient record of a follow-up period of at least 1 year was pre-defined before enrollment. In all, 153 patients were enrolled. AFP levels were recorded for all patients at the time of diagnosis, 1 month after RFA and at 3-month intervals afterward. Patients were divided according to pretreatment AFP level into 3 groups: group 1: AFP <20 ng/mL, group 2: AFP 20-200 ng/mL and group 3: AFP >200 ng/mL.

Results: Pretreatment AFP is not significantly correlated with age, baseline lesion number or size, baseline Child score or class, post RFA recurrence or death. The overall survival rates were 95%, 75.6%, 55.6%, 48.8%, and 48.8% at 1,2,3,4, and 5 years respectively. On comparing the 3 groups on disease-free survival, there was no statistically significant difference among the three classes. Child class A patients showed statistically significant better survival after RFA than those with Child class B. The ROC curve showed that AFP had inadequate accuracy to discriminate survivors and deceased patients and to discriminate patients with recurrence from those without recurrence.

Conclusion: AFP level could not be used as a good predictor of either death or recurrence of HCC after RFA

INTRODUCTION

Alpha-fetoprotein (AFP) is considered the most thoroughly investigated marker for diagnosing hepatocellular carcinoma (HCC). However, it has a limited diagnostic performance for the surveillance of HCC. Two reasons may explain this; first, high AFP levels could be seen in patients with chronic hepatitis and liver cirrhosis **[1]**, second, only a small proportion of early-stage HCCs (10–20%) present with increased AFP levels **[2]**.

The American Association for the Study of Liver Diseases (AASLD) guidelines for HCC diagnosis and treatment, however, has recently eliminated AFP measurement from the surveillance armamentarium because of its poor sensitivity and specificity for the diagnosis of HCC **[3]**.

Also, AFP assessment is not included in The Barcelona Clinic Liver Cancer (BCLC) classification system, although it has been identified by several studies as an overall independent predictor of survival **[4]**.

However, most of studies about the prognostic value of AFP have included heterogeneous cohorts of patients, thus preventing a proper evaluation of its performance as a prognostic marker in a selected subset of patients **[5]**.

In this study, we aimed at evaluating the prognostic role of AFP in patients with HCC treated with radiofrequency ablation (RFA).

MATERIALS AND METHODS

This retrospective study was conducted at the HCC and Hepatology clinics of the Tropical and Internal Medicine Departments, Ain Shams University Hospitals, Cairo, Egypt.

All patients with HCC who were diagnosed and underwent radiofrequency ablation in the period between January 2012 and December 2016 were reviewed and the data from the patients who fulfilled the inclusion criteria were retrospectively retrieved from their files.

The minimum data set within the patient record with a 1-year follow-up period was predefined before collection of data to be included as a record in this retrospective study. This study was confirmed to meet the standards of the Declaration of Helsinki and current ethical guidelines and was approved by the Research and Ethics Committee of Ain Shams University, Cairo, Egypt, in accordance with local research governance requirements.

Incomplete files or patients who did not complete a follow-up period of 1 year were excluded from the study.

The main characteristics of the database have been previously reported. Our database includes patient demographics, main biochemical and hematological parameters, etiology and stage of liver disease, the presence of comorbidities, baseline and serial measurements of AFP, HCC stage and treatment, patient survival, and mortality.

Among all treated HCC patients from January 2012 to December 2016, patients who fulfill the following criteria were only included:

1. Patient's age >18 years,
2. Child-Pugh class A and B cirrhosis,
3. Confirmed diagnosis of HCC according to AASLD guidelines **[3]**,
4. Who underwent RFA for HCC depending on the BCLC staging system with no eligibility to undergo liver transplantation or resection, and
5. Who achieved complete tumor response 1 month after RFA according to modified RESICT criteria **[6]**, i.e. complete disappearance of intra-tumoral enhancement in all target lesions using dynamic imaging ; either CT or MRI.

Patients with advanced liver disease (Child-Pugh class C) or those with extra-hepatic metastasis or gross vascular invasion and those patients with any previous HCC treatment were excluded from this study.

We calculated Disease-free survival (DFS) from the time of complete response to a curative procedure to the time of disease recurrence. Overall survival (OS) was calculated from the time of intervention to the date of death or that of the last follow-up visit (December 2017).

Analysis of survival was done yearly after treatment. The maximum tumor diameter was the proposed tumor size. In case of multiple tumors, the size was measured as the sum of the maximum diameters of all tumors.

Follow up of all patients, with the measurement of serum AFP and CTs, was done every 3 months in the first year after treatment, and then every 6 months for the next 4 years.

Patients' informed consent to the study was not a requirement because their records were reviewed retrospectively and the clinical data that were obtained after each patient agreed to RFA by informed written consent before intervention.

Statistical analysis:

Statistical analysis was performed with SPSS software (SPSS Inc., Chicago, IL, USA). Data were expressed as the mean ± SD, median or count and percentage. Differences in continuous variables between the different groups' data were assessed by independent t-test. Mann-Whitney U tests, Kruskal–Wallis tests or $\chi 2$ tests were used to compare non-parametric variables. A level of significance (p) less than 0.05 was significant. One-way analysis of variance (ANOVA) was applied to compare all groups on quantitative variables to determine significant differences. Pearson correlations were used to assess the correlation between parameters of interest. Pearson correlation coefficients point to a direct correlation, while negative values point to an inverse correlation and were considered significant at the 0.05 level. Univariate regression analysis was used to assess the correlations of the predictors of death or recurrence. Life tables and Kaplan-Meier curves were used to present survival. The log-rank test was used to compare survival times between the different groups.

The probability of the AFP level predicting death or recurrence was used to construct receiver operating characteristic (ROC) curve. The efficacy of each panel was assessed by using area under the curve (AUC). As the AUC of AFP predicting death or recurrence did not reach a statistically significant level, no optimal cut-off values were selected.

RESULTS

According to pretreatment AFP level, patients were divided into 3 groups; group 1 included 59 patients (49 males and 10 females) with AFP less than 20 ng/ml, group 2 included 54 patients (43 males and 11 females) with AFP levels of 20-200 ng/ml and group 3 included 40 patients (30 males and 10 females) with AFP above 200 ng/ml.

Table 1 shows the main demographic, clinical and tumor characteristics of the 153 study patients.

The patients' mean age was 56.43 ± 7.02 years, and approximately 80 % of them were males. Hepatitis C virus was the main underlying etiology of liver cirrhosis (n=142, 92.8%). Around two-thirds of the patients were of Child-Pugh class A, and three-quarters of the patients had a single lesion.

The maximum diameter of the HCC lesion was ≥ 3 cm in 124 patients (81%). Serum AFP levels were within the normal range (< 20 ng/ml) in 59 patients (38.6%), mildly elevated (20-200 ng/ml) in 54 patients (35.3%), and markedly elevated (>200 ng/ml) in 40 patients (26.1%).

In the present study, tumor recurrence was recorded in 88 cases (57.5%), and 53 (34.6%) patients died during follow-up.

Comparison between the 3 groups on gender, age, lesion number, size of the largest lesion, Child class and score, recurrence and death revealed no significant differences between the three groups for any of the parameters as shown in table (2).

The correlation between AFP and other variables (age, size, number of lesions, Child score, and class, recurrence, and death) revealed that pretreatment AFP was not significantly correlated with age, baseline lesion number or size, baseline Child score or class, post RFA recurrence or death.

The overall survival intervals (time to death or end of the study in months), were 95%, 75.6%, 55.6%, 48.8%, and 48.8% at 1,2,3,4 and 5 years, respectively. The mean survival interval was 33.6 months in group 1, 34.3 months in group 2, and 28.6 months in group 3 with no evidence of significant differences between the three groups (p=0.207).

Figure (1) shows the Kaplan-Meier survival curves of all groups. No significant differences was noticed among the three AFP classes in overall survival ($\chi 2$= 1.846, P = 0.397).

The median recurrence-free interval was 28, 28 and 35 months in group 1, 2 and 3; respectively (p=0.777).

On comparing the 3 groups on disease-free survival, there was insignificant differences among the three AFP classes ($\chi 2$= 1.859, P= 0.3975) as shown in figure (2).

The Kaplan-Meier overall survival curves of the 153 studied patients, subdivided according to their Child class (Child A and Child B) at the time of diagnosis of HCC, revealed that Child class A patients showed a better survival after RFA than those with class B ($\chi 2$= 34.613, P = 0.000).

The Kaplan-Meier overall survival curves of the studied patients, subdivided according to their lesion size at the time of HCC diagnosis (<3 and >/= 3 cm), revealed no statistically significant differences ($\chi 2$= 0.305, P = 0.581). Similarly, comparison of the patients in their lesion number at the time of diagnosis of HCC (uninodular and multinodular), the Kaplan-Meier survival curves showed insignificant differences ($\chi 2$= 0.001, P = 0.979).

Alpha-fetoprotein had an inadequate accuracy in discriminating survivors and deceased patients (AUC 0.435, 95% CI 0.338-0.531) (Figure 3). Also, AFP had an inadequate accuracy to discriminate patients with recurrence from those without recurrence (AUC = 0.476, 95% CI 0.378-0.573) (Figure 4).

Table (1): Baseline Characteristics of Patients (N= 153)

Variable	N (%)
Age (years)	
Mean ± SD	56.43 ± 7.02
Range	42 – 76
Sex	
Male	122 (79.7)
Female	31 (20.3)
Viral infection	
HCV positive	142 (92.8)
HBV positive	5 (3.3)
HBV/HCV co-infection	4 (2.6)
Both negative	2 (1.3)
Child-Pugh class	
Class A	104 (67.9)
Class B	49 (32.1)
Child-Pugh score	
5	55 (35.9)
6	49 (32.1)
7	28 (18.3)
8	19 (12.4)
9	2 (1.3)
Level of AFP (ng/ml)	
< 20	59 (38.6%)
20 - 200	54 (35.3%)
> 200	40 (26.1%)
Tumor Characteristics	
Number of lesions	
Single lesion	115 (75.2)
Multiple lesions	38 (24.8)
Diameter of the largest lesion (cm)	
< 3 cm	29 (19)
≥ 3 cm	124 (81)

Table (2): Comparison between the 3 groups regarding different parameters

		AFP level (ng/ml)						χ^2	Sig
		< 20		20 - 200		> 200			
		Count	%	Count	%	Count	%		
Gender	Male	49	40.2%	43	35.2%	30	24.6%	0.957	0.620
	Female	10	32.3%	11	35.5%	10	32.3%		
Child class	A	38	36.5%	39	37.5%	27	26.0%	0.791	0.673
	B	21	42.9%	15	30.6%	13	26.5%		
Child score	5	22	40.0%	18	32.7%	15	27.3%	0.063	0.969
	6	16	32.7%	21	42.9%	12	24.5%		
	7	12	42.9%	8	28.6%	8	28.6%		
	8	8	42.1%	7	36.8%	4	21.1%		
	9	1	50.0%	0	0.0%	1	50.0%		
Lesion number	Single tumor	49	42.6%	37	32.2%	29	25.2%	3.395	0.183
	Multinodular	10	26.3%	17	44.7%	11	28.9%		
Recurrence	Disease-free	23	35.4%	21	32.3%	21	32.3%	2.224	0.329
	Recurrence	36	40.9%	33	37.5%	19	21.6%		
Death	Alive	39	39.0%	33	33.0%	28	28.0%	0.825	0.662
	Dead	20	37.7%	21	39.6%	12	22.6%		
		Mean	**±SD**	**Mean**	**±SD**	**Mean**	**±SD**	**F**	**Sig**
Age (years)		57.153	6.7487	56.444	7.6938	55.350	6.4987	0.782	0.459
Size of largest lesion (cm)		3.290	0.9484	3.426	0.8785	3.407	0.8669	0.370	0.691

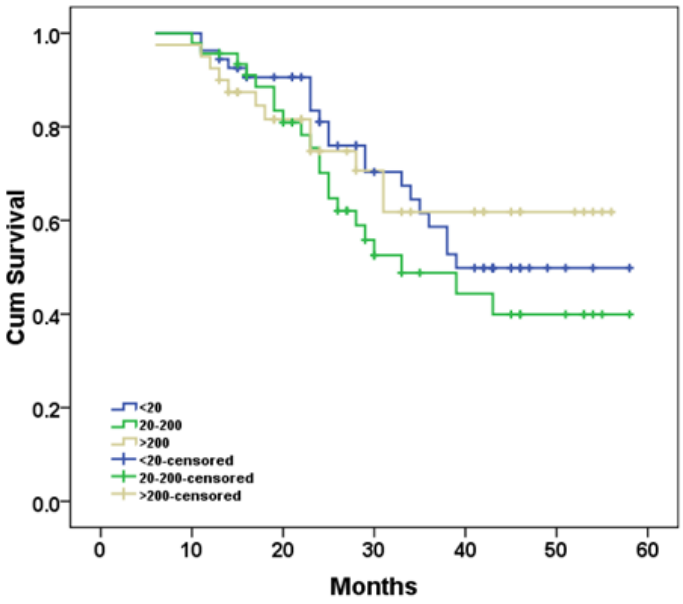

Figure (1): Kaplan-Meier survival curves showing the overall survival of the 153 studied patients subdivided according to their alpha-fetoprotein serum levels at the diagnosis of HCC (<20ng/ml; 20-200ng/ml; >200ng/ml)

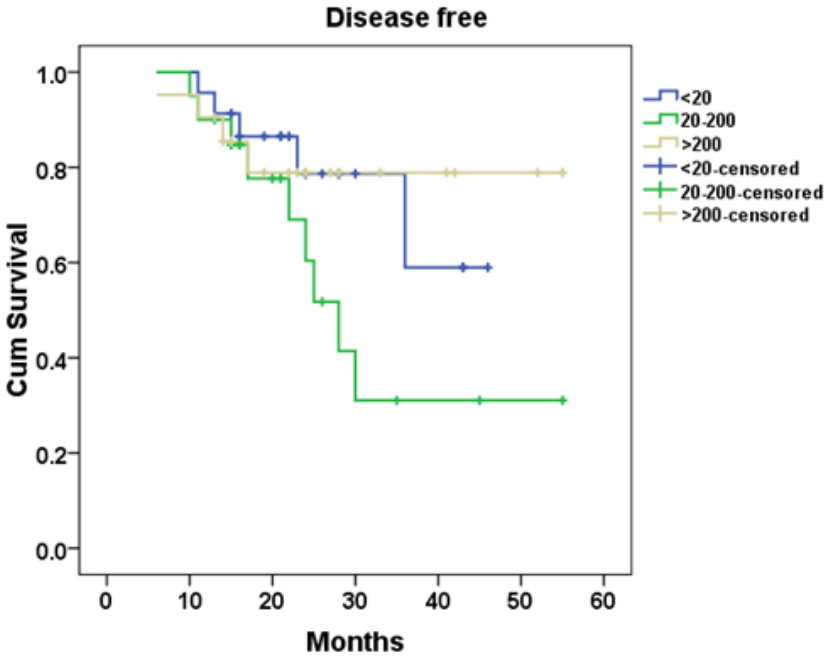

Figure (2): Kaplan-Meier survival curves showing the disease-free survival of the 153 studied patients subdivided according to their alpha-fetoprotein serum levels at the diagnosis of HCC (<20ng/ml; 20-200ng/ml; >200ng/ml)

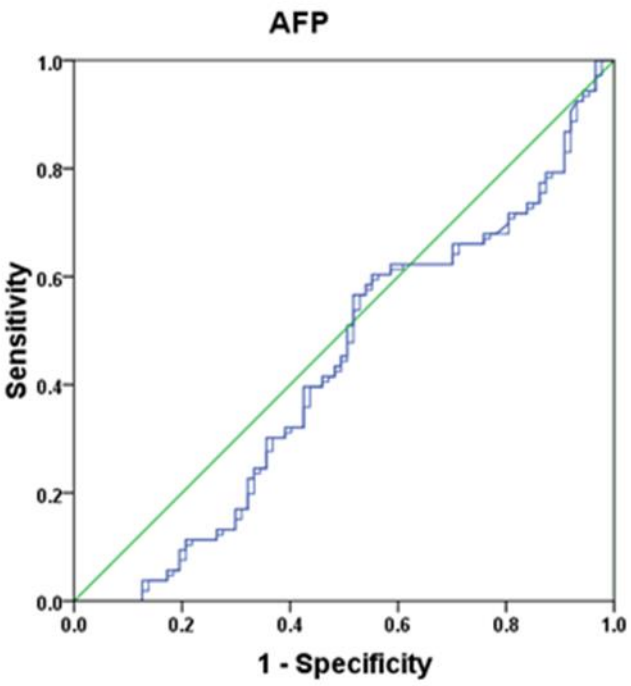

Figure (3): ROC curve showing the overall accuracy of alpha-fetoprotein serum levels for discriminating between survivors and deceased patients.

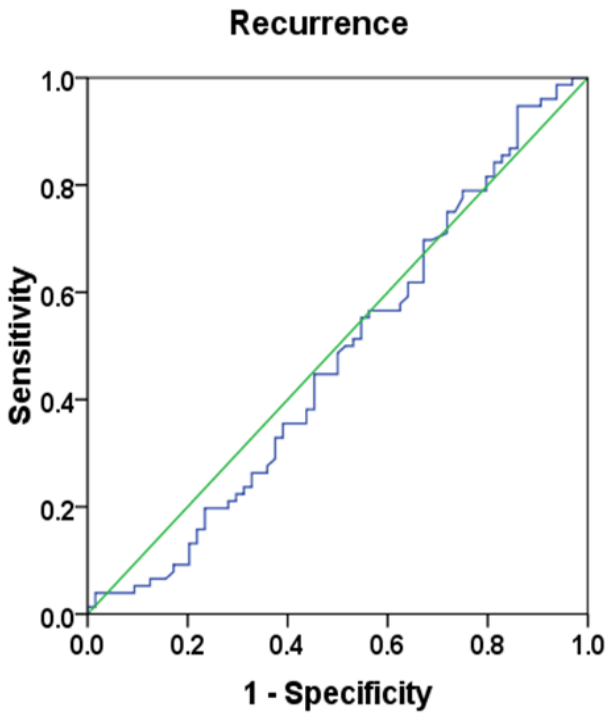

Figure (4): ROC curve showing the overall accuracy of alpha-fetoprotein serum levels for discriminating between recurrence and non-recurrence patients.

DISCUSSION

Serum AFP levels tend to be nonspecifically elevated in patients with chronic liver disease, and the diagnostic levels of this tumor marker are seldom observed in patients with small HCCs **[1]**.

Consequently, updated AASLD guidelines for the diagnosis and management of HCC dropped AFP as a routine marker for HCC surveillance in patients with cirrhosis, but this decision was debatable **[3]**.

Several studies have reported the ability of AFP response to predict response to therapy and survival outcomes **[7]**. However, there is no consensus yet regarding the magnitude of the decrease in AFP that defines AFP response **[8]**.

In a systematic review of 72 studies of prognostic markers in HCC, Tandon and Garcia-Tsao **[4]** identified AFP as one of the most robust prognostic indexes, although they noticed that the appropriate cutoff level and the group of patients in which this serum marker may be beneficial has to be established. Thus, we deemed it of interest to evaluate the prognostic value of AFP in patients who might benefit most from the curative treatment and, therefore, are those for whom prognostication should be of utmost importance.

Among cases treated with RFA, HCC recurred in 88 cases (57.5%) in the current study. Recurrence of HCC after RFA is neither uncommon nor specific to this therapy. Even patients treated with hepatic resection showed recurrence rates >70% 5 years after surgery **[9]**.

A large study demonstrated that the etiology of liver disease is an important predictor of long-term survival and distant intrahepatic recurrence after RFA and identified a role for chronic hepatitis C virus (HCV) in survival **[10]**. In another study, it was shown that patients with HCV-related cirrhosis who achieved sustained virological response to antiviral therapy have a substantially lower rate of HCC recurrence and accompanying higher survival rate **[11]**.

92.8% of the patient cohort in our study was HCV-positive and this status may have played a role in the high recurrence rate.

In the present study, regarding overall survival, Child class A patients could achieve a better survival after RFA than those with Child class B ($\chi2$= 34.613, P = 0.000). Similar to our results, Lee et al. **[12]** found that Child-Pugh class B (relative risk= 2.43, P= .011) is one of the significant predictive factors for poor overall survival.

The development and recurrence of HCC may be attributed to the severity of the underlying liver disease, and thus reinforces the importance and the role of liver function in hepato-carcinogenesis **[11]**.

Kikuchi et al. **[13]** agree with our results as they found that the survival was associated with the Child-Pugh only at a statistically significant level using multiple Cox regression analysis.

Because most HCCs arise in the context of liver cirrhosis, the established liver dysfunction may already represent generally a poor prognosis. Indeed, HCC treatment has no impact on the outcome for Child-Pugh class C patients **[3]**.

In patients with Child-Pugh class B, however, HCC treatment can be beneficial, but the outcome is not consistent. Asymptomatic HCC patients and without decompensated cirrhosis are categorized as patients with ascites, encephalopathy, and/or coagulopathy **[13]**. In the present study, Child-Pugh class B patients comprised 32.1% of the study population.

Recently in 2017, although performed on patients after hepatectomy, Shinozuka et al. **[14]** concluded that Child-Pugh class (A or B) before RFA was a significant predictor of long-term survival.

In the present study, Kaplan-Meier overall survival curves of the studied patients, who were subdivided according to their lesion size at the time of diagnosis of HCC (<3 and >/= 3 cm), revealed no statistically significant difference ($\chi2$= 0.305, P= 0.581). This finding is consistent with the results of Giannini et al. **[15]** who found that there was no significant survival difference associated with the size of the HCC (≤ 2 or 2-3 cm).

In the present study, the survival rate at 5 years was 48.8%, while in the study by Giannini et al. **[15]** the 5-year survival rate was approximately 60% in both patients with AFP serum levels below and above 200 ng/ml. The higher survival rate in the study of Giannini et al. **[15]** comes from the different study population. In their study, patients with compensated liver cirrhosis (Child-Pugh class A) were included and an Eastern Cooperative Oncology Group Performance Status of 0 who were diagnosed with a single, small (i.e., ≤3 cm) HCC, and they used all curative modalities including orthotopic liver transplantation, hepatic resection, percutaneous ethanol injection and

RFA. In our study, we included patients with Child class A and B, and approximately 80% of our patients had lesions ≥3 cm and all our patients were treated with RFA only.

It seems that the predictive ability of AFP depends mainly on tumor size and treatment modality, being more evident in patients with advanced HCC and in those who received a palliative treatment, and less evident in patients with small tumors and in those who underwent curative treatment **[16]**.

Indeed, the prognostic role of AFP was dramatically diluted in studies excluding patients with advanced liver disease and/or advanced HCC **[17]**. These considerations are also supported by the evidence in our series that there was no ‘‘therapeutic disparity,’’ and that causes of death were evenly distributed across patients with normal, mildly, and markedly elevated AFP levels, likely ruling out the presence of other possible prognostic confounding factors.

In some studies, it was shown that the rate of increase in serum AFP levels may have prognostic role in HCC patients awaiting liver transplantation; yet, these studies couldn't identify a role for static AFP levels as a predictor of survival or HCC recurrence after liver transplantation **[18,19]**.

Overall survival (time to death or end of the study in months), was 95%, 75.6%, 55.6%, 48.8%, and 48.8% at 1,2,3,4 and 5 years respectively. The mean survival interval was 33.6 months in group 1, 34.3 months in group 2, and 28.6 months in group 3 with no significant differences among the three groups (p=0.207).

In the study by Farinati et al. **[20]**, the mean survival time for the group of treated patients with AFP levels (<20 ng/ml) was 39 months, while it was 31months and 20 months for the patients with AFP levels of (21- 400 ng/ml) and (>400 ng/ml), respectively, with a significant link between AFP level and survival time.

This difference could be attributed to several factors, such as the larger study population in the study of Farinati et al. **[20]**. Additionally, their study population was not homogenously distributed among the three groups as patients were subdivided into 3 AFP groups: normal (<20 ng/ml) [46% of the study population], elevated (21–400 ng/ml) [36% of the study population], and diagnostic (>400 ng/ml) [18% of the study population].

In the present study, the ROC curve showed that AFP had inadequate accuracy in discriminating survivors and deceased patients (AUC 0.435, 95% CI= 0.338-0.531). These results are consistent with that of Giannini et al. **[15]** (AUC 0.536, 95% CI= 0.465-0.606).

From our results, we demonstrated that AFP level could not be used as a good predictor of either death or recurrence. This finding agrees with the study by Kiriyama et al. **[21]** who found that serum AFP levels did not have value in predicting recurrence or death. Shim et al. **[22]** found that the time-dependent risks of recurrence and cancer-specific death were similar in patients with AFP-producing HCC and AFP-nonproducing HCC who were treated by liver resection.

In contrast to our results, it was reported by Park et al. **[23]** that patients who showed an AFP response had significantly longer overall survival and progression-free survival than AFP non-responders.

Contrary to our results, a recent study by Zhang et al. **[24]** concluded that tumor size, albumin, prothrombin time, and α- fetoprotein levels were independently associated with mortality after RFA for HCC, while tumor size and HBV-DNA were independently associated with recurrence.

The difference between our results and those of Zhang et al. **[24]** can be attributed to many factors. First, they included only patients with high AFP before treatment, while we included all patients with different levels. Second, the viral status of their patients was HBV, while in our study; approximately 93% of our patients were HCV-positive.

The prognostic role of alpha-fetoprotein reported in other studies may be due to the heterogeneous liver and tumor-related characteristics, as well as different modalities of HCC treatment in the studied populations **[9]**.

A major limitation of the current study is that it is a retrospective study with a relatively small number of patients. Furthermore, the feasibility of RFA is mainly dependent on the operator’s technique, the experience, and the equipment available at the center. Moreover, the findings in the current study were obtained from a single-center cohort and cannot be compared to clinical experience at other treatment centers, due to the heterogeneity of selection and patient management, physician expertise, the indication for additional treatments, and the institution’s volume of care.

In conclusion, our results demonstrated that AFP level could not be used as a good predictor of either death or recurrence after RFA in HCC cases.

Conflicting Interest: No conflict of interest.

Institutional Review Board Statement and Ethical Committee Approval: This study involved human participants and was reviewed and approved by the Ethics Committee of Ain Shams University Hospitals.

Informed consent statement: Patients were not required to give informed consent to the study because the records of patients were reviewed in a retrograde manner and the clinical data that were obtained after each patient agreed to treatment by written consent.

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Efficacy and Adverse Effects of Sofosbuvir plus Daclatasvir Therapy in Chronic HCV Patients in Sharkia Governorate, Egypt

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Key words:
Sofosbuvir; Daclatasvir; Ribavirin; Sustained virological response; Chronic hepatitis, Cirrhosis

Background and study aim: Hepatitis C is the most pressing public health challenge in Egypt with variable prevalence rates among different age groups. This study aimed to detect the efficacy and adverse effects of sofosbuvir plus daclatasvir therapy in treatment of chronic HCV patients in Sharkia governorate.

Patients and Methods: One hundred and ten patients were included in this study, divided into 4 groups; group I: 55 treatment naïve patients receiving (sofosbuvir + daclatasvir) for 12 weeks, group II: 36 treatment naïve patients receiving (sofosbuvir + daclatasvir +ribavirin) for 12 weeks, Group III: 9 treatment experienced patients receiving (sofosbuvir + daclatasvir + ribavirin) for 24 weeks and Group IV: 10 chronic HCV patients not receiving anti-viral therapy. Patients were followed by clinical and laboratory evaluation monthly during treatment and for 3 months after end of treatment. In addition, the virological response and adverse effects were reported.

Results: The rate of SVR response was equal in the three treated groups. There was statistically significant increase in nausea and headache in groups I and II while arthralgia, myalgia and fatigue were more frequent in group I. There was also statistically significant improvement in Child score among treated cirrhotic patients after treatment.

Conclusion: Daclatasvir plus sofosbuvir with or without ribavirin for 12 or 24 weeks is highly effective in treatment of naïve or experienced Egyptian HCV patients in Sharkia governorate. This combination is well tolerated in both cirrhotic and non-cirrhotic patients with mild adverse effects.

INTRODUCTION

Hepatitis C is the most pressing public health challenge in Egypt. According WHO, Egypt has the highest prevalence of hepatitis C virus (HCV), where the results of blood screening and testing for the Egyptian blood donors showed 20% positivity for HCV antibody **[1].** A published Egypt Health Issues Survey (EHIS) in 2015 on a nationally representative sample showed that 10% of Egyptians between 15 – 59 years of age had been infected with HCV, while 7% are chronic active hepatitis C patients **[2].**

Sofosbuvir is a nucleotide analogue NS5B polymerase inhibitor approved by FDA in 2013, for the treatment of chronic HCV infection as a component of a combination antiviral treatment regimen. Sofosbuvir-based regimens provide a higher cure rate, fewer side effects, and a two- to four-fold reduced duration of therapy **[3].**

Daclatasvir is an HCV NS5A replication complex inhibitor that inhibits intra-cellular HCV RNA synthesis as well as inhibiting virion assembly and release. In vitro, daclatasvir demonstrated potent pangenotypic antiviral activity against HCV genotypes 1-6 **[4]**.

This study aimed to compare the efficacy and safety of sofosbuvir + daclatasvir regimen with or without ribavirin in non-cirrhotic and cirrhotic naive and treatment experienced HCV patients in Sharkia governorate, Egypt.

PATIENTS AND METHODS

This prospective cohort study was conducted in Tropical Medicine Department, Zagazig University Hospitals, Egypt during the period between May 2016 and February 2017. A total of one hundred and ten patients were included in this study.

Inclusion criteria:

Patients with chronic HCV infection evidenced by positive HCV RNA quantitative PCR with at least twice elevation of liver enzymes (more than 2 times upper limit of normal for the laboratory) in the previous 6 months with or without cirrhosis were included. Diagnosis of cirrhosis was based on combined clinical, laboratory and imaging data.

Exclusion Criteria:

Total serum bilirubin >3mg/dl, Serum albumin <2.8 gm/dl, INR ≥1.7, Platelet count <50000/mm³, Intra or extahepatic malignancy, Pregnancy or inability to use effective contraceptive, In-adequately controlled diabetes mellitus (HbA1c >9%), Age below 18 years or over 75 years, Patients who didn't give consent to participate in the study, Patients with Child C cirrhosis.

The patients were divided according to National Committee for Control of Viral Hepatitis **(NCCVH)** protocol update on November 2015**[5]** into four groups:

- Group I: Fifty five treatment naïve patients treated by sofosbuvir 400 mg/day +daclatasvir 60 mg/day for 12 weeks.
- Group II: Thirty sex treatment naïve patients treated by sofosbuvir 400mg +daclatasvir 60 mg/day +ribavirin 600mg/day for 12 weeks.
- Group III: Nine treated experienced patients were retreated by sofosbuvir 400mg/day +daclatasvir 60mg/day +ribavirin 600 mg/day for 24 weeks.
- Group IV: Ten chronic HCV patients matched for age, sex and Child classification as the treatment group and are not receiving anti-viral therapy at the time of the study (control group).

All patients were subjected to:

- Full history taking, Thorough clinical examination
- Laboratory investigations: Complete blood picture (CBC), liver functions (S. bilirubin, SGOT, SGPT, total protein and S. albumin), coagulation profile (PT, INR), kidney function (S. creatinine), Viral markers (HBsAg, HBc IGM and HCV IgG), Alpha-feto protein (α-FP), Blood sugar,HBA1C for diabetics, HCV PCR for detection of HCV RNA.
- Abdominal ultra-Sonography (U/S): Ultrasound used for assessment of the liver, diagnosis of cirrhosis, detection of ascites and exclusion of hepatic focal lesions [6,7].

Follow up:

Patients were followed up through treatment by clinical evaluation, CBC, liver functions and kidney functions after 1 week and 2 weeks of treatment then every month till end of treatment and PCR for HCV RNA after 4 weeks, end of treatment (EOTR) and 3 months after stoppage of therapy. The primary efficacy end point was the percentage of patients in each group with sustained virological response (SVR), defined as HCV RNA <15 IU/mL 12 weeks after stoppage of treatment **[8].** Patients in all groups were followed up monthly during treatment and for 3 months after end of treatment for any developed adverse effects with complete analysis including types, onset, course, duration, association, frequency, if the patient asked for medical advice, took any medications and if had been admitted to hospital for these side effects. Grading of these adverse effects was done according to the common terminology criteria of adverse events 2010**[9].**

The Common Terminology Criteria for Adverse Events (CTCAE):

A descriptive terminology which can be utilized for Adverse Event (AE) reporting. A grading (severity) scale is provided for each AE term.

CTCAE Terms: An Adverse Event (AE) is any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medical treatment or procedure

Grades: Grade refers to the severity of the AE. The CTCAE displays Grades 1 through 5 with unique clinical descriptions of severity for each AE based on this general guideline:

- Grade 1 Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
- Grade 2 Moderate; minimal, local or non-invasive intervention indicated; limiting age-appropriate instrumental activities of daily life (ADL).
- Grade 3 Severe or medically significant but not immediately life-threatening; hospitalization

or prolongation of hospitalization indicated; disabling; limiting self-care ADL.
- Grade 4 Life-threatening consequences; urgent intervention indicated.
- Grade 5 Death related to AE.

End points
The primary efficacy end point was SVR. The second end point is the development of treatment related side effects.

Statistical analysis:
The collected data were computerized and statistically analyzed using SPSS program (Statistical Package for Social Science) version 20.0.Chi-square test was used to examine the relation between qualitative variables. Quantitative data were expressed as mean ± SD. Paired Anova and Kruskal wallis test were used to compare quantitative data and simple t test and Wilcoxon test were used to compare the changes before and after the course of treatment for parametric and non-parametric variables respectively . Significance was defined as P<0.05**[10]**.

RESULTS

Study population
This study included 110 HCV patients from Sharkia governorate, Egypt. Their age ranged from 22 to 76 years old, 74 males (67.3%) and 36 females (32.7%), 74 patients were cirrhotic (67.3%), 36 patients were non cirrhotic (32.7%), 67 cirrhotic patients were child A (90.5%) and 7 were child B.

The patients included in this study were treated according to the national Egyptian guidelines developed by NCCVH.

There were no significant differences in demographic,clinical,and sonographic findings among the studed groups at base line as shown in Table 1.

Virological response
The combination of sofobruvir/dacltasvir is very potent in treatment of HCV patients both cirrhotic and non cirrhotics with and without use of ribavirin for 12 or 24 weeks. This noticeable by achievement of 100% response both EOT and SVR. In this study as regard virological response for all treated patients (group 1, group 2 and group 3), there was negative PCR for HCV RNA at week 4 with 100% response in all treated patients which is still negative till end of treatment and 3 months after end of treatment.

Biochemical and clinical paremeters
It seems that sofosbruvir/daclatasvir regmins used in this study did not adversly affect the biochemical parameters used in routine practice (Table 2). CBC, liver enzymes, bilirubin and renal biochemistry did not show any significant change when baseline figure were comapred with figures during (data not shown) and after treatment.

Furthmore, all the treated crirhotic patients in this study showed clinical and biochemical benefits from sofobruvir/dacltasvir based rejiemns and this is reflected by improvements noticed in their Child scores by the end of treatment (Table 3).

Adverse events
As shown in Table 4, the combination of sofosbruvir/daclatasvir with or without RBV seems tolerable because there were no significant differences among the studied groups as regard all listed side effects. And when the side effects were graded into grades (1,2,3 as mild, moderate and severe respectively) according to CTCAE grading the reported side effects were mild (grade 1).

No serious or life threatning adverse events were reported. When the groups are viewed separately, nausea, headache, myalgia and arthralgia and fatigue were significantly higher at end of treatment than at beginning in group I. In group II only nausea and headache showed significant rise in the frequency by the end of treatment. In Group III no symptom had significant higher frequency by end of treatment.

Table (1): Comparison of clinico-demographic findings among different groups

		Group IV N=10	Group I (N=55)	Group II (N=36)	Group III (N=9)	P value
Age(years) (range)		42.5 (29-56)	50 (22-67)	51.5 (24-66)	50 (40-63)	0.132
Sex	Female	5 (50.0%)	14 (25.5%)	13 (36.1%)	4 (44.4%)	0.338
	Male	5 (50.0%)	41 (74.5%)	23 (63.9%)	5 (55.6%)	
Spleen size (in cm)	Average	5 (50.0%)	41 (74.5%)	18 (50.0%)	5 (55.6%)	0.080
	Enlarged	5 (50.0%)	14 (25.5%)	18 (50.0%)	4 (44.4%)	
PV diameter (in mm)	Average	10 (100.0%)	54 (98.2%)	33 (91.7%)	9 (100.0%)	0.294
	Dilated	0 (0.0%)	1 (1.8%)	3 (8.3%)	0 (0.0%)	
Hepatic state	Non-cirrhotic	3 (30.0%)	24 (43.6%)	8 (22.2%)	1 (11.1%)	0.069
	Cirrhotic	7 (70.0%)	31 (56.4%)	28 (77.8%)	8 (88.9%)	
Child Class	A	6 (85.7%)	31 (100.0%)	24 (85.7%)	6 (75.0%)	0.092
	B	1 (14.3%)	0 (0.0%)	4 (14.3%)	2 (25.0%)	

Table (2): Comparison between changes in laboratory parameters before and after treatment in the treated groups

	Group I (N=55)	Group II (N=36)	Group III (N=9)	P
Hb, g/dl	-1 ± 1.5	-01.2 ± 1.7	-1.1 ± 1	0.728
PLT, x109L	18.7 ± 58	33.3 ± 57.3	25.5 ± 41.9	0.442
WBC, x109L	0.1 ± 2	-0.1 ± 2.2	-0.8 ± 1.8	0.980
Bil, mg/dl	-0.2 ± 0.3	0 ± 0.4	-0.7 ± 0.1	0.751
Alb, mg/dl	0 ± 0.5	-0.1 ± 0.5	-0.5 ± 0.6	0.968
INR	-0.1 ± 0.1	-0.1 ± 0.2	-0.2 ± 0.1	0.692
AST, IU/l	-18 ± 24.1	-30.7 ± 23.2	-34.7 ± 30.2	0.798
ALT, IU/l	-21 ± 44.2	-38.3 ± 42.5	-41.5 ± 32.2	0.487
Cr, mg/dL	0 ± 0.2	0 ± 0.2	0 ± 0.1	0.755

Table (3): Comparison between changes in Child score before and after treatment in the treated groups

	Child score		P value
	Baseline Mean ± SD	EOT Mean ± SD	
Group I (N=31)	5.5 ± 0.5	5.4 ± 0.5	0.013
Group II (N=28)	5.7 ± 0.7	5.5 ± 0.5	0.012
Group III (N=8)	5.8 ± 0.8	5.2 ± 0.4	0.051

Table(4): Frequency of Adverse Events in the studied groups during receiving treatment

Adverse Events	Group IV (N=10)	AVT Regimen			P Value
		Group I N=55	Group II N=36	Group III N=9	
Nausea	2 (20.0%)	8 (14.5%)	11 (30.6%)	2 (22.2%)	0.190
Vomiting	1 (10.0%)	4 (7.3%)	5 (13.9%)	0 (0.0%)	0.243
Constipation	1 (10.0%)	3 (5.5%)	1 (2.8%)	1 (11.1%)	0.607
Diarrhea	1 (10.0%)	1 (1.8%)	4 (11.1%)	1 (11.1%)	0.135
Dry mouth	0 (0.0%)	3 (5.5%)	2 (5.6%)	1 (11.1%)	0.827
Dyspepsia	3 (30.0%)	11 (20.0%)	7 (19.4%)	4 (44.4%)	0.286
Pruritus	1 (10.0%)	3 (5.5%)	5 (13.9%)	2 (22.2%)	0.203
Headache	3 (30.0%)	11 (20.0%)	10 (27.8%)	2 (22.2%)	0.692
Insomnia	1 (10.0%)	5 (9.1%)	2 (5.6%)	1 (11.1%)	0.772
Depression	0 (0.0%)	1 (1.8%)	0 (0.0%)	0 (0.0%)	0.548
Arthralgia	2 (20.0%)	14 (25.5%)	7 (19.4%)	2 (22.2%)	0.797
Myalgia	2 (20.0%)	14 (25.5%)	7 (19.4%)	2 (22.2%)	0.797
Bone pain	1 (10.0%)	14 (25.5%)	6 (16.7%)	1 (11.1%)	0.433
Fatigue	3 (30.0%)	16 (29.1%)	7 (19.4%)	3 (33.3%)	0.506
Cough	2 (20.0%)	9 (16.4%)	4 (11.1%)	2 (22.2%)	0.647
Flu-like	3 (30.0%)	10 (18.2%)	7 (19.4%)	3 (33.3%)	0.606

Table (5): Comparison between grades of Adverse Events before and after treatment in the studied groups

Adverse Events	Grade	Group I N=55		P	Group II N=36		p	Group III N=9		P value
		Baseline	EOT		Baseline	EOT		Baseline	EOT	
Nausea	Grade1 Grade2	0(0%) 0(0%)	6(10.9%) 2 (3.6%)	0.0134 (s)	3(8.3%) 0(0%)	8(22.2%) 3(8.3%)	0.0413 (s)	1(11.1%) 0(0%)	1(11.1%) 1(11.1%)	0.5866
Headache	Grade1 Grade2	1(1.8%) 0(0%)	7(12.7%) 4(7.2%)	0.0086 (s)	2(5.5%) 0(0%)	7(19.4%) 3(8.3%)	0.0326 (s)	1(11.1%) 0(0%)	0(0%) 2(22.2%)	0.2158
Arthralgia	Grade1 Grade2	4(7.2%) 0(0%)	10(18.1%) 4(7.2%)	0.0217 (s)	2(5.5%) 0(0%)	5(13.9%) 2(5.5%)	0.1586	2(22.2%) 0(0%)	1(11.1%) 1(11.1%)	0.5134
Myalgia	Grade1 Grade2	2(3.6%) 0(0%)	9(16.3%) 5(9.1%)	0.0041 (s)	3(8.3%) 0(0%)	6(16.6%) 1(2.8%)	0.3233	2(22.2%) 0(0%)	0(0%) 2(22.2%)	0.1353
Fatigue	Grade1 Grade2	4(7.2%) 0(0%)	10(18.1%) 6(10.9%)	0.0062 (s)	4(11.1%) 0(0%)	6(16.6%) 1(2.8%)	0.4613	1(11.1%) 0(0%)	1(11.1%) 2(22.2%)	0.3189

EOT: end of treatment. **Grade 1**: mild symptoms; clinical or diagnostic observations only; intervention not indicated. **Grade 2**: Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily life (ADL).

DISCUSSION

Based on data from the 2015 Egyptian Health Issue Survey [EHIS], approximately 3.7 million persons in Egypt were estimated to have HCV viremia **[2]**. In Egypt most of our infections are due to difficult to treat genotypes with genotype 4 is the prevalent one and also genotype 1 occurs in some patients and that is why potent antiviral regimens are needed to eliminate the infection **[11]**.

Following approval of sofosbuvir as a backbone in treatment regimen of HCV and in November 2015 NCCVH protocol was updated to add new regimens in HCV treatment including daclatasvir in combination with sofosbuvir with or without ribavirin, ledipasvir in single tablet with sofosbuvir with or without ribavirin and Paritaprevir/ ritonavir/ ombitasvir with or without ribavirin [5] and the local industry was licensed to manufacture generic products of the potent antivirals including sofosbuvir and daclatasvir at affordable prices and that is why they were used in this study.

In our study there was male predominance highlighted the high exposure rate and characteristics of the blood donor population who are presumably healthy adult males who seek medical assistance after being diagnosed in blood banks. A similar male predominance was reported by Gad et al. **[12]** and Mabrouk et al. **[13]**.

The 100% SVR reported in our study re-enforces the potency of sofosbuvir/daclatasvir combination therapy and is in agreement with Wyles et al. **[14]** who found that treatment naïve and treatment experienced patients with HCV genotype 3 or 4 infection who received daclatasvir plus sofosbuvir for 12 weeks, the SVR 12 rate was 100 %.

These results were also similar to that reported by EASL which found that combination of sofosbuvir and daclatasvir in patients genotype 1 without cirrhosis with 24 weeks of therapy, the SVR rates were 100% (14/14 and 15/15, without and with ribavirin, respectively) in treatment-naïve patients, and 100% (21/21) and 95% (19/21) without and with ribavirin, respectively, in patients who did not respond to the combination of pegylated IFN-a, ribavirin, and either telaprevir or boceprevir. With 12 weeks of therapy, SVR was achieved in 98% (40/41) of treatment-naïve patients without ribavirin **[8]**.

In addition Leroy et al. **[15]** detected that chronic HCV genotype 3 receiving sofobuvir and daclatasvir plus ribavirin for 12 weeks, the SVR 12 weeks was 100 % in patients with advanced fibrosis, SVR 12 was 86% in patients with cirrhosis and SVR 12 was 87% in patients with treatment experienced. This difference may be due to selection criteria as most of our patients were Child A and difference in HCV genotyping because most of Egyptian patients are genotype 4 and that is why in Egypt genotyping is not routinely performed before initiation of anti-viral therapy.

Many clinical side effects were detected during treatment of our patients. However, most of adverse effects were of grade 1 severity and some of grade 2 severity according to the CTCAE grading. Adverse effects were mild without intervention or affection of the daily activity or quality of life. Furthermore, no serious adverse events were detected and no patient stopped treatment due to side effects with 100% compliance rate.

The most commonly reported adverse events were fatigue, bone pain, myalgia and headache. Frequency of all adverse events was not statistically significant between the studied groups. The frequency of reported side effects were not different from those reported in the literature and were found in agreement with Landis et al. **[16]** and Hezode et al. **[17]** who found that headache affects (18.5%) of daclatasvir plus sofosbuvir recipients versus (27.2%) of daclatasvir plus sofosbuvir and ribavirin and fatigue affect 2.8 % and 15.3% respectively. Also, EASL reported that the most common adverse reactions are fatigue, headache and nausea among daclatasvir plus sofosbuvir recipients. When sofosbuvir and daclatasvir were administered with ribavirin, the most frequent adverse drug reactions were consistent with the known safety profile of ribavirin **[8]**.

Another review by Keating **[18]** reported that the majority of adverse events were of mild to moderate severity. The most commonly reported adverse events were headache 18.5% of daclatasvir plus sofosbuvir recipients and 27.2% of daclatasvir plus sofosbuvir and ribavirin recipients, nausea (14.4% and 15.8 %) and fatigue (2.8% and 15.3%). A similar tolerability profile was seen in patients with or without cirrhosis who received daclatasvir plus sofosbuvir and daclatasvir plus sofosbuvir and ribavirin that was also generally well tolerated in patients with chronic HCV genotype 1 or 3 infection and post-transplant recurrence and this is consistent.

Also, Paul et al. **[19]** who reported that daclatasvir plus sofosbuvir (± ribavirin) was well tolerated in clinical trials with no treatment-related deaths, discontinuations as a result of adverse events or treatment-related serious adverse events were reported. Across these trials, adverse events reported in these trial were headache more than 10 % of patients included (20% and 24 % of patients in ALLY-3 and ALLY-3+ respectively), fatigue (19% and 26 %), nausea (12 % and not reported) and insomnia (6% and 30 %).

In addition to the excellent antiviral activity (100% SVR) and accepted safety profile (mild adverse events) of Daclatasvir/sofosbuvir regimen in treatment of our cohort with HCV, the biochemical parameters and clinical items were also improved and this noticed in improvements of the Child scores reported after the end of treatment. These improvements of biochemical and clinical parameters were also reported by other authors from the local Egyptian community **[20-22]** and by international authors **[23]**.

When also viewed from the economic side with affordable price in the Egyptian market the

combination of Daclatasvir/sofosbuvir is prioritized in treatment of or Egyptian patients.

Our study has limitations: first; patients were followed up for 3 months after the end of treatment, so cannot detect any relapse if it developed. Second; this is one center study . Third; small numbers of patients.

In Conclusion Daclatasvir plus sofosbuvir with or without ribavirin for 12 or 24 weeks is highly effective in treatment of naïve or experienced Egyptian patients with or without cirrhosis with SVR rates of 100%. Daclatasvir plus sofosbuvir with or without ribavirin for 12 or 24 weeks is tolerable in both cirrhotic and non cirrhotic patients with mild adverse effects.

Ethical consideration:
Ethical approval was obtained from the Committee of Research, Publications and Ethics of the college of Medicine, Zagazig University, Egypt. All procedures were explained to patients and a written or thumb-printed informed consent was obtained

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Conflict of interest: None.

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Current Evaluation of Sepsis among Patients with Liver Cirrhosis

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Key words:
sepsis, cirrhosis, infection

Background and study aim: patients with liver cirrhosis have high incidence of sepsis. Spontaneous bacterial peritonitis and urinary tract infections are the most common infections among patients with liver cirrhosis. New criteria including quick qSOFA and sepsis-3 criteria are used for diagnosing sepsis in patients with liver cirrhosis. These criteria appear to be more accurate than SIRS. The aim of this study is to evaluate the existing scoring systems in our patients with liver cirrhosis to identify patients with sepsis.

Patients and Methods: This prospective study included 288 consecutive patients previously diagnosed to have liver cirrhosis and suspected to have bacterial/fungal infections. Quick Sequential (sepsis-related) organ failure assessment (qSOFA) criteria and sepsis-3 criteria were used to identify patients with organ dysfunction due to sepsis.

Results: qSOFA and sepsis-3 criteria are more accurate than SIRS in detecting sepsis among patients with cirrhosis (The area under the receiver operating characteristic curve (AUROC) value for a model with qSOFA and sepsis-3 was AUROC: 0.77 and 0.76), while AUROC for SIRS was 0.66.

Conclusion: Sepsis-3 and qSOFA are more accurate than SIRS criteria in early detection of sepsis among patients with cirrhosis. Patients with positive criteria need intensive management due to high risk of in-hospital mortality.

INTRODUCTION

Patients with decompensated cirrhosis have high incidence of sepsis. The prevalence of sepsis is about 30-50% in patients hospitalized for acute hepatic decompensation **[1]**. Spontaneous bacterial peritonitis and urinary tract infection are the most common infections among patients with cirrhosis, followed by chest infection, cellulitis and spontaneous bacteremia **[2]**. These different types of infections induce excessive systemic inflammation that may lead to decompensation, organ failure and acute-on-chronic liver failure in patients with cirrhosis **[3]**.

Diagnosis of sepsis is still challenging in general population. There is no standard diagnostic test that allows easy and accurate diagnosis of sepsis **[4]**. Systemic inflammatory response syndrome (SIRS) criteria (at least two of the following: body temperature <36°C or >38°C; heart rate >90 bpm, respiratory rate >20/min, white blood cells [WBC] <4.000/µL or >12.000/µL or immature neutrophils >10%) were used for diagnosing sepsis in cirrhosis **[5]**. However, it may be difficult to prove infection in addition to the previous criteria or the results are delayed. Also, patients with cirrhosis may have leucopenia because of hypersplenism, tachypnea because of hepatic encephalopathy or ascites, and/or bradycardia because of the use of beta-blockers which make the clinical judge is more difficult **[1]**. Thus, SIRS criteria are ineffective for diagnosing sepsis in cirrhosis. Recently, organ dysfunction due to sepsis is defined as a change in sequential organ failure assessment (SOFA) score ≥ 2 points or positive quick SOFA (at least two of the following: alteration in mental status, systolic blood pressure ≤100 mm Hg or respiratory rate ≥22/min). Both scores are used recently instead of systemic inflammatory response syndrome (SIRS) criteria **[5]**. Both Sepsis-3 criteria and qSOFA were

shown to be more accurate than SIRS criteria in predicting in-hospital mortality in patients with cirrhosis and bacterial infections **[6]**. As rapid diagnosis of sepsis in cirrhotic patients is a critical issue, there is a need for diagnostic scoring systems to facilitate early detection of sepsis. So, we aimed in this study to evaluate the existing scoring systems in our patients with liver cirrhosis to identify patients with sepsis in the context of available clinical and laboratory data.

PATIENTS AND METHODS

This prospective study included 288 consecutive patients previously diagnosed to have liver cirrhosis and suspected to have bacterial/fungal infections. They were selected among patients with cirrhosis were admitted to Intensive Care Unit (ICU) of tropical medicine and anaesthia departments, Zagazig University hospitals during the period between January 2018 and May 2018. Variables for sepsis criteria were calculated within 24 h after the admission. Quick Sequential (sepsis-related) organ failure assessment (qSOFA) criteria and sepsis-3 criteria were used to identify patients with organ dysfunction due to sepsis. Baseline SOFA was assessed using preadmission data. Acute changes in SOFA score of 2 points or more represent organ dysfunction.

Systemic screening for infections included all the followings :

1. Thorough history taking and clinical examination.
2. Complete blood counting.
3. Liver function including coagulation profile and kidney function tests.
4. Ascetic fluid sampling for total and differential leucocytes counting.
5. Urine analysis and measurement of urine output
6. Different body fluids culture (urine, blood, sputum, ascites) according to clinical setting.
7. Arterial blood gases (ABG) and electro-cardiography (ECG)
8. Chest x ray
9. Ultrasonography on abdomen and pelvis.

After infection was suspected, patients were meticulously evaluated and promptly treated with empirical broad-spectrum antibiotic combination, depending on the site of infection, known colonization and previous antibiotic treatment. Antifungal therapy was added if fungal infection was suspected or documented. Antimicrobial treatment was narrowed after identification of the responsible pathogen. The patients were followed up until discharge or death.

Sequential [Sepsis-Related] Organ Failure Assessment Score :

System	0	1	2	3	4
PaO2/FIO2, mmHg	≥400 (53.3)	<400 (53.3)	<300 (40)	<200 (26.7) with respiratory support	<100 (13.3) with respiratory support
Platelets×10/uL	≥150	<150	<100	<50	<20
Bilirubin, mg/dL	<1.2	1.2-1.9	2-5.9	6-11.9	>12
MAP	MAP≥70	MAP<70	Dopamine <5 or dobutamine (any dose)	Dopamine 5.1-15or epinephrine ≤0.1or norepinephrine≤0.1	Dopamine >15or epinephrine >0.1or norepinephrine>0.1
Glasgow Coma Scale score	15	13-14	10-12	6-9	<6
Creatinine mg/dL	<1.2	1.2-1.9	2-3.4	3.5-4.9	>5

Statistical analysis :

The SPSS version 16 was used for statistical analysis. Data were expressed as mean ± standard deviation (SD) or number (%) as appropriate. To identify the risk of mortality we used the area under the receiver operating characteristic (AUROC) curve.

RESULTS

A total of 432 patients with decompensated cirrhosis were admitted to ICU of tropical and anaesthia departments during the period of the study, among these patient 288 were suspected to have bacterial/fungal infections. Gender, age, the cause cirrhosis, the types of infection, positive cultures and type bacterial infection, all are represented in table (1).

Overall, 16 patients (6%) had 0 SIRS criteria, 81 patients (28%) had 1, 125 patients (43%) had 2, 50 patients (17%) had 3 and 16 patients (6%) had 4 with their characters of systemic inflammatory response (SIRS) criteria are shown in table (2). On the other hand, 56 patients (20%) had 0 qSOFA score, 145 patients (50%) had 1, 61 patients (21%) had 2 and 26 patients (9%) had 3 with their characters of qSOFA score are shown in table (3). Regarding sepsis-3 criteria, patients who have positive sepsis-3 criteria due to infection were 95 patients (33%).

Regarding mortality, The number of patients who died was 3,8,13,13, and 12 for 0,1,2,3 and 4 SIRS criteria, while those of qSOFA are increased with higher scores 4, 11, 15 and 19 for 0,1,2 and 3 qSOFA scores (Figs. 3,4). The mortality percentage in patients with SIRS criteria and qSOFA with 2 or more points is compared in table (4).

The area under the receiver operating characteristic curve (AUROC) value for a model with qSOFA was AUROC: 0.77 (95%CI, 0.68-0.85) (Fig. 5) and that for sepsis-3 criteria was AUROC: 0.76 (CI; 0.68-0.85) (Fig. 6), while AUROC value for a model with SIRS criteria was lower AUROC: 0.66 (95%CI, 0.57-0.75) (Fig. 7).

Table (1) : Demographic and clinical data

Variables	No (total: 288, %)
Age	**61.5 (35-90)**
Gender	Male: 138 (48%) Female: 150 (52%)
Cause of cirrhosis; Chronic hepatitis B Chronic hepatitis C Combined chronic B, C Undetermined	 30 (10.5%) 159 (55%) 20 (7%) 79 (27.5)
Infection type;	UTI: 86 (30%) SBP: 75 (26%) Chest infections: 55 (19%) Bacteremia: 29 (10%) Cellulitis: 15 (5%) Secondary peritonitis and rupture umbilical hernias: 28 (10%)
Positive cultures – no (%)	218 (76%)
No of bacteria per patient-n (%); Monomicrobial Polymicrobial Culture negative Multi-drug resistant	 160 (56%) 40 (14%) 70 (24%) 18 (6%)

Table (2) : Characters of systemic inflammatory response (SIRS) criteria among the studied patients

SIRS ≥ 2	**191 (66%)**
1- Temperature >38°C or <36°C	**130 (45%)**
2- Heart rate >90 bpm	**159 (55%)**
3- White blood cell count >12 000/μL or <4000/μL or >10% immature Bands	**96 (33%)**
4- Respiratory rate >20/ min or PaCO2 <32 mm Hg	**150 (52%)**

Table (3) : Characters of qSOFA model among the studied patients

qSOFA ≥ **2**	87 (30%)
Respiratory rate/min ≥22/min	70 (24%)
Altered mental status, Glasgow coma scale score ≤ 13	152 (53%)
Systolic blood pressure ≤100	123 (43%)

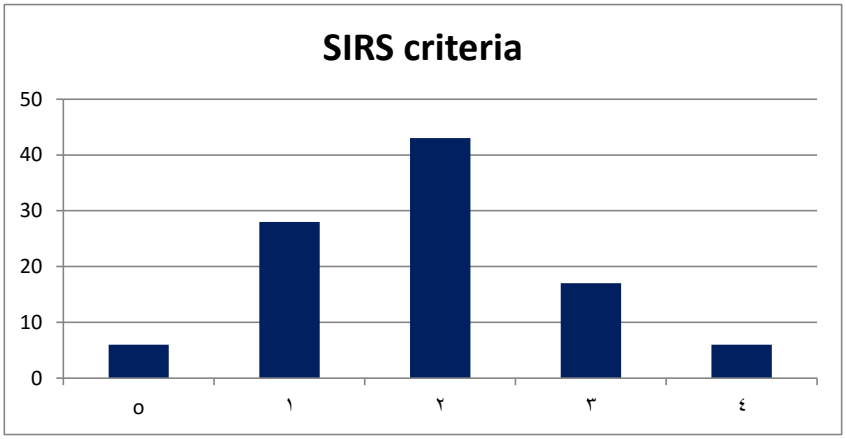

Figure **(1) :** Distribution of patients by SIRS criteria

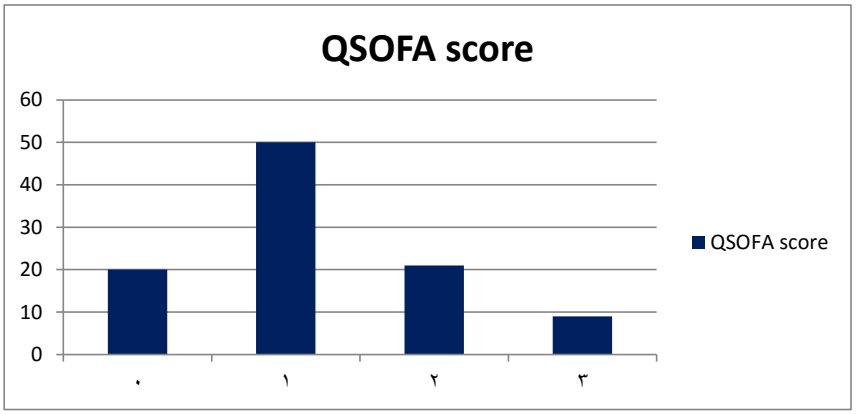

Figure **(2) :** Distribution of patients by qSOFA score.

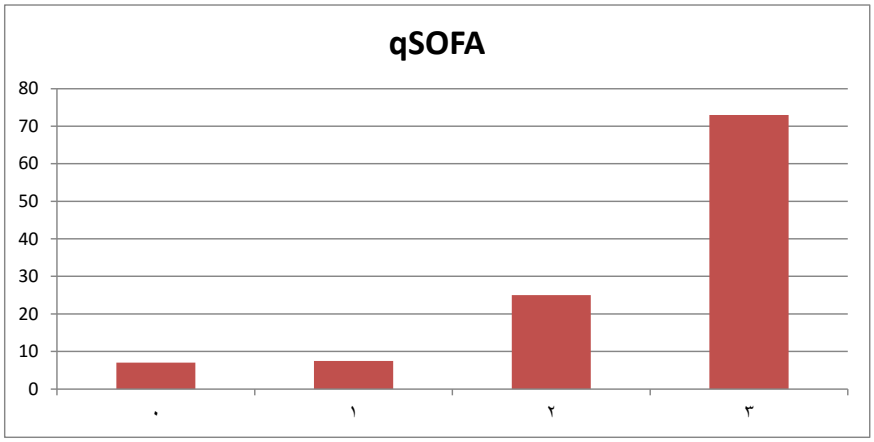

Figure **(3) :** Observed mortality rate according to qSOFA score

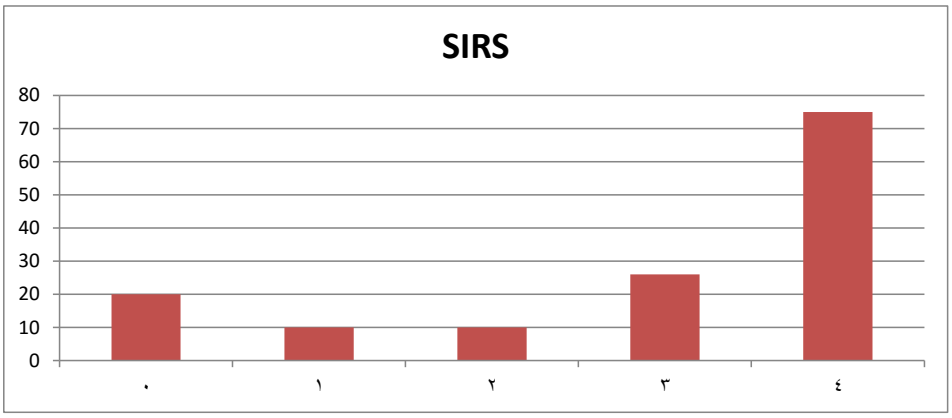

Figure **(4) :** Observed mortality rate according to SIRS criteria

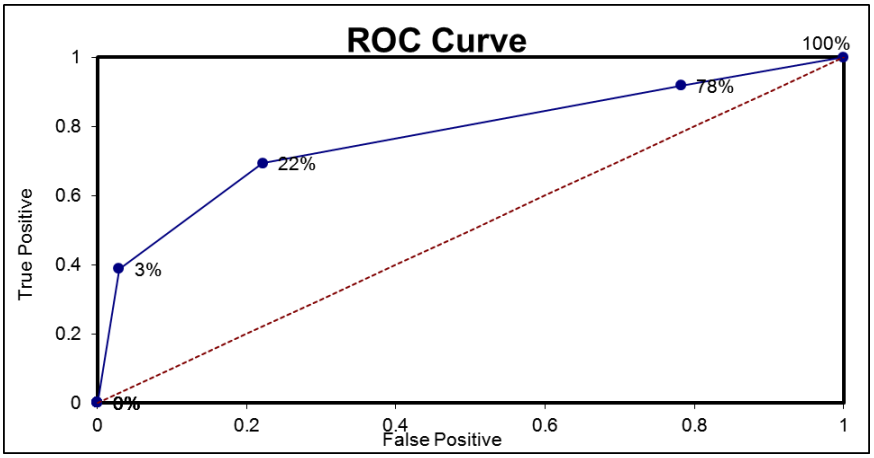

Figure **(5) :** Roc curve for qSOFA; AUROC:0.77 (95%CI, 0.68-0.85)

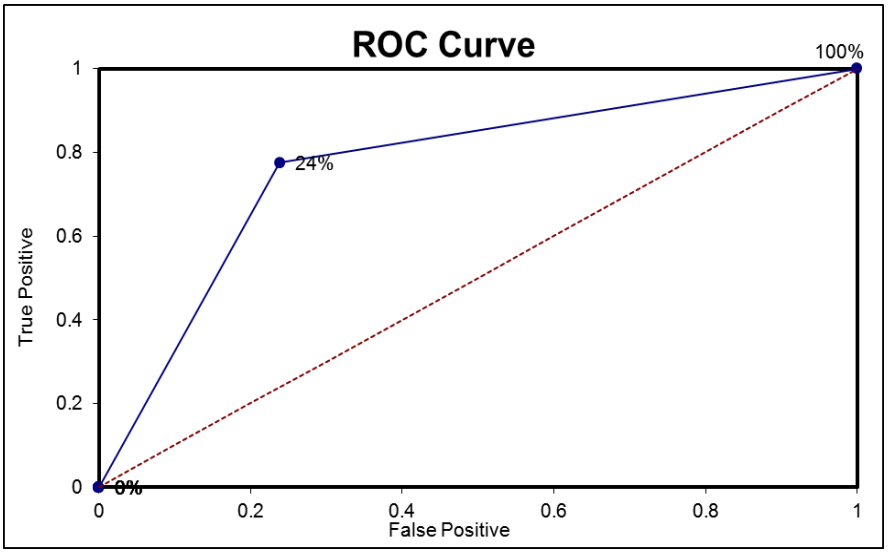

Figure **(6) :** ROC curve for sepsis3:AUROC:0.76(CI; 0.68-0.85)

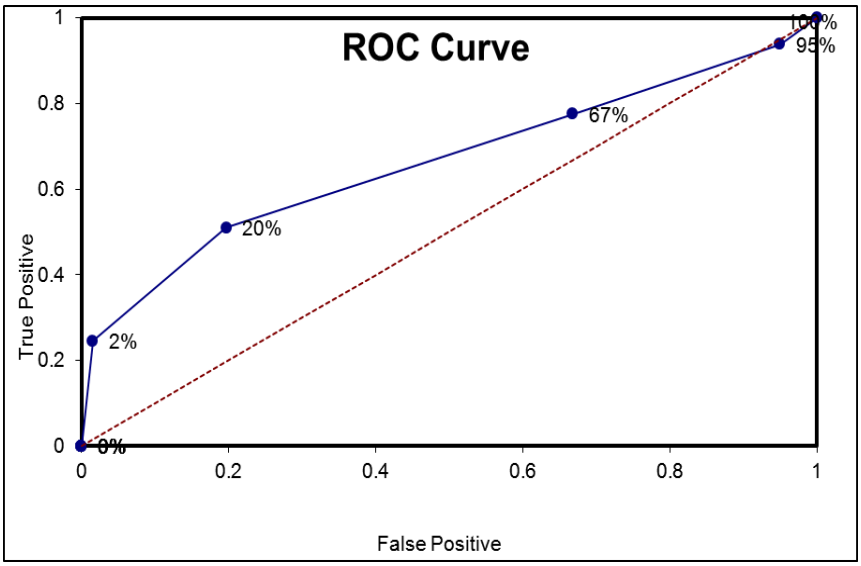

Figure **(7) :** ROC curve for SIRS; AUROC:0.66 (95%CI, 0.57-0.75)

DISCUSSION

Sepsis is defined as life-threatening organ dysfunction due to a dysregulated host response to infection **[4]**. There is no gold standard test for diagnosis of sepsis but several clinical and laboratories data are used to identify patients with sepsis. Sepsis-3 criteria and qSOFA are widely used for prediction of sepsis in general population. However, their use in patients with cirrhosis is not yet adequately studied. Piano et al. **[6]** were the first to study the predictive validity of both sepsis-3 criteria and qSOFA in patients with cirrhosis and bacterial/fungal infections. They reported that both sepsis-3 criteria and qSOFA are superior to SIRS criteria in predicting severity of infection and mortality among patients with cirrhosis. Our study was aimed at evaluation of these criteria in identification of sepsis and to detect the predictive validity in-hospital mortality in patients with cirrhosis.

The patients included in this study have decompensated cirrhosis based on clinical, laboratory and radiological data. The underlying cause of cirrhosis in most patients was chronic viral hepatitis (B and C), due to its high prevalence in the Egyptian community. Urinary tract infection and spontaneous bacterial peritonitis were the most common infections. The body fluid cultures were positive in 76% of patients, Sepsis-3 and qSOFA criteria were positive in 33% and 30% of patients respectively. In Piano et al. **[7],** the underlying cause of cirrhosis in most patients was alcohol. The cultures were positive in 57% of patients and qSOFA criteria were positive in 23% of patients. This discrimination may be attributed to high incidence of bacterial rather than fungal or viral infections in our cirrhotic patients (alcoholics are more immune-compromised).

Sepsis -3 and qSOFA criteria had significantly greater predictive validity for in-hospital mortality (area under the receiver operating characteristic curve (AUROC) =0.768 and 0.770 respectively) than SIRS (AUROC=663). These results are consistent with that observed by Piano et al. **[7].** AUROC for sepsis-3 and qSOFA are 0.784 and 0.732 respectively, while AUROC for sepsis was 0.606. This high discrimination validity of both sepsis-3 and qSOFA criteria allow the use of these criteria as a bed side tool for early detection of cirrhotic patients with poor outcomes and hence apply more intensive management. Although high validity of both scores in detecting high risk patients, the need for objective laboratory markers may confer better detection of sepsis. So, further studies focusing on laboratory markers are needed.

CONCLUSION

Sepsis-3 and qSOFA are more accurate than SIRS criteria in early detection of sepsis among patients with cirrhosis. Patients with positive criteria need intensive management due to high risk of in-hospital mortality.

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Ethical approval: The study protocol was reviewed and approved by the Institutional Review Board of the faculty of Medicine, Zagazig University, Egypt.

Conflict of interest: None

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Risk Factors of Hepatitis C in the Suez Canal Region, Egypt

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Key words: *risk factors, HCV, Suez Canal, Egypt*

Background and study aim: Egypt has very high prevalence of Hepatitis C virus (HCV) infection Aim: To identify possible risk factors of HCV in Suez Canal region of Egypt.

Materials and Methods: HCV positive individuals in 5 different hospitals and control blood donors were subjected to anti HCV tests and interview questionnaire to identify risk factors.

Results: A total of 1176 subjects were studied for HCV, of which 539 were HCV-positive and637 HCV-negative donors. Subjects who achieved less than university education, unemployed or gaining less than 600 Egyptian pounds monthly had an increased risk of HCV (OR= 4.18, CI3.28-5.34, p 0.000), (OR= 3.26, CI 2.55-4.16, p 0.000), and (OR= 3.32, CI 2.59-4.26, p 0.000). Informal male circumcision doubled the risk of HCV (OR= 2.08, CI 1.53-2.83, p 0.000). Shaving at a barber and sharing razors increased HCV risk 2 times, while sharing tooth brushes increased it 7 times (OR = 7.23, CI 2.74-18.79, p 0.000). HCV risk increased after endoscopy (OR =3.62, CI2.02-6.52, p 0.000), blood transfusion (OR 3.47, CI 2.18-5.54, p 0.000), and injection treatment(OR= 1.41, CI 1.02-1.95, p 0.040). Any delivery and dental care in governmental clinic were independent risk factors (OR 2.57, CI 1.25-5.30, 0.011), (OR 1.46, CI 1.08-1.97, p 0.014). Schistosomiasis parenteral treatment doubled the HCV risk (OR= 2.09, CI 1.35-3.23, p 0.001) and chronic kidney disease patients were more at risk (OR= 2.95, CI 1.40-6.24, p 0.005).

Conclusion: Infection control in medical practice and behavioral modifications in this region is essential to prevent HCV transmission.

INTRODUCTION

Infection by hepatitis C virus (HCV) is now recognized as a major world public health problem. The global prevalence of anti HCV increased from 2.3% to 2.8 % between 1990 and 2005 **[1]**. While regions like central and east Asia and North Africa/middle East are estimated to have high prevalence (>3.5%), areas like South Asia, Latin America and Australia have moderate prevalence (1.5-3.5%), whereas Asia Pacific, Tropical Latin America, and North America have low prevalence (<1.5%) **[1]**. Infection with HCV is a major health problem in Egypt **[2]**. The Egyptian Demographic Health Survey (DHS) 2015 estimated that overall 6.3% had ever been infected with hepatitis C virus while only 4.4% had active hepatitis C. These numbers were lower than the 14.7% overall prevalence of positive patients for antibody to HCV found in the 2009 DHS survey **[2]**. Certain governorates in Egypt are identified as high prevalent areas like the interface between the governorate of Beni Suef and Minya, Faiyum, Dakahlia, Kafr el Sheikh, Monufia, and Minya **[3]**. Apart from the usual modes of transmission, such as intravenous drug usage, the main risk factors for transmission in Egypt historically have included the parenteral antischistosomal therapy (PAT), shared or reused needles, poorly sterilized surgical or dental equipment, and blood transfusions **[4]**. Contrary to this historical view, in a cluster-based analyses a rather weak and statistically

not significant association between HCV prevalence and previous PAT was found. In Suez, despite there was no reported PAT exposures nor a history of PAT campaigns, HCV prevalence was nearly as high as the national HCV prevalence **[3]**. Few data are available about the risk factors associated with HCV infection in the Suez Canal region in Egypt. There is need for proper program to control spread of the disease and to alleviate the suffering of the people. It is essential to understand the dynamics of its transmission that can be utilized to guide screening procedures as well as provide insight into the control and prevention of the disease. Furthermore, there is need to identify the risk factors that are responsible for the continued endemic transmission of HCV in Egypt. This work studied the behavioral aspects and life style of HCV patients in the Suez Canal region in Egypt compared to controls from the same region.It also describes the demographic characteristics of HCV infected patients in the Suez Canal region. This study was conducted during the years 2014-2017.

MATERIALS AND METHODS

A case-control study was conducted among HCV positive individuals referred to the Suez Canal University Hospital in Ismailia governorate, the Communicable Diseases Research and Training Center in Suez governorate, and three Fever Hospitals in Port-Said, Ismailia, and Suez Governorates from October 2014 to September 2017. On the basis of a specially designed protocol, standard commercially available tests and physical examinations were performed. The analysis included data of medical history, physical examination and serological evaluation. All subjects were evaluated using a face-to-face questionnaire about demographic and socioeconomic aspects, parenteral exposure to blood or blood products, social and sexual behavior, occupational exposure, intravenous drug use, tattooing, acupuncture, surgery, previous hospitalization and parenteral administration of drugs, personal history of jaundice or hepatitis or history of these diseases in the cases' and controls' families. The control group consisted of blood donors referred to the Regional Blood Transfusion clinics. None of the control group subjects were HBsAg positive, HIV-positive or have any signs or symptoms of hepatitis. Antibodies to HCV were detected employing a commercially available second-generation enzyme immunoassay (Organon/ Teknica UB/HCV EIA). Positive serum specimens were retested using a second-generation recombinant immunoblotassay (RIBA-2) and a polymerase chain reaction for HCV RNA(Abbott Lab., Abbott Park, IL, USA).

Reported risk factors among infected subjects ("HCV- positive") were compared to those of subjects never exposed ("HCV-negative") to HCV.

Statistical analyses:

Collected data were coded, analyzed and computed, using the Statistical Package for Social Sciences (SPSS) version 10 (SPSS Inc., Chicago, IL, USA). Simple statistics such as frequency, and standard deviation were used. Chi-square and Student's t-tests were used for comparison.

We conducted age adjusted multivariate logistic regression analysis to identify risk factors associated with HCV infection.

RESULTS

A total of 1176 subjects were studied for HCV, of which 539 were HCV-positive and 637were HCV-negative donors comprised the control group. 1.6% of the patients had both HCV and HBV infections. Mean age of the patients was 48.3 (SD 11.00) years. Of the 539 patients, 276 (52.21%) were males, and 71.79% were from urban areas. Of the studied patients and their families, 6.13% of the spouses and 0.56% of their children had HCV. Demographic factors significantly affected the risk of HCV transmission as we found that patients who achieved less than university education, unemployed, or gaining less than 600 Egyptian pound monthly income had an increased risk of HCV (OR= 4.18, CI 3.28-5.34, p 0.000), (OR= 3.26, CI 2.55-4.16, p 0.000), and (OR= 3.32, CI 2.59-4.26, p 0.000). People who ever travelled abroad was almost 2 times more prone to infection than those who didn't (OR= 1.64, CI 1.26-2.14, p 0.000). Marriage was found one of the risk factors of HCV transmission among our study group as we found that subjects who ever married were about 5 times more likely to have HCV (OR= 5.45, CI 3.64-8.17, p 0.000). Male circumcision by informal health providers doubled the risk of HCV (OR= 2.08, CI 1.53-2.83, p 0.000). Shaving at a barber, sharing razors and tooth brushes were also risky (OR = 1.81, CI 1.27-2.58, p 0.001), (OR = 2.43, CI 1.54-3.84, p 0.001), and (OR = 7.23, CI 2.74-18.79, p 0.000). Ear piercing increased the risk of

HCV transmission (OR= 3.11, CI 2.23-3.35) while other practices like Smoking hubble-bubble and tattoo formation were of insignificant risk (p= 0.440, and 0.184). Hospital admissions and major surgical procedures weren't identified as independent risks of HCV (p 0.861, p 0.086), but risk increased after endoscopy (OR =3.62, CI 2.02-6.52, p 0.000), blood transfusion (OR 3.47, CI 2.18-5.54, p 0.000), and injection treatment inside hospitals (OR= 1.41, CI 1.02-1.95, p 0.040). Any delivery was a risk factor (OR 2.57, CI 1.25-5.30, 0.011), Caesarean section (CS) delivery or episiotomy almost doubled the risk (OR 2.50, CI 1.42-4.39, p 0.001), and home delivery was also identified as a risk factor (OR 0.36, CI 0.20-0.65, p 0.001). Dental care in governmental clinic only was of a significant risk (OR 1.46, CI 1.08-1.97, p 0.014). HCV infection was also more common among persons having history of schistosomiasis infection (OR= 1.74, CI 1.29-2.35, p 0.000), especially among those received injections for treatment (OR= 2.09, CI 1.35-3.23, p 0.001). An increased risk of infection was found in chronic kidney disease patients (OR= 2.95, CI 1.40-6.24, p 0.005) but not in diabetics receiving frequent injection (p 0.319).

Table (1): Demographic characteristics of the study group

	HCV positive Cases (n=539)		**HCV negative Controls (n=637)**	
	N	**%**	**N**	**%**
Age (years)				
< 30	35	6.49	244	38.30
> 30	504	93.51	393	61.70
Mean age years ± SD	48.3 ± 11		34.28 ±10.9	
Gender				
Male	276	51.21	486	76.29
Marital status				
Never married	31	5.75	159	24.96
Previously married	68	12.62	27	4.24
Currently married	440	81.63	451	70.80
Educational attainment				
No education	203	37.66	97	15.23
Read and write	147	27.27	97	15.23
Less than University	156	28.94	318	49.92
University and more	33	6.13	125	19.62
Work status				
Not working	283	52.50	161	25.27
Workman	150	27.83	254	39.87
Employee	106	19.67	222	34.86
Monthly income				
<200	86	15.96	24	3.77
200-400	97	17.99	56	8.79
400-	92	17.07	76	11.93
600-	107	19.85	107	16.79
800-	115	21.34	175	27.47
+ 1000	42	7.79	199	31.25
Place of residence				
Urban	387	71.79	350	54.95
Rural	152	28.21	287	45.05
Previously travelled abroad	162	30.06	132	20.72

Table (2): Demographic and behavioral risk factors associated with HCV transmission

Variable	Cases=539		Controls=637		OR	95% CI	P value
	N	%	N	%			
Less than university education	348	64.56	194	30.46	4.18	3.28-5.34	0.000*
Unemployment	283	52.50	161	25.27	3.26	2.55-4.16	0.000*
Low economic status (<600 LE/mo.)	270	50.09	148	23.23	3.32	2.59-4.26	0.000*
Ever travelled abroad	162	30.06	131	20.57	1.64	1.26-2.14	0.000*
Ever married	508	94.25	478	75.04	5.45	3.64-8.17	0.000*
Males circumcised by informal health care provider	222	41.19	121	19.00	2.08	1.53-2.83	0.000*
Tattoo	25	1.69	17	2.67	0.78	3.67	0.184
Shaving at barber	453	84.04	494	77.55	1.81	1.27-2.58	0.001*
Sharing razors	84	15.58	46	7.22	2.43	1.54-3.84	0.001*
Sharing tooth brush	37	6.8	6	0.94	7.23	2.74-18.79	0.000*
Ear piercing	207	39.13	82	12.87	3.11	2.23-3.35	0.000*
Smoking hubble-bubble	82	15.21	94	14.76	0.86	0.59-1.26	0.440

OR:Odds Ratio (Age-adjusted OR based on multivariate logistic-regression model)
CI:Confidence Interval, * statistically significant.

Table (3): Health care risk factors associated with HCV infection

Variable	Cases=539		Controls=637		OR	95% CI	P value
	N	%	N	%			
Hospital and clinic exposures							
i. Admissions	272	50.46	239	37.52	1.26	0.77-1.36	0.861
ii. Injections in the hospital	167	30.98	112	17.58	1.41	1.02-1.95	0.040*
iii. Major surgical procedures	285	52.88	280	43.96	1.11	0.84-1.46	0.476
iv. Endoscopy	92	17.07	17	2.67	3.62	2.01-6.52	0.000*
v. Blood transfusion	131	24.30	30	4.71	3.47	2.18-5.54	0.000*
Obstetric exposures (women)							
1. Any delivery	239	92.64	98	78.40	2.57	1.25-5.30	0.011*
2. Surgical delivery (CS or episiotomy)	110	44.00	30	31.91	2.50	1.42-4.39	0.001*
3. Home delivery	74	30.71	36	37.89	0.36	0.20-0.65	0.001*
Dental treatment							
A. Any treatment	476	88.64	453	71.34	1.43	0.98-2.08	0.064
B. Private clinic	251	46.57	310	48.67	0.86	0.66-1.14	0.303
C. Governmental clinic (e.g., MOH)	227	42.12	143	22.45	1.46	1.08-1.97	0.014*
Chronic medical diseases							
a. History of schistosomiasis	241	44.71	134	21.04	1.74	1.29- 2.35	0.000*
b. Schistosomiasis injections treatment	146	27.09	34	5.34	2.09	1.35-3.23	0.001*
c. Diabetics receiving frequent injection	49	9.09	12	1.88	1.43	0.71-2.86	0.319
d. Chronic kidney disease	48	8.91	11	1.73	2.95	1.40-6.24	0.005*

OR:Odds Ratio(*Age-adjusted OR based on multivariate logistic-regression model)*
CI:Confidence Interval, * statistically significant.

DISCUSSION

Hepatitis C infection is a raising problem and its long term complications like cirrhosis and hepatocellular carcinoma is considered a particular health burden **[5]**. Route of transmission varies between developed and developing countries. In developed countries after the World War II and up until 1980s parenteral exposure to contaminated blood and blood products and the use of injectable drugs were the most common source of infection **[6]**. The routine screening of HCV in the donated blood eliminated this source of infection in most of the developed countries leaving the illicit use of injectable drugs the most common rout of transmission **[7]**. In the developing countries, the nosocomial transmission of HCV is the main source of new HCV infection due to the reuse or under sterilization of medical equipment **[8,9, 10]**. In our study we found the prevalence of Co infection of both hepatitis B and C virus was 1.6%. This might be explained by the fact that both infections share common routes of infection. Nevertheless, this prevalence was lower than what an Italian study reported of almost 7% of hepatitis B positive patients with anti HCV as well **[11]**. We found that anti HCV were more common in urban than rural areas in the Suez Canal region (71.79%, and 28.21% respectively),which was the contrary of what the latest DHS survey reported of whole Egypt **[2]**. Of our studied patients and their families, 6.13% of the spouses and 0.56% of their children had HCV. Although some studies found a higher HCV sero-prevalence of family member **[12,13]**, others found only 1.33% **[14]**. Similar to our study among the household contacts of HCV seropositive index cases, spouses were found to be the most affected but still this can't be explained by the sexual transmission only as partners tools sharing is a risky behavior by itself **[15]**. Illiteracy, unemployment, and low economic status increased the risk of HCV by 3 to 4 folds. In the same way, we previously identified socioeconomic status and knowledge as risk factors of hepatitis in Ismailia as one of the governorates in Suez Canal area **[16]**. Others also found that HCV in Egypt were common among low educated patients and that mothers of low education tend to share personal tools **[17]**. Sharing tools was repeatedly identified as a risk factor of HCV transmission **[10,17]**. It increased the risk in our group up to 7 times. In our study, shaving at a barber, sharing razors and tooth brushes were significant predictors of HCV infection. In two previous studies conducted in Egypt one study identified barber shaving as a risk factor **[17]** while the other one didn't **[10]** although the former one was more recent. Like many other studies **[10,17,19]** we found that circumcision by informal health care provider was a predictor of HCV infection. Our results revealed that major surgical procedures didn't significantly increase the risk of HCV, but risk increased after endoscopy, blood transfusion, and injection treatment inside hospitals. Blood transfusion is identified as a current risk factor of HCV transmission in the developing countries like Egypt **[20]**. Interestingly, when Upper Egypt and The Nile Delta was compared, unlike Upper Egypt blood transfusion was not a risk of HCV infection in the Nile Delta what might be explained by the younger age in the latter as the donor anti HCV screening was started in Egypt in 1991 **[20]**. Several invasive procedures were associated with acquiring new HCV infections as they were identified as the most common risk factor of HCV transmission even in developed countries **[21-23]**. In Egypt the specific increase in the prevalence of new HCV infection after endoscopy procedures might be explained by the high number of patients with chronic hepatitis B and schistosomiasis requiring endoscopy what could be minimized with following the recommended sterilization techniques **[24-26]**. Our finding went with what repeatedly found by others regarding the parenteral anti schistosomiasis therapy risk of causing HCV **[27-32]**; as we found that history of schistosomiasis increased the risk by 2.5 folds, and those who reported a history of parenteral therapy for schistosomiasis were almost three times as likely to have anti-HCV than those who did not. Obstetric exposure was a predictor of HCV infection in our study group but was not a significant factor in other studies **[10,17]**. In Suez Canal area, dental care in governmental centers only was still a significant risk of HCV. In Alexandria any dental care was identified risky **[19]** while dental care in Upper Egypt and Sharkia governorates wasn't a risk factor of HCV transmission **[10,17]**. Health care exposure was also identified as the most important risk factor of the ongoing HCV transmission; probably due to the lack of decontamination techniques before the minor procedures **[33]** what might explain the increased risk of HCV in patients with chronic kidney disease in our study.

CONCLUSION

Our data indicate that history of blood transfusion; endoscopy and multiple injections are important risk factors for HCV infection in Egypt. Therefore, focusing on medical practices and infection control in health facilities is essential for HCV transmission prevention. Furthermore, Improvements in certain lifestyle patterns and customs such as sharing razors and tooth brushes especially among HCV patients and their contacts should be taken in consideration through community education program.

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Ethical approval

Consent for an interview was taken from each participant, who was assured about the confidentiality of his information. The Ethics Committee of the Ministry of Health and Population approved the study on 12/12/2012 (No: 35-2012/1).

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Conflicts of interest

There are no conflicts of interest.

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The Association between Helicobacter pylori and Graves' Disease

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Key words:
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Background and study aim: *Helicobacter pylori* (*H. pylori)* infection is a very common health problem associated with both gastric and extra gastric manifestations. Its association with autoimmune thyroid diseases (ATD) including Graves' Disease (GD) was suspected and needed to be furtherly investigated.

Patients and Methods: This case - control study included 43 patients with GD and a control group of 47 healthy volunteers. Hormonal diagnosis of GD was achieved by decreased level of thyroid stimulating hormone (TSH) and elevated levels of tri-iodothyronine (FT3) and free thyroxine (FT4) and serological diagnosis was achieved by positive titers of auto-antibodies against thyroglobulin (TG Abs), thyroid peroxidase (TPO Abs) and thyrotropin receptor (TR Abs). *H. pylori* infection was diagnosed by detecting *H. pylori* antigens in stool using an amplified enzyme immunoassay (amplified EIA). The antibodies against Cytotoxin-associated gene A (Cag-A) were assessed in serum samples using the enzyme-linked immunosorbent assay method (ELISA). The results were statistically analyzed using Fisher's test and the respective Odd's ratio (OR).

Results: No significant difference in prevalence of *H. pylori* infection was found between GD patients and the control group.

Conclusion: No association between *H. pylori* infection and Graves' disease could be detected.

INTRODUCTION

Helicobacter pylori (H. pylori) is a worldwide chronic infection **[1,2]**. It is a Gram - negative spiral pathogen that inhabits the gastric mucosa causing multiple gastric diseases such as chronic gastritis, peptic ulcers, and gastric malignancies **[3,4]**. It was also incriminated in both organ specific and non-organ specific autoimmune disease **[5]**. *H. pylori* was also involved in many extra gastric diseases such as nonalcoholic fatty liver diseases and metabolic syndrome **[6,7]**. Many available data links autoimmune thyroid diseases (ATD)- particularly Graves' disease (GD)- with *H. pylori* infection **[8]**. A strong association between thyroid auto- antibodies and immunoglobulin G (IgG) anti-*H. pylori* antibodies was reported. Radical treatment of *H. pylori* infection was found to be associated with a gradual drop in the levels of thyroid auto-antibodies, however these data are still controversial **[9-11]**. Moreover, thyroid nodules were found to be associated with *H pylori* infection in people with normal thyroid functions **[12]**. Cytotoxin-associated gene A (Cag A) strain accounts for most of *H. pylori* seropositive infection (89.2%) **[13]**. The mechanism by which *H. pylori* infection induces ATD was supposed to be a cross - reactivity between bacterial and thyroid antigens **[14]**. The ability of *H. pylori* infection to imitate the antigenic pattern of thyroid cell membrane has been suggested **[15,16]**. Multiple data - about similarities between amino acid sequence of *H. pylori* Cag-A and thyroid peroxidase (TPO) - have been published **[17]**. Typical markers of GD include the assessment of autoantibodies against thyroid peroxidase (TPO Abs), thyro-globulin (TG Abs) and thyrotropin receptor (TR Abs) **[18]**.

This work aims at investigating the relationship between GD and *H. pylori* infection and whether there is any association in between.

PATIENTS AND METHODS

This is a collaborate work between Tropical Medicine Department, Internal Medicine Department and Clinical Pathology Department, Faculty of Medicine Zagazig University. A one-year study was conducted in the period from January, 2017 to January, 2018.

A total number of 43 patients with GD (7 males and 36 females) and a control group of 47 healthy individual (27 females and 20 males) were enrolled in a case- control study over one year. The inclusion criteria included any patient with GD, while exclusion criteria included the presence of other diseases, history of antimicrobial drugs use for at least the preceding three months, presence of gastric diseases and dyspeptic symptoms (nausea, heartburn, and epigastric pain). Both groups were of the same body – mass index (BMI), socioeconomic status and inclusion criteria.

GD patients were selected based on their positive hormonal hyperthyroidism profile - including suppressed thyroid stimulating hormone (TSH), elevated free tri-iodothyronine (FT3), elevated free thyroxine (FT4) and positive titers of TPO Abs, TG Abs and TR Abs. They were measured by electrochemiluminescence immunoassay (ECLIA) on cobas immunoassay analyzers. Anti TG titer above 50 ng/ml and anti TPO above 200 IU/ml were considered positive. The control group individuals were confirmed to have normal TSH, FT3 and FT4, and to be free from autoantibodies against TPO, TG and TR. Also, thyroid ultrasound was done to assess the presence of goiter and to exclude thyroid nodules. Both groups were subjected to thorough history taking and clinical examination, then they underwent the following:

- *H. pylori* antigens were detected in fresh stool samples by amplified enzyme immunoassay (Amplified IDEIA *H. pylori* StAR, Oxoid, United Kingdom). Positive results were confirmed for the presence of *H. pylori* with an absorbance value >0.150 using a dual wavelength (450/ 620 to 650 nanometers).
- Anti - Cag-A IgG antibodies were detected in fresh serum samples by the enzyme-linked immunosorbent assay method (ELISA, Radim, Pomezia, Italy, sensitivity 93.7%, specificity 100%). An IgG level >15 units/mL was considered positive.

Statistical analysis:

The statistical analysis of results was carried out by an experienced epidemiologist. It was achieved using the SPSS version 19 considering *P* value <0.05 as statistically significant. The Chi-square or Fisher exact test were used to assess differences in proportions among categorical data. The independent impact of *H. pylori* on GD was assessed by multivariate analysis. On univariate analysis, variables with a *P* value of <0.2 were included in the multivariate analysis using Logistic regression for this purpose. Odds ratios (OR) with a confidence interval (CI) of 95% were reported. Median and interquartile range (IQR) were used to represent continuous variables.

RESULTS

A collaborate work was conducted over one year between Tropical Medicine Department and Internal Medicine Department, Faculty of Medicine Zagazig University. Forty-three GD patients were included in the study along with 47 healthy control individuals.

The study groups were classified into age and sex subgroups (range= 18-65 years). No statistically significant difference was found between both groups regarding age or sex (Table 1).

The mean, median and range of the body mass index showed no significant differences between the GD group and the control group (Table 2).

The prevalence of *H. pylori* +ve stool Ag was found to be more among GD patients' group (46.5%) than the control group (42.6%), but the difference was statistically insignificant. The prevalence of Cag A antibodies among the study groups was found to be more among *H. pylori* +ve stool Ag of the control group (30%) than the *H. pylori* +ve stool Ag of GD patients' group (20%), but the difference was statistically insignificant (Table 3).

H. pylori was more prevalent among the age subgroup of 40- 59 years of both GD patients and the control group but the difference was statistically insignificant (Table 4).

The prevalence of *H. pylori* was not associatedwith GD (OR 1.02, 95% CI 0.57–1.83, *P*=0.95), but the family thyroid malfunction was found to be a risk factor independently associated with GD (OR =3.93, 95% CI was 1.86- 618, and *P* < 0.001) (Table 5).

Table (1): Classification of patients and control groups according to age and sex.

Sex / Age	GD group (n= 43)		Control group (n= 47)		*P*
	Female n= 36	Male n= 7	Female n= 27	Male n= 20	
18- 20 years	7 (19.4%)	0 (0 %)	7 (25.9%)	4 (20%)	0.159
>20- 39 years	10 (27.7%)	3 (42.8%)	9 (33.3%)	7 (35%)	
40- 59 years	8 (22.2%)	1 (14.2%)	6 (22.2%)	5 (25%)	
60- 65 years	11 (36.6%)	3 (42.8%)	5 (18.5%)	4 (20%)	

GD: graves' Disease.

Table (2): Comparison between the two study groups regarding the body- mass index (BMI).

Study groups / BMI	GD N=43	Control N=47	*P*
Mean (SD)	25.5 ± (5.4)	25.2 ± (5.2)	0.801
Median (IQR)	24.6 (21.5- 28)	23.9 (21.2-28.9)	
Range	16.8- 44.4	16.4- 43.7	

IQR = interquartile ratio SD = standard deviation. GD: graves' Disease. BMI: body- mass index

Table (3): Comparison between the two study groups regarding the distribution of *H. pylori* stool Ag and serum Cag A-antibodies

H. pylori / Study groups	Stool Ag +ve	Stool Ag –ve	Anti- Cag A +ve	Anti- Cag A -ve	+ve Anti- Cag A (among the total no. of studied groups)
GD n= 43	20 46.5%	23 53.5%	4/20 **20 %**	16/20 80 %	4/43 9.3 %
Control n= 47	20 42.6%	27 55.4%	6/20 **30 %**	14/20 70 %	6/47 12.7 %
P	0.63	Not calculated	0.352	Not calculated	0.41

Ag: antigen. Cag A: cytotoxin-associated gene A. GD: Graves' Disease.

Table (4): Comparison between different age subgroups regarding the prevalence of *H. pylori* infection

H pylori / Age	GD group N=43	Control group N=47	*P*
18- 20 years	3/7 (42.8 %)	4/11 (36.3 %)	> 0.5
>20- 39 years	6/13 (46.1 %)	7/16 (43.7 %)	> 0.8
40- 59 years	5/9 (55.5 %)	6/11(54.5%)	> 1.0
60- 65 years	6/14 (42.8 %)	3/9 (33.3%)	>0.7

GD: Graves' Disease.

Table (5): Multivariate analysis of risk factors associated with GD.

Risk factors	OR (95% CI)	*P*
Univariate H. pylori	1.14 (0.66- 1.96)	0.63
Multivariate * - H pylori	1.02 (0.57- 1.83)	0.95
- Family history of thyroid diseases.	3.93 (1.86- 618)	< 0.001*

CI: confidence interval OR: odd ratio Graves' Disease * adjusted for age

DISCUSSION

H. pylori is a Gram-negative motile organism that colonizes and infects the gastric mucosa causing multiple pathological conditions. Its virulence is mainly encountered with the Cag A antigen strains **[19]**. *H. pylori* - among other genetic or environmental factors - was involved in ATD **[11,20,21]**. ATD include GD, atrophic thyroiditis, Hashimoto's thyroiditis (HT), subacute lymphocytic thyroiditis (postpartum thyroiditis, PPT), painless thyroiditis (PT), or silent thyroiditis (ST) **[22]**. Accumulating information connected H. pylori infection with ATD, especially with GD **[8]**, and thyroid nodular formation in euthyroid patients **[12]**. This study aimed at evaluating the association between *H. pylori* infection and GD.

This study showed that the prevalence of *H. pylori* was more among GD patients' group than the control group, but the difference was statistically insignificant. Interestingly, the prevalence of Cag A antibodies was even found to be more among the control group than the GD group, but the difference was statistically non- significant. These results agree with that of Tomasi et al. Franceschi et al and Novikova et al **[23,27,28]** who found no correlation between *H pylori* prevalence and ATD in their studies directed on participants of different ages and different environmental conditions.

On the other hand, these results do not agree with that of some studies **[8,11,13,15,24,29,37]** who found marked correlation between *H. pylori* infection- especially the Cag A positive strains- and ATD especially GD. This can be explained be the different genetic or environmental factors of the different studies. In developing countries, the incidence of *H. Pylori* infection starts at earlier ages with eventual higher rates of prevalence than in developed countries that can mask the suggested association **[8,24,25]**. Moreover, certain HLA antigens were suspected to predispose for ATD following *H. Pylori* infection **[26]**. However, this association - in some studies - was not between GD and *H. Pylori* infection per se, but it was mainly between Cag A antibodies level and GD **[25]**. A cross reactivity was suggested between Cag A antibodies and TPO Abs explaining the over estimation of *H. pylori* infection among GD patients in these studies **[14,17]**. Some studies adopted the estimation of *H. pylori* antibodies as a clue for prevalence of *H. pylori* infection **[11,29]**. Their over estimation of *H. pylori* infection among GD patients can be attributed in some cases to a previous and not the current infection.

In this study, GD was found to be significantly associated with a family history of thyroid diseases. This finding coincides with that of other studies which mentioned that the incidence of GD clusters in families usually occurs separately or in association with HT **[29-32]**. This can be explained by sharing the same genetic or environmental factors by the family members.

There was no significant difference in *H. pylori* prevalence among the different age subgroups of both GD and control groups, coinciding with the study result of Haim et al who found no associated between age and *H. pylori* prevalence **[33]**. However, these results do not agree with some authors **[34-36]** who mentioned that *H. pylori* infection is usually acquired during childhood and increases with age, with rare possibility to attract new infection in adulthood. Again, this controversy can be explained by the different genetic and environmental factors under which the different studies were conducted.

Conclusion: There is no association between *H. pylori* infection and Graves' Disease.

Ethical approval:
Informed consent was taken from each patient. The research protocol was duly approved by the ethical committee of Zagazig University Hospitals.
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Significance of screening antibodies to hepatitis B core antigen among chronic hepatitis C patients before antiviral therapy

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Background and study aim: Anti-hepatitis B core (anti-HBc) sero-positivity in general population in Egypt is reported to be 10-13%. This study was performed to determine the prevalence of anti-HBc among chronic hepatitis C patients before antiviral therapy.

Patients and Methods: A total of 178 consenting patients with chronic HCV infection eligible for treatment with DAAs according to program of National Committee for Control of Hepatitis Infection in Portsaid, Egypt from April to October 2017. All of the patients were screened for anti-HBs and anti-HBc. Anti-HBc–positive patients were assayed for HBV DNA.

Results: Out of 178 chronic hepatitis C patients, Eighty four patients (47.2%) were treated with triple therapy (Sofosbuvir/ Daclatasvir/Ribavirin) and ninety four patients (52.8%) with dual therapy (Sofosbuvir/Daclatasvir). A65 patients (36.5%) were reactive for anti-HBc. Of 84 patients, 34 (40.5%) who treated with triple therapy were reactive for anti-HBc. Of 94 patients, 31 (33%) who treated with dual therapy were reactive for it. All patients were negative for anti-HBs and anti-HBc–positive patients were no detected HBV DNA at baseline and 12 weeks after DAAs.

Conclusion: Our results suggest including anti-HBc as an additional screening test for chronic hepatitis C patient in Egypt who are eligible for DAAs to reduce the risk of HBV reactivation and fulminant hepatitis after DAAs.

INTRODUCTION

Hepatitis B virus (HBV) and Hepatitis C virus infection are leading causes of chronic liver disease worldwide, affecting 350-400million and 170 million people, respectively **[1]**. HCV is currently the most significant public health problem in Egypt **[2]**. The recently published Egyptian Demographic and Health Survey (EDHS) in 2015 estimated an overall antibody to hepatitis C virus (anti-HCV) prevalence of 6.3% **[3]**. Anti-hepatitis B core (anti-HB_C) sero-positivity in general population in Egypt is reported to be 10-13% **[4]**.

HBV and HCV share common modes of transmission, thus simultaneous infection is quite frequent, particularly where both viruses are endemic as among people with a high risk for parenteral infections **[5]**. HCV infection has a suppressive effect on the replication of HBV, shown by the loss of replicative markers as HBV-DNA [6]. The extensive application of sensitive molecular tests such as polymerase chain reaction (PCR) and real-time PCR has enabled HBV-DNA to be detected in specimens from individuals without serological evidence of chronic HBV infection **[7]**.

Occult HBV infection (OBI) can be defined by the presence of HBV-DNA in the serum of patients who are negative for HB_S Ag **[8]**. In the last decade, OBI pattern has been documented and frequently identified in patients with chronic hepatitis C (CHC) infection **[9]**. The prevalence of OBI in chronic HCV patients was higher in subjects having either anti-HBs or anti-HB_C or both **[4]**. Sero-

logical findings in patients with OBI and HCV co-infection revealed that 35% of people were anti-HBs positive, 42% were anti-HB_C IgG positive and 22% were negative for both **[10]**.

Treatment of chronic hepatitis C virus (HCV) infection has been revolutionized in the last few years by the introduction of highly effective and well-tolerated DAAs able to achieve high rates of sustained virological response(SVR) in many groups of patients **[11]**. In past years, HBV reactivation occurring in HBV/HCV-co-infected patients treated with IFN-based therapy has been reported, probably as a consequence of an unbalanced HBV replication caused by treatment-related suppression of HCV, although a direct immune-modulatory effect of IFN might also be advocated for either on- or off-treatment HBV reactivation **[12].**

In contrast, DAAs have no effect on HBV replication, but such therapies may release HBV from HCV suppressive effects, resulting in HBV reactivation in CHC patients with a concomitant overt or occult HBV Infection, leading to acute hepatitis with the risk of liver failure both on- or off-treatment **[13,14]**. Despite this, up to 2015 EASL and AASLD guidelines on HCV treatment did not provide specific indications for the management of OBI during or after HCV clearance by DAAs **[11]**.

This study was performed to determine the prevalence of anti-HBc and frequencies of hepatitis B virus (HBV) DNA and antibodies to hepatitis B surface antigen (anti-HBs) among chronic hepatitis C patients before antiviral therapy.

PATIENTS AND METHODS

Type of Study: Follow up descriptive study.

Site of Study: Port-Said center for treatment of viral hepatitis in Port-Said Fever Hospital.

Study Population:
Chronic hepatitis C patients treated with Sofosbuvir- based regimens.

Criteria of selection:
All 178 patients enrolled in this study were previously diagnosed as chronic hepatitis C patients aged 18-70 years. All patients which had decompensated liver diseases, hepatocellular carcinoma, extra-hepatic malignancy and uncontrolled diabetes mellitus (HbA1c >8%) were excluded. All these criteria were according to the protocol provided by national committee for control of viral hepatitis in Egypt (NCCVH) in December 2016.

Study methods:
Patients who enrolled into the study assessed anti-HB_C in serum of CHC patients before starting Direct Acting Anti-viral(DAAs) treatment regimen if positive assess HBV-DNA at baseline, end of treatment and at SVR12weeks.

A- Data collected by personal interview included:

1- Personal interviewing: for age, sex, residence, special habits, education, job, marital status , duration of liver disease since diagnosis.
2- History suggestive of etiology (blood transfusion ,dental extraction).
3- Presence of any other chronic illness (e.g. diabetes mellitus, hypertension, thyroid disorder, cardiac patient,…etc).
4- History of encephalopathy or hepatocellular carcinoma HCC.
5- In treatment experience patients type of IFN and date of last dose taking.
6- History of schistosomal infection ,if present type of treatment taking.
7- Clinical examination of patients which include:

B- General examination: with special emphasis on vital signs and the presence of signs of chronic liver disease such as darking of the face, wasting of temporalis and maseter muscles and prominent zygomatic bone, bilateral parotid enlargement, jaundice, fetor hepaticus, palmar erythema, lower limb edema, flapping tremors and impaired level of consciousness.

C- Local examination**:** withspecial emphasis on liver examination and detection of ascites.

D- Laboratory investigations:

1. Fasting blood sugar and if diabetic HbA1c
2. complete blood count.
3. Aspartate aminotransferase(AST), alanine aminotransferase (ALT).
4. Serum creatinine.
5. Prothrombin concentration or INR.
6. Serum albumin.
7. Serum total and direct bilirubin.
8. AFP.
9. HBs Ag.
10. HCV Ab and PCR for HCV if positive.
11. Anti-HB_c and HBV-DNA if positive at baseline and at SVR12weeks.

E- Assay of Anti-HB_c: Antibody to Hepatitis B virus Core Antigen Elisa Kit was provided by *Wkea med supplies corp*.

1- Sample Preparation**:** 10ml blood taken from patients.2ml in EDTA tube for CBC and HbA1C and 8ml in a plain tube for all other tests. Samples are allowed to clot for 10-20 mins at room temperature before centrifugation for 20mins at the speed of 2000-3000 r.p.m. Remove serum in eppendorf centrifugal tubes and store it at -20C until used.

2- Principle of The Assay: This kit is based on solid phase, one step incubation competitive principle ELISA method. Anti-HBc if present in the sample, compete with monoclonal anti-HBc conjugated to horse radish peroxidase (HRP-Conjugate) for a fixed amount of purified HBcAg pre-coated in the wells. When no anti-HBc present in the sample, the HRP-conjugated anti-HBc will be bound with the antigens inside the wells and any unbound HRP-conjugate is removed during washing. Chromogen A and B solutions are added into the wells and during incubation, the colorless Chromogens are hydrolyzed by the bound HRP-Conjugate to a blue-colored product. The blue color turn yellow after stopping the reaction with sulfuric acid. No or low color developing suggests for presence of antibodies to HBcAg in the sample.

Statistical analysis

Statistical analyses were performed using the statistical software package SPSS for Windows, version 19 (SPSS, IBM Inc., NC, USA). Baseline demographic and clinical characteristics were analyzed descriptively for all patients. Categorical variables were expressed as frequency and percentage while the continuous variables were expressed as mean and standard deviation. The normality of distribution for all variables was tested by Shapiro- Wilk test. We used the Mann–Whitney *U*-test, the Fisher's exact test and student's t test where appropriate.

RESULTS

A Total of 178 chronic hepatitis C patients initiated treatment with DAAs. Eighty four patients (47.2%) were treated with difficult treatment (SOF/DAC/RBV) and ninety four patients (52.8%) with easy treatment (SOF/ DAC). one hundred and seventy patients were treated for 12 weeks and eight treated for 24 weeks (Table 1). There were no significant differences between chronic hepatitis C patients with positive HBcAb vs. those with negative HBcAb regarding sex, age, history of smoking, Hemoglobin, and HCV RNA. However, positive HBcAb patients had significantly diabetes mellitus (Table 2). There was no significant correlation between liver enzymes and HBcAb seropositivity. However ALT and AST had reasonable sensitivity around 70% at cut off points 29.5 and 32.5 respectively the specificity dropped to25.4%and 40.4%, respectively (Figure 1).

Table (1): Baseline demographics, clinical characteristics and laboratory values in all patients

Parameter	Overall patients (178)
Age (years)	54.3±8.3
Sex (No., %)	
Males	93(52.2%)
Females	85(47.8%)
Smokers (No., %)	53(29.8%)
Diabetes mellitus (No., %)	21(11.8%)
Previous treatment failure (No., %)	
NO	170 (95.5%)
SIM/SOF	1(0.6%)
SOF/RBV	7(3.9%)
Hemoglobin (g/dl)	13.4±1.6
White blood cell count (/mm^3)	6.1±2.0
Platelets (X 10^3/mm^3)	173.6±72.3
ALT (IU/ml)	49.8±35.7
AST (IU/ml)	51.0±36.6
Bilirubin (mg/dl)	0.8±0.4
Albumin (g/dl)	3.9±0.5
INR	1.1±0.1
AFP (ng/ml)	8.6±10.5
Fib 4 score	2.9±2.4
HBsAg (negative)	178(100.0%)
HbcIgG	
Negative	113(63.5%)
Positive	65(36.5%)
HCV RNA (log 10 IU/ml) (mean ± SD)	3.3±11.0
HBV DNA (log10iu/ml)	0.0±0.0
Splenomegaly (No., %)	36(20.2%)

(ALT) alanine amino-transferase, (AST) aspartate amino-transferase, (INR) international normalized ratio, (AFP) alpha-fetoprotein, (HCV) hepatitis C virus, (SD) standard deviation .

Table (2): Baseline data for patients with positive HBcAb vs. negative HBcAb

Sociodemographic data	HbcAb		P value
	Positive (64) Mean ± SD	Negative (114) Mean ± SD	
Age	54.6±7.8	53.9±8.7	0.644
Sex (No., %)			
Males	34(53.1%)	59(51.8%)	0.861
Females	30(46.9%)	55(48.2%)	
Smoking (No., %)			
Nonsmoker	86(75.4%)	45(70.3%)	0.457
Smoker	28(24.6%)	19(29.7%)	
Diabetes mellitus (No., %)	12(18.5%)	9(8.0%)	0.037*
Previous treatment failure (No., %)			
NO	62 (96.9%)	108 (94.7%)	
SIM/SOF	0(0.0%)	1(0.9%)	0.675
SOF/RBV	2(3.1%)	5(4.4%)	
Splenomegaly (No., %)	17(26.2%)	19(16.8%)	0.135
Platelets	161.2±63.1	180.6±76.3	0.146
Hb	13.2±1.5	13.4±1.6	0.417
WBC	6.0±2.1	6.1±1.9	0.532
AST	48.8±29.4	52.3±40.1	0.850
ALT	46.3±27.3	51.8±39.6	0.697
INR	1.1±0.1	1.1±0.1	0.244
T. bilirubin	0.8±0.3	0.8±0.4	0.107
S. albumin	3.9±0.5	3.9±0.5	0.534
AFP	7.9±5.5	9.0±12.4	0.130
Fib 4 score	3.2±2.7	2.7±2.2	0.250
HBV DNA (log 10 IU/ml)	0	0	
HCV RNA (log 10 IU/ml)	5.8±15.8	1.8±6.3	0.056

* Statistically significant at $p<0.05$

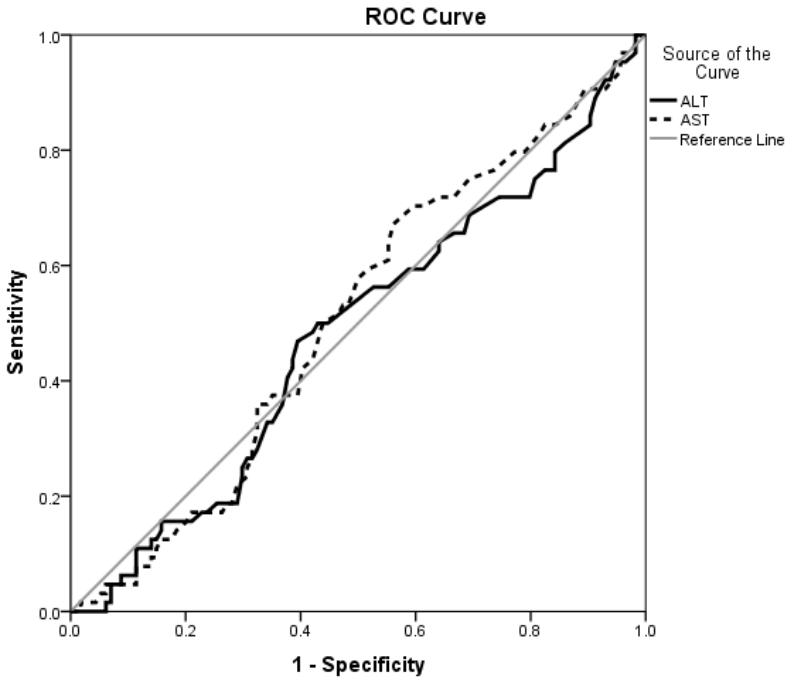

Figure (1): ROC curve and determine optimum cutoff value for ALT&AST to start screening for occult hepatitis B before DAAs

Area Under the Curve					
Test Result variables	Area	Std. Error	P value	95% Confidence Interval	
				Lower Bound	Upper Bound
ALT	0.48	0.045	0.697	0.394	0.571
AST	0.51	0.044	0.850	0.421	0.596

	Cut off value	Sensitivity	Specificity
ALT	29.50	71.9%	25.4%
AST	32.50	70.3%	40.4%

DISCUSSION

In this study OBI seroprevalence tested by HBcIgG Elisa has positive for sixty four patients(35%)with mean age of 54.6years old ,more in males (53.1%) than females (46.9%), type 2 diabetes mellitus (18.5%), highly Fib 4 index >3.25 and highly HCVRNA($5.8x10^5$ IU/ml). The EDHS 2015 included tests for anti- hepatitis B core antibody (anti-HB) and hepatitis B surface antigen (HBsAg) in addition to testing for HCV. The age prevalence of anti-HBc (indicating exposure to hepatitis B virus [HBV] infection) mirrors the age prevalence of anti-HCV in both males and females.

The incidence of OBI in HCV patients varies greatly, ranging from 0% to 52**%** [15]**.** In this study; 35% of HCV patients were sero-positive for OBI. Our results were in agreement with those reported among Mediterranean countries **[16]**.

Furthermore, two similar studies Fukuda et al. and Liu et al., reported that the serum titer of HCV-RNA was visibly higher in patients with concurrent HBV and HCV infection than those with HCV mono-infection, this reports agreed with this study **[17,18]**. Additionally, Mrani et al., also found that HCV viral load was significantly higher in HBV-DNA positive than in negative patients **[1]**. Also, Emara et al. **[15]** reported that this seemed to be applicable to genotype 4, where HBV-DNA positive patients in their study showed higher baseline HCV viral load than HCV mono-infected patients.

Also, Chen et al.**[19]** reported that patients with both OBI and HCV infection had lower ALT

levels, liver histology activity index and fibrosis scores than those with HCV mono-infection. The clinical impact of OBI on anti-HCV therapy outcome is still controversial.

Few studies however evaluated the impact of occult hepatitis B infection on the current standard treatment of HCV infection and viral replication of HBV. European Medicines Agency (EMA, 2016) reported all the cases of OBI patients should be interpreted with caution before establishing a clear correlation between effective DAAs treatment and HBV reactivation, due to the presence of at least one possible confounding factor.

In recent study, De Monte et al., reported HBV reactivation in an HIV/HCV co-infected male who discontinued TDF 14 months before starting DAAs due to bone toxicity **[20]**. In this case the role of HIV infection and/or the immune reconstitution after effective HAART cannot be excluded as causes of the HBV reactivation, not to mention discontinuation of TDF that is effective on HBV.

Also, there is a recent Asian study in 124 HCV infections with OBI patients treated with DAAs showing no cases of HBV reactivation **[21]**.

Yeh et al., observed a minimal impact of anti-HBc seropositivity on HCV efficacy and safety, while the risk of reactivation was present for CHC patients with current infection **[22]**. Similarly, Belperio et al., and Sulkowski et al., affirmed the rarity of HBV reactivation after DAA, even in the setting of isolated anti-HBc **[23,24]**.

So, it is important to screen all CHC patients for HBV markers (HBsAg, anti-HBc and anti-HBs) before starting DAAs .OBI patients serum HBV DNA should be assessed with a very sensitive test at baseline and monitored during and after DAAs in those patients with positive baseline. Whereas no periodic monitoring of serum HBV DNA or HBsAg during and after DAAs treatment is recommended in patients with undetectable.

Baseline serum HBV DNA. In the latter patients periodic monitoring of ALT may be enough to detect hepatic flare reflecting HBV reactivation to be treated with anti-HBV therapy. Current EASL guidelines even suggest starting concurrent HBV nucleoside/nucleotide analogue therapy if HbsAg is present or HBV-DNA is detectable in OBI **[11]**.

However, further prospective studies in large cohorts of OBI patients better characterized from the virological point of view at baseline and during and after EOT are needed to quantify and stratify the risk of HBV reactivation in parallel with HCV eradication, and to standardize the management of such patients in order to avoid the risk of fatal complication.

CONCLUSION

In conclusion, occult hepatitis B Infection is highly prevalent in chronic hepatitis C infection and not depend on HBs Ag only in diagnosis for HBV infection. In future we need to check for HBsAg, anti HBc, and if positive screen for HBVDNA PCR in chronic hepatitis C infection treated with oral direct acting antiviral drug in initial visit, end of treatment and after 12 weeks of treatment for fear of reactivation of HBV infection.

Ethics:
The study confirmed to the 1975 Declaration of Helsinki and was approved by the Ethics Committee of Suez Canal University Faculty of Medicine in February 2016. Written, informed consent was obtained from each patient included in this study.

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No Conflict of Interest.

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Efficacy and Safety of Peg-Interferon/Sofosbuvir/Ribavirin VS Sofosbuvir/Simeprevir in Egyptian chronic hepatitis C patients

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Key words: *Sofosbuvir, simeprevir, HCV RNA, Interferon*

Background and study aim: Recently, multiple regimens of different direct-acting antiviral agents (DAAs) have been emerged. We aimed to assess the efficacy, safety and improvement of liver profile for patients treated with regimens of direct acting-antivirals in Egypt.

Patients and Methods: A retrospective observational study was conducted at Suez governorate, including a simple random sample of 76 patients treated with directly-acting antiviral therapy and came to our center to enroll our follow up program after antiviral therapy from November 2015 to May 2016. Sustained viral response (SVR) was established at week 12 after end of treatment.

Results: A total of 76 chronic hepatitis C patients initiated treatment with DAAs. Forty patients (52.6%) were treated with triple therapy and thirty-six patients (47.3%) with dual therapy. All patients were treated for 12 weeks. According to Intention to treat analysis, 35 of 40 patients (87%) who treated with triple therapy achieved SVR while 32 of 36 patients (88.9%) treated with dual therapy achieved SVR. However, the difference between responders after both regimens wasn't statistically significant (p= 1). In the group treated with triple therapy, significantly more patients had anemia, leukopenia and thrombocytopenia with no serious side effects leading to discontinuation of therapy.

Conclusion: Both regimens had similar efficacy, but the dual therapy was more tolerated with less side effect profile.

INTRODUCTION

Hepatitis C virus (HCV) is a worldwide problem. Globally, it was estimated that in 2005, more than 185 million people had hepatitis C virus (HCV) antibodies (prevalence of 2.8 percent) **[1]**. The condition has been worsened in Egypt as it has the highest HCV prevalence in the world **[2]**. In 2008, an Egyptian Demographic Health Survey (EDHS) determined a prevalence of 14.7% for sampled population 11125 had positive antibodies to HCV, however only 9.8% were found to have HCV RNA **[3]**. Recently, Egyptian Health issues Survey (EHIS) in 2015 estimated a prevalence of 10% for sampled population 26172 had positive antibodies to HCV, however only 7% were found to have HCV RNA **[4]**. HCV also represents an economic burden in Egypt which will continue over the next decade **[5]**.

As far as 2011, the combination of pegylated interferon and ribavirin for 48 weeks was the effective treatment for chronic hepatitis C, but several HCV direct-acting antiviral agents (DAAs) have been approved in 2014 for HCV infection so many DAA agents have been licensed as the standard treatment now **[6]**.

We aimed to assess the efficacy, safety and improvement of liver profile for patients treated with Peg- triple therapy (Peg-Interferon/Sofosbuvir/Ribavirin) VS dual therapy (Sofosbuvir/Simeprevir) regimens. These regimens represented the first early experience of Egyptian chronic hepatitis C patients with DAAs.

PATIENTS AND METHODS

Study design:
A retrospective observational study was conducted in Communicable Diseases Research and training Center (affiliated with Suez Canal University hospitals) at Suez governorate from November 2015 to May 2016.

Population and sample:
Target population:
Chronic hepatitis C patients treated with Sofosbuvir-based regimens included dual therapy, Simeprevir & Sofosbuvir and triple therapy, Pegylated interferon, Sofosbuvir and Ribavirin in Egypt.

Study sample:
A simple random sample of 76 patients was selected from total 559 patients treated with directly-acting antiviral therapy and came to our center to enroll our follow up program after antiviral therapy.

Criteria of selection:
All patients enrolled in this study were previously diagnosed as chronic hepatitis C patients aged 18-70 years and had Fib 4 more than 2.5.All patients which had decompensated liver diseases, hepatocellular carcinoma, extra-hepatic malignancy and uncontrolled diabetes mellitus (HbA1c >8%) were excluded. All these criteria were according to the protocol provided by national committee for control of viral hepatitis in Egypt (NCCVH) in May 2015.

Assessment
HCV RNA was assessed at all patients 12 weeks and 24 weeks after therapy completion using Cobas AmpliPrep/Cobas TaqMan HCV Test, Version 2.0, Real-Time PCR assay, Roche Molecular Systems, with a Low detection limit of 15 IU/mL and a linear amplification range of HCV RNA from approximately 15 to 10 000 000 IU/ mL. CAP/CTM HCV v2.0 assays.

Ethics
The study was approved by the Ethics Committee of Suez Canal University Faculty of Medicine. Written, informed consent was obtained from each patient included in this study.

Statistical analysis
Statistical analyses were performed using the statistical software package SPSS for Windows, version 19 (SPSS, IBM Inc., NC, USA). Baseline demographic and clinical characteristics were analyzed descriptively for all patients. Categorical variables were expressed as frequency and percentage while the continuous variables were expressed as mean and standard deviation. We used the Mann–Whitney *U*-test, the Fisher's exact test and student's t test where appropriate. Differences were considered statistically significant when $P<0.05$. Statistical analysis will be performed using SPSS version 19 (SPSS, IBM Inc., NC, USA).

RESULTS

Treatment response, predicators and side effect profile
Baseline characteristics between patients treated with both regimens who achieved SVR and non-responders were described and there were no significant differences between them. (Table 1,2)

According to Intention to treat analysis, 35 of 40 patients (87%) who treated with triple therapy (Peg-IFN/SOF/RBV) achieved SVR. While 32 of 36 patients (88.9%) treated with dual therapy (SIM/SOF) achieved SVR. However, the difference between responders after both regimens wasn't statistically significant (p= 1) (Table 3).

Multivariate logistic regression analysis was done in patients treated with triple therapy (Peg-IFN/SOF/RBV) and dual therapy (SIM/SOF); with the failure of response was dependent variable. In patients treated with triple therapy, male gender, patients with previous interferon therapy and viral load >600 000 IU were 1.1, 7.2 and 5.2 times less likely to respond, respectively. Moreover, in patients treated with dual therapy, males, patients with previous interferon therapy and viral load > 600 000 IU were 1.2, 2.2 and 1.8 times less likely to respond, respectively (Table 4). However, the model was statistically insignificant for both groups (P = 0.056 and P = 0.872, respectively).

In the group treated with triple therapy, significantly more patients had anemia, leukopenia and thrombocytopenia with no serious side effects leading to discontinuation of therapy (Table 5).

Follow up laboratory assessment during and 12 weeks after therapy
In patients treated with triple therapy (Peg-INF/ SOF/RBV), ALT levels improved significantly until week 12 post-treatment (89.05 ± 41.87 IU/ ml at baseline vs. 46.15 ± 39.91 IU/ml at week 12 post-treatment; P<0.05). Moreover, AST levels also improved significantly at the same time point (93.05 ± 32.83 IU/ ml at baseline vs. 50.87

± 41.11 IU/ml at week 12 post-treatment; P = 0.106). However, the levels of Hemoglobin steeping decreased significantly after therapy (13.59 ± 1.51 g/L at baseline vs. 11.62 ± 1.51 g/L at week 12 post-treatment; P<0.05). Moreover, the levels of platelet count significantly decreased as follow: (151.35 ± 32.46 × 10^3/mm^3 at baseline vs. 125.5 ± 37.85 × 10^3/mm^3 at week 12 post-treatment; P < 0.05). Also, WBCs count as follows: (6.12 ± 1.75 /mm^3 at baseline vs. 4.77 ± 1.87 /mm^3 at week 12 post-treatment; P<0.05). (Figure 1 & 2).

In patients treated with dual therapy (SIM/SOF), ALT levels normalized during and after therapy (67.78 ± 35.14 IU/ ml at baseline vs. 21.94 ± 10.71 IU/ml at week 12 post-treatment; P < 0.05). Also, AST levels normalized as follow: (71.5 ± 35.57 IU/ ml at baseline vs. 25.58 ± 8.72 IU/ml at week 12 post-treatment; P<0.05). Moreover, the Platelet count improved significantly during therapy until week 12 post-treatment (108.47 ± 38.56 × 10^3/mm^3 at baseline vs. 120.64 ± 45.14 × 10^3/mm^3 at week 12 post-treatment; P<0.05). However, the hemoglobin levels decreased significantly after therapy as follow: (13.58 ± 1.27 g/L at baseline vs. 12.68 ± 1.78 g/L at week 12 post-treatment; P<0.05) (Figure 3 & 4).

Table (1): Sustained virological response (SVR12) and predicators of response in patients treated with triple therapy (Peg-INF/SOF/RBV).

Parameter	SVR n= 35 (87.5%)	Non-SVR n= 5 (12.5%)	P value
Age (years) (mean ± SD)	56.86±6.35	53.2±4.82	.103
Sex [male : female]	19:16	3:2	1
Body mass index (Kg/m^2) (mean ± SD)	30.63±4.75	30.2±2.49	.751
Smokers (%)	2 (5.7)	1 (20)	.338
Diabetes mellitus (%)	12 (34.3)	1 (20)	1
Previous treatment failure (%)	4 (11.4)	1 (20)	.507
Current type of Interferon (INF) INF alpha-2a (%) INF alpha-2b (%)	 16 (45.7) 19 (54.3)	 3 (60) 2 (40)	.654
Hemoglobin (g/dl) (mean ± SD)	13.63±1.5	13.28±1.43	.605
White blood cell count (/mm^3) (mean ± SD)	5.98±1.77	7.08±1.27	.157
Platelets(X 10^3/mm^3) (mean ± SD)	153.4±31.69	137±37.98	.228
ALT (IU/ml) (mean ± SD)	91.57±42.07	71.4±40.34	.298
AST (IU/ml) (mean ± SD)	96.31±32.74	70.2±25.52	.113
Bilirubin (mg/dl) (mean ± SD)	0.93±0.28	0.79±0.41	.449
Albumin (g/dl) (mean ± SD)	4.02±0.34	3.84±0.22	.244
INR	1.15±0.17	1.17±0.05	.498
AFP (ng/ml) (mean ± SD)	10.29±6.86	14.54±11.82	.498
Fib 4 score	3.89±1.03	3.53±0.85	.498
Splenomegaly (%)	14 (40)	2 (40)	1

(SVR) sustained virological response, (ALT) alanine aminotransferase (AST) aspartate aminotransferase, (INR) international normalized ratio, (AFP) alpha-fetoprotein, (HCV) hepatitis C virus, (SD) standard deviation

Table (2) : Sustained virological response (SVR12) and predicators of response in patients treated with dual therapy (SIM/SOF)

Parameter	SVR n= 32 (88.9%)	Non-SVR n= 4 (11.1 %)	P value
Age (years) (mean ± SD)	57.78±7.45	58.5±3	1
Sex [male : female]	21:11	3:1	1
Body mass index (Kg/m^2) (mean ± SD)	30.33±5.18	27.25±2.75	.208
Smokers (%)	5 (15.6)	1 (25)	.535
Diabetes mellitus (%)	12 (37.5)	0	.278
Previous treatment failure (%)	4 (12.5)	0	1
Hemoglobin (g/dl) (mean ± SD)	13.58±1.35	13.55±0.59	.610
White blood cell count ($/mm^3$) (mean ± SD)	4.66±1.47	4.65±1.93	1
Platelets(X $10^3/mm^3$) (mean ± SD)	108±39.66	107.25±33.12	.942
ALT (IU/ml) (mean ± SD)	64.31±31.29	95.5±55.99	.248
AST (IU/ml) (mean ± SD)	66.37±27.2	112.5±67.79	.19
Bilirubin (mg/dl) (mean ± SD)	0.95±0.42	1.45±1.07	.366
Albumin (g/dl) (mean ± SD)	3.77±0.58	3.52±0.46	.393
INR	1.24±0.18	1.29±0.11	.315
AFP (ng/ml) (mean ± SD)	10.42±6.07	19.17±12.79	.173
Fib 4 score	5.2±3.09	6.54±2.76	.340
Splenomegaly (%)	25 (78.1)	1 (25)	.057

(SVR) sustained virological response, (ALT) alanine aminotransferase, (AST) aspartate aminotransferase, (INR) international normalized ratio, (AFP) alpha-fetoprotein, (HCV) hepatitis C virus, (SD) standard deviation

Table (3): Sustained virological response in all patients according to received regimens of antiviral therapy

Type of Regimen	SVR 12 (%)		P value
	SVR	Non-SVR	
Peg-INF/SOF/RBV	35 (87)	5 (12)	1
SIM/SOF	32 (88.9)	4 (11.1)	

(SVR) sustained virological response, (SOF) Sofosbuvir, (INF) Interferon, (RBV) Ribavirin, (SIM) Simeprevir

Table (4): In multivariate logistic regression, in which dependent variable is the treatment failure in patients treated with both regimens

Baseline Characteristics	Triple therapy		Dual therapy	
	OR (CI 95 %)	P value	OR (CI 95 %)	P value
Male gender	1.1 (.07- 16.9)	0.93	1.2 (.09 – 15.4)	0.87
Previous treatment failure	7.2 (.8 – 64.3)	0.07	2.2 (.16 – 32.1)	0.55
Viraemia > 600 000 IU	5.2 (.4 – 64.7)	0.2	1.8 (.17 – 20.2)	0.62

Table (5) : Side effect profile in all patients according to received regimens of antiviral therapy

Side effect	Overall (%)	Peg-INF/SOF/RBV (%)	SIM/SOF (%)	P value
Pseudo-flu syndrome	3 (3.9)	3 (7.5)	0	.242
Respiratory disorders	6 (7.9)	6 (15)	0	.026
Headache	2 (2.6)	2 (5)	0	.495
Skin rash	1 (1.3)	1 (2.5)	0	1
GI Symptoms	4 (5.3)	4 (10)	0	.117
Anemia	30 (39.5)	24 (60)	6 (16.7)	.000
leukopenia	26 (34.2)	22 (55)	3 (8.3)	.000
Thrombocytopenia	22 (28.9)	19 (47.5)	4 (11.1)	.000
Elevated bilirubin	13 (17.1)	7 (17.5)	6 (16.7)	1
Elevated ALT	6 (7.9)	5 (12.5)	1 (2.8)	.204
Elevated AST	1 (1.3)	1 (2.5)	0	1

(SOF) Sofosbuvir, (Peg-INF) Pegylated Interferon (RBV) Ribavirin, (SIM) Simeprevir, (ALT) alanine aminotransferase, (AST) aspartate aminotransferase

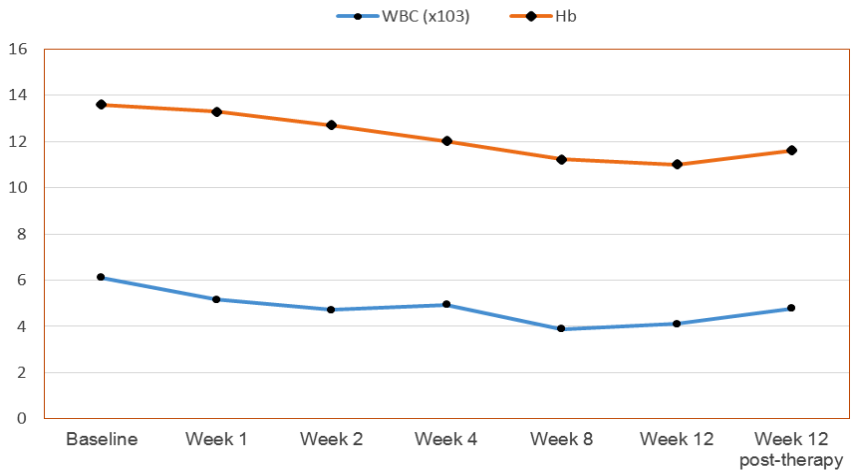

Figure 1. Hematological changes regarding WBC (x103) & Hemoglobin levels (gm/dl) at baseline and follow-up assessments during and 12 weeks post-end of Peg-INF/RIB/ SOF therapy (N=40)

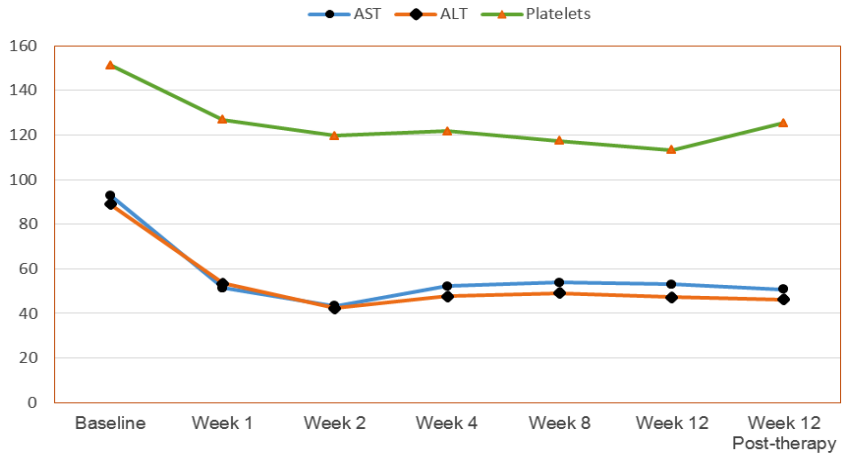

Figure 2. Changes in AST, ALT & Platelets (x103) at baseline and follow-up assessments during and 12 weeks post-end of Peg-INF/RIB/ SOF therapy (N=40)

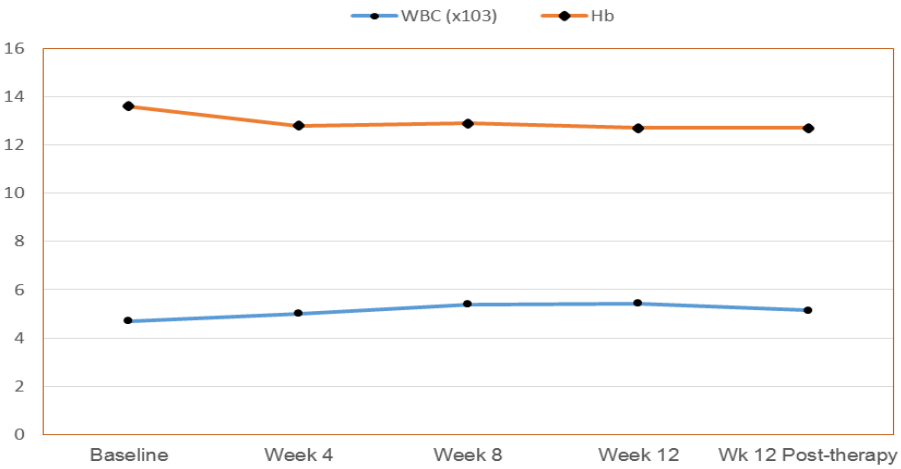

Figure 3. Hematological Changes regarding WBC (x103) & Hemoglobin level (gm/dl) at baseline and follow-up assessments during and 12 weeks post-end of SIM/SOF therapy (N=36)

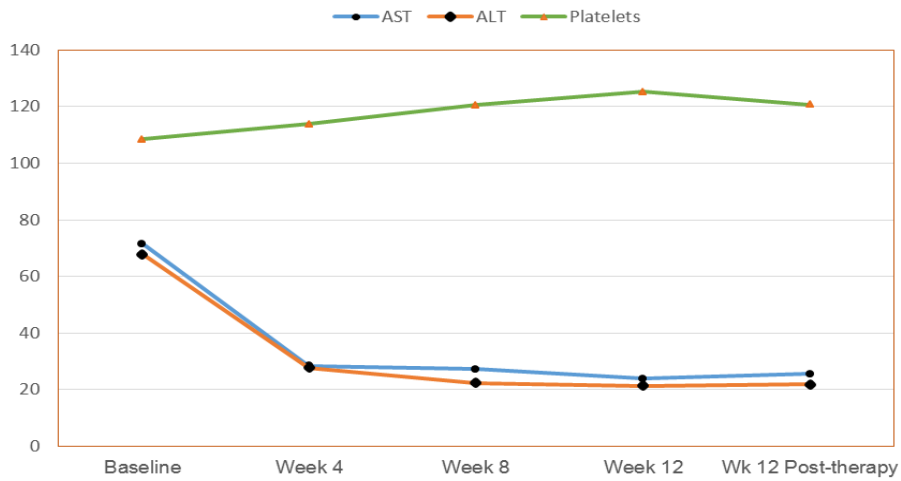

Figure 4. Changes in AST, ALT & Platelets (x103) at baseline and follow-up assessments during and 12 weeks post- end of SIM/SOF therapy (N=36)

DISCUSSION

In this study 76 patients with proven chronic hepatitis C which were treated for 12 weeks by DAAs. Forty patients (52.6%) were treated with triple therapy (Peg-INF/SOF/RBV) and SVR12 rate was 87% while thirty-six patients (47.3%) were treated with dual therapy (SIM/SOF) and SVR12 rate was 88.9%.

A retrospective multicentric study conducted in Egypt on 8742 chronic hepatitis C patients with compensated cirrhosis treated with triple therapy (Peg-INF/SOF/RBV) for 12 weeks and the SVR12 rate was 94%. The SVR12 in this cohort is slightly higher than my study as all 76 patients included in my study have high FIB4 (3.85 ± 1) in comparison to this cohort (3.08 ± 7.6) **[7]**.

A single centered, nonrandomized, uncontrolled phase 2 study conducted at Texas on 47 treatment-experienced patients with HCV genotypes 2 and 3. All these patients treated for 12 weeks with triple therapy (Peg-INF/SOF/RBV) and the overall SVR12 rate was 89%, but for genotype 3, the SVR 12 was 83% **[8]**.

In the phase 2 trial (LONESTAR-2 trial) using a 12 week regimen of triple therapy (Peg-INF/SOF/RBV), SVR12 rate was 89% among 47 treatment-experienced patients with chronic HCV genotype 2and 3 infection, so that the EASL guidelines 2015 recommended this combination of as a first option in patients with genotype 3 **[9]**.

A randomized multicenter phase 2 trial (ATOMIC) reported that 82% of patients with genotype 4 who treated with triple therapy achieved SVR12 **[10]**. Moreover, a randomized, double-blind trial (phase 2) in USA stated that SVR12 rate among 47 patients with genotypes 1, 2 and 3 was 91% **[11]**. It is notable that our study had SVR12 close to SVR12 rate of these phase 2 clinical trials. However, the NEUTRINO study (phase 3) reported higher SVR12 than our study which was 96% among patients with genotype 4 **[12]**.

A multicenter cohort study in Egypt stated that the overall SVR12 rate was 95% in 583 chronic hepatitis patients treated with 12 week regimen of dual therapy (SIM/ SOF). Moreover, The SVR12 rates in both naïve patients and those with previous interferon treatment were 94% to 99% for mild to moderate fibrosis (F1-F3) and 80% to 90% for advanced fibrosis (F4), respectively **[13]**.

Another Egyptian cohort study conducted on 6211 chronic HCV genotype 4 patients found the SVR 12 rate was 97% after 12 week regimen of dual therapy (SIM/SOF). Moreover, SVR 12 rates in easy and difficult to treat (patients with Fib 4 index >3.25 and METAVIR score F3- F4) groups were 96% and 93% respectively **[14]**. In our study had close SVR 12 rate to the patients with advanced fibrosis in the previous two Egyptian studies **[13,14]**.

A retrospective study from Netherlands assessed the SVR 12 for 53 patients with genotype 4 HCV infection treated with dual therapy (SIM/SOF) with or without ribavirin which was 92% **[15]**. This SVR12 was slightly higher than our result which might be explained by addition of ribavirin in the Dutch study as they reported that all relapsed patients didn't receive ribavirin **[15]**.

The OSIRIS trial studied effectiveness of simeprevir plus sofosbuvir for eight or 12 weeks in 63 chronic hepatitis C virus (HCV) genotype 4 patients with METAVIR F0-F4 fibrosis. Accordingly, the overall SVR was 92% while the SVR in 23 patients with compensated cirrhosis (METAVIR F4) received 12 weeks of treatment was 100% which higher than my study **[16]**.

The OPTIMIST-1 reported efficacy of dual therapy (SIM/SOF) for 12 weeks in 133 chronic hepatitis C virus (HCV) genotype 1 patients without cirrhosis (METAVIR F0-F3). Hence, the SVR12 was 97% which higher than our study due to lower fibrosis stage in patients included in the OPTIMIST-1 **[17]**.

However, the efficacy of Simeprevir 150 mg and Sofosbuvir 400 mg once daily for 12 weeks in 103 chronic hepatitis C virus (HCV) genotype 1 patients with cirrhosis was accessed in the OPTIMIST-2 study **[18]**. Accordingly, the SVR12 was 83% in the OPTIMIST-2, which close to my study.

A single center study conducted at Miami, US reported the SVR12 for 86 chronic hepatitis C virus (HCV) genotype 1 patients with confirmed cirrhosis (About 60% of them was cirrhotic and METAVIR score was F4) treated with dual therapy (SIM/SOF) and 12 other patients treated with triple therapy (Peg-INF/SOF/RBV). Accordingly, the SVR12 for dual therapy was 88%, which agree with our results. However, the SVR12 for triple therapy was 50% which was much lower than our results **[19]**.

Also, a single center study at Atlanta, US found that chronic hepatitis C virus (HCV) genotype 1 patients treated with dual therapy (SIM/SOF) for 12 weeks had a significantly higher rate of SVR12 than those treated with triple therapy (Peg-INF/SOF/RBV) **[20]**. In this study, a 12 week regimen of dual therapy also had a higher rate of SVR12 than a 12 week regimen of triple therapy, but unfortunately not significant.

In our current study, males, patients with previous Interferon therapy and high viral load less likely to achieve SVR12, but all of these baseline characteristics were statistically insignificant as predicators for treatment failure in both univariate and multivariate analysis. Similar results were reported by Christina et al, who reported baseline characteristics included age, BMI, high viral load, cirrhosis, prior treatment and ethnicity were no longer predicative factors for treatment failure except male gender in directly acting antiviral therapy **[21]**.

In contrast, female gender, higher baseline platelets and grades of fibrosis were predicators for SVR12 in 583 chronic hepatitis C genotype 4 patients treated with dual therapy (SIM/SOF) **[13]**. Also, a large cohort observational study in Egypt reported that male gender, higher baseline viraemia and previous treatment failure were predicative factors for treatment failure in 14, 409 chronic hepatitis C genotype 4 patients

treated with triple therapy (Peg-INF/SOF/RBV) **[7]**.

In this study, liver enzymes improved significantly in patients treated with both regimens while the platelet count improved only in patients treated with dual therapy (SIM/SOF). This agreed with German study which reported improvement of liver function during directly acting antiviral therapy regardless of HCV genotype **[22]**. Moreover, Dutch study stated that platelet count improved in decompensated hepatic fibrosis patients following SVR after interferon-based therapy which correlated with decreased spleen size as portal pressure might be improved by HCV eradication **[23]**.

In our study, Dual therapy (SIM/SOF) and triple therapy (Peg-INF/SOF/RBV) were well tolerated in patients evaluated. Notable side effects in patients treated with triple therapy included pseudo-flu syndrome in 7.5%, respiratory disorders in 15%, headache in 5%, skin rash in 2.5%, GI symptoms in 10%, anemia in 60%, thrombocytopenia in 47.5%, and hyper-bilirubinemia in 17.5%. Furthermore, mild side effects also noted in patients treated with dual therapy included anemia in 16.7%, thrombo-cytopenia in 11.1%, and hyperbilirubinemia in 16.7%.

Similar side effect profile reported in other studies which evaluated the patients treated with triple therapy also included pseudo-flu syndrome, headache, skin rash, GI symptoms, anemia, thrombocytopenia, and hyperbilirubinemia (Lawitz et al., 2013a; Lawitz et al., 2015a; Wu et al., 2015). Moreover, the most reported adverse events in patients treated with dual therapy included anemia, thrombocytopenia and hyper-bilirubinemia with no serious adverse effects leading to discontinuation of treatment **[13,17]**.

In conclusion, both regimens had similar efficacy, but the dual therapy was more tolerated with less side effect profile.

Ethical consideration

This research was approved by the Ethics committee at Faculty of Medicine, Suez canal university in February 2016

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Hepatic Fascioliasis, Unexpected Diagnosis and Atypical Presentation

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INTRODUCTION

Hepatic *Fascioliasis* affects about 17 million people worldwide and out-breaks occur mostly in developing areas of the Caribbean **[1]**. In Egypt, the prevalence is variable. It is about 2-17% in different villages in Nile delta **[2]**. Humans are accidently infected after ingestion of encysted metacercaria contaminating watercress or water. Metacercaria are then developed into larva which penetrate duodenal wall to reach the liver. The disease has two phases; parenchymatous (acute/ hepatic) phase; characterized by fever, right upper quadrant pain, nausea, vomiting, jaundice, hepatomegaly and eosinophilia. The second phase is biliary (chronic), characterized by cholangitis and cholestasis **[3]**.

Detection of *Fasciola* eggs in the stool is the most definitive test for diagnosis. Immunodiagnostic tests including enzyme immunoassays (EIA) and indirect haemagglutination assay (IHA) are useful in early phases of infection **[4]**. Imaging studies include abdominal ultrasonography and Computed tomography (CT) scanning may reveal characteristic lesions in the liver. Such findings may be confused with alternate diagnosis such as malignancy **[5]**. Abdominal ultrasonography findings in the parenchymal phase may include focal hypo-echoic or hyper-echoic lesions or diffuse involvement of the liver, while in the ductal phase there may be ductal dilatation which appears as hypo-echoic lines parallel to portal areas. Computed tomography (CT) findings in the parenchymal phase include multiple branching hypo-dense lesions, while in ductal phase there are dilated biliary ducts **[6]**.

Here we present a case with hepatic focal lesions caused by *Fasciola hepatica*, misdiagnosed initially by her physician as hepatocellular carcinoma (HCC), due to its high incidence in our community, aiming at increasing the awareness of the physicians with such presentation.

CASE REPORT :

A 35 years old female living in a village belongs to Zagazig, a city in the Nile delta, Egypt, presented to another health institute with upper abdominal pain, anorexia for one month. Her blood results were as follow; normal complete blood count (CBC), increased alanine aminotransferase (ALT) and aspartate aminotransferase (AST) more than twice the upper limit of normal, alkaline phosphatase (ALP): 185 U/L, gamma-glutamyl transpeptidase (GGT): 115 U/L and bilirubin: 1.5 mg/dl. Abdominal ultrasonography showed hypo-echoic focal lesions with ill-defined borders which were confirmed by abdominal CT. Her physician gave a probable diagnosis of HCC (based on high incidence of HCC in our community) and referred to our hospital. On admission the patient complained of upper abdominal pain. On examination there was no fever, no jaundice, and no organomegaly. Repeated laboratory tests showed normal CBC, ALT; 55 U/L, AST; 43U/L, ALP; 143 U/L, GGT; 96 U/L, bilirubin; 2 mg/dl and Alfa

fetoprotein (AFP); 6 ng/ml. Abdominal CT imaging confirmed the presence of multiple branching hypo-dense focal lesions in segments 7 and 8 of the liver but no cirrhosis (Fig. 1). Serology for viral hepatitis (B,C) was negative. However, repeated stool analysis revealed *Fasciola* eggs. Nitazoxanide was given for one month. Her symptoms resolved and liver biochemistry improved. However, CT abdomen showed regression of the lesions but didn't disappear completely, so, Triclabendazole 10mg/kg was given once. Two months later, there was resolution of the focal lesions completely.

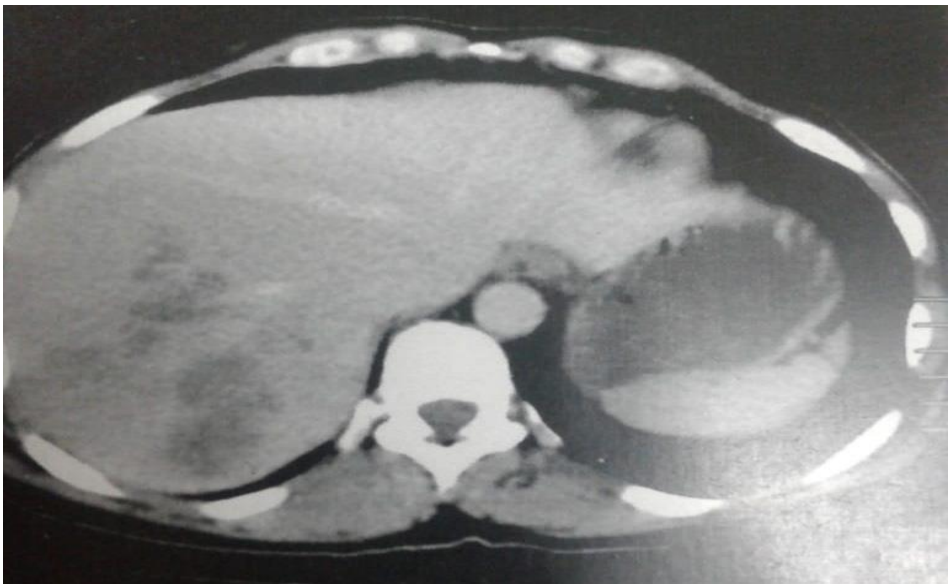

Figure (1) : Abdominal CT showed hypo-dense branching focal lesions in segments 7 and 8 in liver

DISCUSSION

Hepatic Fascioliasis is a neglected infectious disease despite its high prevalence in the developing countries. It is caused by the trematode *Fasciola hepatica* which is a parasite of herbivores (sheep, cattle) and human is accidentally infected. Human Fascioliasis has two important clinical phases; the hepatic or acute phase is characterized by fever, right upper quadrant pain, jaundice and eosinophilia, while the biliary or chronic phase is characterized by recurrent cholangitis or biliary obstruction **[7]**. Although our case presented with relevant clinical symptoms and signs, her laboratory tests didn't reveal eosinophilia. This may be attributed to high endemicity of the disease and acclimatization of the immune system to the parasite due to exposure through the whole life, or it may be due to immuno-suppression, a state which wasn't encountered in our case.

Although detection of the *fasciola* eggs in the stool is the gold standard for the diagnosis **[8]**, initial stool analysis of our case couldn't detect any ova. This may be attributed to presentation of the patient in the acute stage when immature worms feed on the liver tissue and no ova release. Also, absence of the ova in stool may be related to intermittent ova release **[9]**. Diagnosis of Fascioliasis should be confirmed by immunological tests including ELISA or HIA in case of absence of the eggs in stool.

Hepatic multiple focal lesions seen on routine abdominal ultrasonography is common in practice in our community due to high prevalence of hepatocellular carcinoma (HCC). However, the young age of the patient and absence of underlying liver disease together with the experience of our consultant with the radiological characteristics of fascioliasis, we made a suspicious of diagnosis. Further evaluation using abdominal computed tomography (CT) revealed characteristic branching hypo-dense lesions in segment 7 and 8 of the liver **[10]**.

Triclabendazole (10-20 mg/kg/day) is the drug of choice for treatment of Fascioliasis **[11]**. Due to unavailability of this drug we used Nitazoxanide

initially which gave some improvement. However, after treatment with Triclabendazole, complete resolution of the lesions was observed denoting its superiority over Nitazoxanide **[12]**.

In conclusion, *Fasciola hepatica* should be kept in mind in the differential diagnosis of hepatic focal lesions, especially in younger patients with no history of chronic liver disease, since our country is endemic for it. We report this case to our physicians to be familiar and oriented with such atypical scenario.

Ethical consideration: Informed consent was taken from the patient.

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Conflict of Interest :None.

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