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Contents

Fibrogenesis Markers and Hepatic Histopathology : What is the Role?

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See article pages:4-11

Background

HCV infection has an estimated prevalence of 3% around the world[1],and Egypt is among the highest prevalence [2]. Asymptomatic HCV patients are underrepresented.Unfortunately, many persons with HCV infection are asymptomatic[3]. Many asymptomatic seropositive donors have clinically significant liver disease[4]. The progression to severe fibrosis and occurrence of HCC were reported [1, 2]. Patients with normal enzymes may have definite chances of chronic hepatitis on histological examination [6].

Percutaneous liver biopsy, is the gold standard for grading and staging liver diseases [7], but it is invasive, has limitations [8], and asymptomatic patientsmay not accept.

The matrix metalloproteinases (MMPs), and their inhibitors are groups of proteins involved in controlling matrix degradation. Therefore, it seems that imbalance between MMPs and TIMPs affects rate of fibrosis progression, and their estimation was correlated with the stage of fibrosis [9].

Aspartate aminotransferase to platelet ratio (APRI Score) was also proved useful to stage liver fibrosis[10].It is an easy and validated predictor of hepatic fibrosis in chronic hepatitis C [11].

Non invasive diagnosis of liver fibrosis and cirrhosis in chronic hepatitis C, is required in pre-treatment and follow up [12].

Summary of paper

The paper entitled " Can fibrogenesis markers reflect early hepatic histopathology in chronic hepatitis C?" published in this issue of the Afro-Egyptian journal of infectious and Endemic Diseases by Abou El-Azm et al., aimed at evaluating individual and combined non invasive indicators of fibrogenesis (MMP-2, T1MP1 and APRI score) to assess early hepatic histopathology, and developing cirrhosis in chronic HCV patients with and without symptoms. The authors enrolled 344 patients (Group I:129 asymptomatic chronic-HCV, Group II: 135 with symptoms and Group III: 80 patients with compensated HCV-related cirrhosis). For each patient, APRI-Score was evaluated. Quantitative immunoassay measured serum MMP-2 and TIMP-1. Guided liver biopsy for histopathology staging and grading was done. The results imply that combination of markers raised the sensitivity, specificity and correlations. It could reflect early hepatic histopathology, developing cirrhosis and potentially could replace liver biopsies in pre-treatment and follow up of chronic HCV.

Comment on the study

The current study showed a significant correlation of AST with the stage of fibrosis in the studied patients. This finding is consistent with the results mentioned that liver fibrosis severity and subsequent cirrhosis were correlated with high AST levels [13].

The current study showed that platelets decreased significantly in severe fibrosis or cirrhosis and these results are in agreement with previous results[14].Decreased platelet count was the earliest indicator of cirrhosis [15].

As regard to direct serum fibrogenesis markers which reflect extracellular matrix turnover, MMP-2 & TIMP-1were measured and correlated to the stage of fibrosis in liver biopsy.There was a significant positive correlation between serum MMP-2 and serum TIMP-1 and the stage of fibrosis. These results are in accordance with the results of Abdel-Samea et al. [14].

APRI score was reported to have correlations with the stages of histological fibrosis [16], in agreement with the present results .While Khairy M et al, showed that APRI score had moderate degree of accuracy [10].Ma et al. considered it

as a tool with limited expense, widespread availability, a promising noninvasive alternative to liver biopsy for detecting hepatic fibrosis and treatment response in patients with chronic hepatitis C [17].

Although the outcome of non-invasive markers in different studies is not the same but multiplicity of markers can give more accuracy. The combined indicators of fibrosis: TIMP-1, MMP-2 and APRI score in the current study showed a higher sensitivity, specificity, and strong correlations with histopathology staging of liver fibrosis.

Recommendations:

Serum markers of hepatic fibrosis are to replace liver biopsy - especially in the presence of obstacles – in assessment of patients with HCV related liver disease and in follow up of these patients post-treatment.

Further serum markers of liver fibrosis are to be determined individually or in combination to replace liver biopsy. These markers include procollagen type III N-terminal peptide (PIIINP) [18], procollagen type I N-terminal peptide (PINP) [19], type IV collagen [20], procollagenV C-terminal peptide (PVCP) [21], hyaluronic acid [22], matrix metalloproteinase 1 (MMP 1) and matrix metalloproteinase 9 (MMP 9) [23].

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Can Fibrogenesis Markers Reflect Early Hepatic Histopathology in Chronic Hepatitis C?

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See editorial pages:1-3

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Key words: chronic hepatitis C; liver biopsy; matrix metalloproteinases; APRI score

Background and study aim: Chronic HCV can progress to cirrhosis, and HCC. Liver biopsy is the best to assess and monitor progression. But it has limitations.The aim of this study was to evaluate noninvasive indicators of fibrogenesis, matrix metalloproteinase-2 (MMP-2), tissue inhibitor of metalloproteinase-1 (T1MP1) and AST/Platelet (APRI-score) for assessment of early hepatic histopathology in chronic-HCV, and cirrhosis.

Patients and methods: A cross section study included 344 participants from Tanta University Hospitals, (GI): 129 asymptomatic chronic-HCV, (GII): 135 with symptoms. (GIII): 80 patients with compensated HCV-related cirrhosis and 30 healthy controls. Investigations proved diagnosis, and excluded associated diseases. APRI-Score was evaluated. Quantitative immunoassay measured serum MMP-2 and TIMP-1, guided liver biopsy for histopathology staging and grading.

Results: Serum MMP-2& TIMP-1 showed significant difference between control& GI, GII, GIII, and GI, GII& GIII (P< 0.001) with significant correlation between GI& GIII, between GII & GIII, while insignificant between G1 & GII. There was significant positive correlation between APRI-score versus Metavir A, Metavir F, Ishak score of fibrosis, serum MMP-2 and serum TIMP-1 (P <0.001). Combined serum MMP-2, TIMP-1 and APRI-score showed high sensitivity, and specificity.

Conclusion: This combination of markers raised the sensitivity, specificity and correlations. It could reflect early hepatic histopathology, developing cirrhosis and potentially could replace liver biopsies in pre-treatment, follow up of chronic-HCV, and screening of asymptomatic patients.

INTRODUCTION

HCV infection has an estimated prevalence of 3% around the world [1], and Egypt is among the highest prevalence [2].

Asymptomatic HCV patients are underrepresented. Unfortunately, many persons with HCV infection are asymptomatic [3]. Many asymptomatic seropositive donors have clinically significant liver disease [4]. The progression to severe fibrosis and occurrence of HCC were reported [1, 2]. Patients with normal enzymes may have definite chances of chronic hepatitis on histological examination [6].

Percutaneous liver biopsy, is the gold standard for grading and staging liver diseases [7], but it is invasive, has limitations [8], and asymptomatic patients may not accept.

The matrix metalloproteinases (MMPs), and their inhibitors are groups of proteins involved in controlling matrix degradation. Therefore, it seems that imbalance between MMPs and TIMPs affects rate of fibrosis progression, and their estimation was correlated with the stage of fibrosis [9].

Aspartate aminotransferase to platelet ratio (APRI Score) was proved also useful to stage liver fibrosis [10]. It is an easy and validated predictor of hepatic fibrosis in chronic hepatitis C [11].

Non invasive diagnosis of liver fibrosis and cirrhosis in chronic hepatitis C, is required in pre-treatment and follow up [12]. So, we aimed to evaluate individual and combined non invasive indicators of fibrogenesis (MMP-2, T1MP1 and

APRI score) to assess early hepatic histopathology, and developing cirrhosis in chronic HCV patients with/without symptoms.

PATIENTS AND METHODS

A cross sectional study included 344 participants: (GI) 129 asymptomatic chronic HCV, (GII) 135 with HCV specific symptoms (abdominal pain, fatigue, tinge of jaundice etc…) and (GIII) 80 patients with compensated HCV-related cirrhosis in addition to 30 proved healthy subjects as control. They were collected between the years 2010 and 2013 from Department of Hepatolog, GIT & Infectious Diseases which is a pooling center for patients with viral hepatitis, and Tanta University blood bank with matched age and sex. Asymptomatic patients were selected from those accidentally discovered blood donors, pre-employment and during check up of cases contacts or pre-travel. They may have mild elevated liver functions or even average.

Exclusion criteria:

Patients proved, with associated diseases as other causes of hepatitis, decompensated liver cirrhosis, collagen diseases, blood diseases and active schistosomiasis were excluded.

Quantitative detection of serum matrix metalloproteinase (MMP-2) in patients with chronic HCV using Immunoassay kits R&D Systems Inc. McKinley Place N.E. Minneapolis, USA [13].

Quantitative detection of human TIMP-1 in the serum of patients, with chronic HCV using Flow Cytomix Human TIMP-1 Simplex Kit, Bender MedSystems GmbH, Campus Vienna Biocenter 2, A-1030 Vienna, Austria [14]. Sample collection and storage: a serum separator tube was used and allowed blood sample to clot 30 minutes. Once clotted, samples are centrifuged at 1000 x g for 10 min. Carefully remove serum and assay immediately or aliquot and store samples at <-20 c. Freeze/ thaw cycles were avoided.

APRI score (AST/Platelet ratio) was calculated after liver functions and blood picture

Guided liver biopsy: After completion of proper abdominal sonographic scanning, and checking the patient for any bleeding tendencies, and patient's consent, A18 G true- cut needle was set on a probe guide and a gun was used for biopsy. The biopsy was preserved in diluted formalin solution and sent for histopathological examination.

Histopathological examination of liver biopsy: The length of each histological specimen was 1.5 to 2.5 cm, and all specimens were placed in 40 g/L methanol for fixation immediately. After dehydration, they were embedded in paraffin, and sections were then stained with Hematoxylin-Eosin and Masson trichrome. The histological changes were examined under light microscopy for type, degree and activity of hepatic affection.

Statistical Analysis:

Was conducted, using the mean, standard deviation, by SPSS® V.16. Values were compared between groups by using the student's t-test. ANOVA test was used for comparison of quantitative data: has significant value: ($P < 0.05$*) and ($P < 0.001$**) is highly significant but insignificant: ($P > 0.05$).

RESULTS

There was insignificant difference between the studied groups as regard to age and sex ($P>0.05$).

Comparison between the studied groups in relation to serum MMP-2 and TIMP-1 (Table: 1 and Figure: 1a, b) showed a significant difference between control & GI, GII and GIII ($P < 0.001$) and G1, GII & GIII ($P < 0.001$) while insignificant between GI &GII ($P > 0.05$).

Correlation between AST/PLAT ratio (APRI score) and different scores of fibrosis, MMP2 and TIMP1 (Table: 2 and Figure 2a, b) showed significant positive correlation between APRI score and Ishak, Metavir A, Metavir F scores of fibrosis, serum MMP-2 and serum TIMP-1 ($P<0.001$*).

Histopathological staging and grading (Figure 3a) showed liver section from chronic-HCV patient showed ground glass, portal inflammation, fibrosis and piece meal inflammation (stage 2, grade 3) and (Figure 3b) showed porto-central fibrosis.

Inividual markers: MMP-2 (table: 3a), TIMP-1 (table: 3b) and APRI score (table: 3c) were compared as regard to AUC (Area under the curve), sensitivity and specificity. TIMP-1 was the highest.

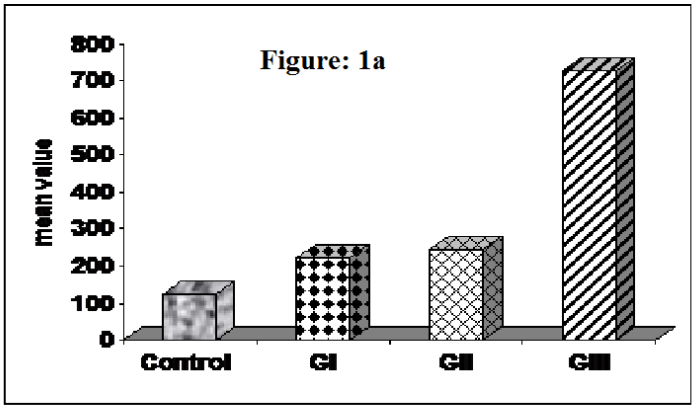
Combined markers: MMP-2 plus TIMP1 against APRI score in table 4a, combined APRI score plus MMP2, and TIMP1 (table: 4b) raised

sensitivity and specificity than any single marker, or combined MMP-2 and TIMP1. AUC indicated excellent test.

Table 1: Comparison between the studied groups in relation to serum MMP-2 and TIMP-1.

MMP-2:	Range	Mean + SD	p. value
Control	93-255	125.4±61.31	
GI	120-415	226.3±57.2	0.001*
GII	130-607	235.1±91.5	
GIII	520-936	727.5±121.5	
P1	0.314		
P2	0.001*		
P3	0.001*		
TIMP-1:			
Control	9-278	139.25±135.63	0.001*
GI	340-940	617.6±1569.04	
GII	200-966	691.3±196.1	
GIII	1400-2610	1878±317.2	
P1	0.517		
P2	0.001*		
P3	0.001*		

Figure 1: Comparison between the studied groups in relation to serum MMP-2 (1a) and TIMP-1 (1b).



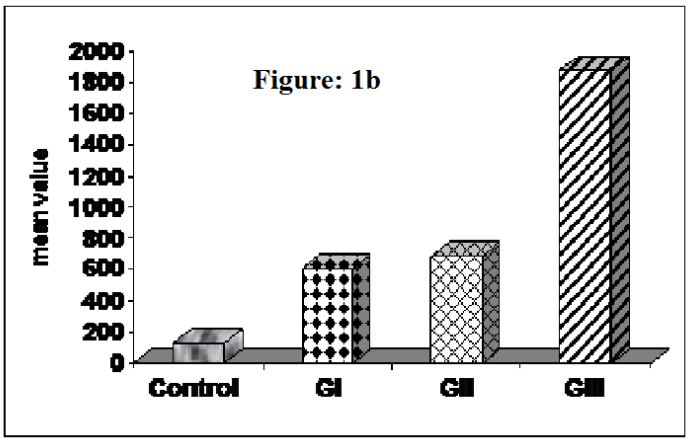
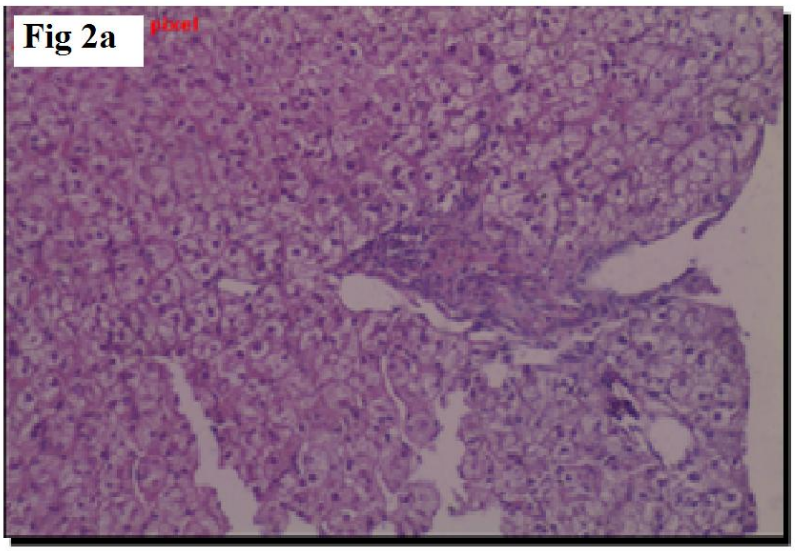


Table 2: Correlation between AST/PLAT ratio (APRI score) and different scores of fibrosis, MMP2 and TIMP1

	AST/Platelets	
	r.	**p. value**
ISHAK	0.514	0.001
METAVIR A	0.621	0.001
METAVIR F	0.471	0.001
MMP2	0.482	0.001
TIMP1	0.564	0.001

Figure 2a: liver section (HX &E) from chronic HCV patient showed ground glass, portal inflammation, fibrosis and piece meal inflammation (stage 2, grade 3) and (**Figure 2b**) showed porto-central fibrosis.



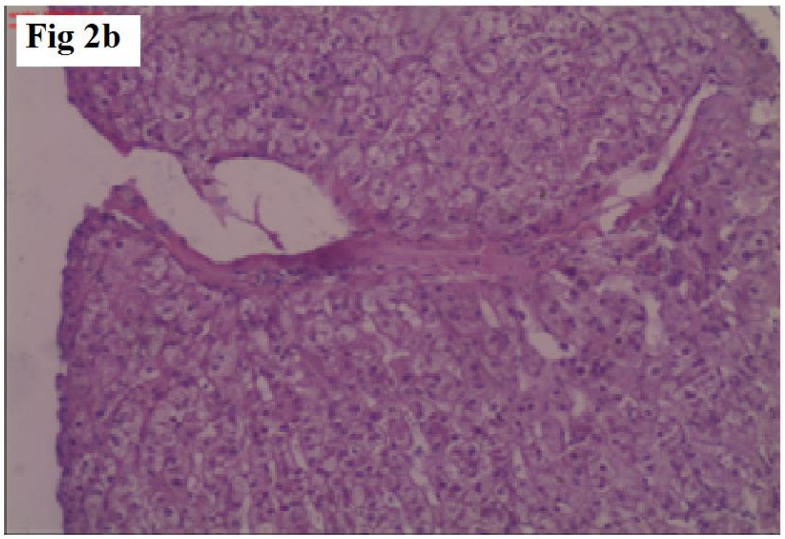
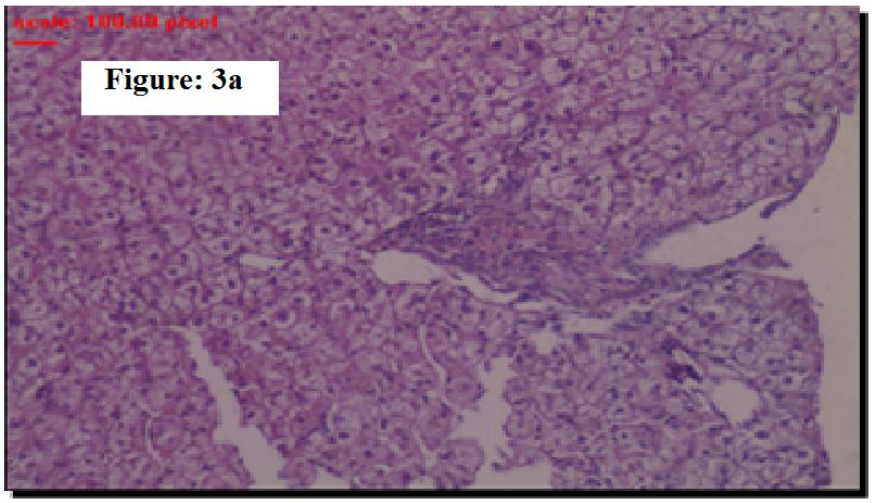


Figure 3a: liver section (HX &E) from chronic HCV patient showed ground glass, portal inflammation, fibrosis and piece meal inflammation (stage 2, grade 3) and (**Figure 3b**) showed porto-central fibrosis.



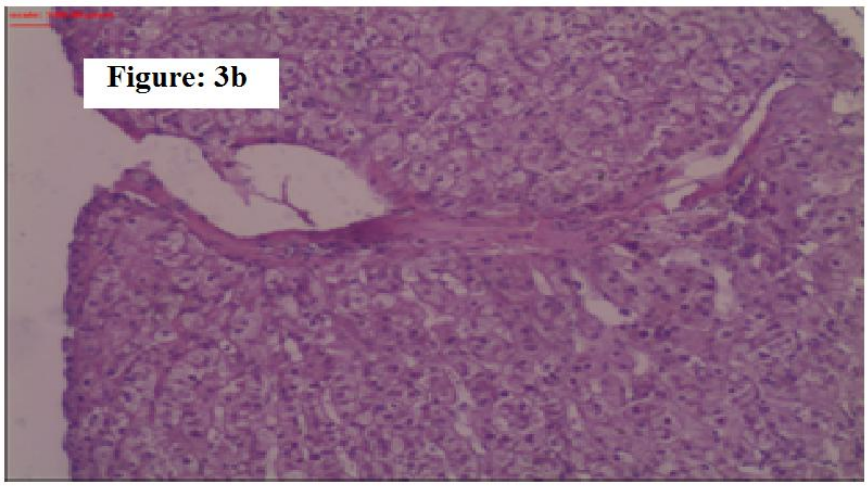


Table 3: AUC, Sensitivity and Specificity of individual markers: APRI score, MMP2 and TIMP-1 & Metavir A and Metavir F

Table 3a: APRI score & Metavir A and Metavir F

	AST/platelets (APRI score)		
	AUC	Sensitivity	Specificity
METAVIR A	0.752	90%	88%
METAVIR F	0.796	92%	86%

Table 3b: MMP-2 & Metavir A and Metavir F

	MMP2		
	AUC	Sensitivity	Specificity
METAVIR A	0.869	89%	85%
METAVIR F	0.874	87%	86%

Table: 3c: TIMP-1 & Metavir A and Metavir F

	TIMP1		
	AUC	Sensitivity	Specificity
METAVIR A	0.925	91%	92%
METAVIR F	0.936	95%	89%

AUC = Area Under the Curve AUC=1.0 indicate perfect test,
0.9 to 0.99 indicate excellent test 0.8 to 0.89 indicate good test
0.7 to 0.79 indicate fair test, 0.6 to 0.69 indicate poor test and 0.5 indicate worthless test

Table 4: AUC, Sensitivity and Specificity of Combined markers:

Table 4a: Showed APRI score & combined MMP2& TIMP1

	AST/platelet (APRI score)		
	AUC	Sensitivity	Specificity
MMP2& TIMP1	0.947	96%	94%

Table 4b: Showed Combined AST/platelets, MMP2 and TIMP1 & Metavir A and Metavir F

	Combined AST/platelets, MMP2 and TIMP1		
	AUC	Sensitivity	Specificity
METAVIR A	0.925	95%	94%
METAVIR F	0.917	96.1%	97%

AUC indicate excellent test with highest sensitivity and specificity

DISCUSSION

In the current study, all groups showed insignificant difference as regard to age, and sex as fibrosis progression was influenced by duration of infection and the age at time of infection [15].

The present results of AST and ALT showed significant difference between GI and both: GII and GIII, also between GII and GIII. This means that asymptomatic patients had lower levels. AST findings disagree to some extent with the results of Wendy et al, [16] who detected no correlation between severity of symptoms and AST levels. This could be related to the younger age of their patients and the possibility of short period of exposure. We detected, also significant correlation of AST, with the stage of fibrosis in the studied patients which may be attributed to reduced clearance of AST by liver as fibrosis progress [17], and/or mitochondrial injury of active necrosis [18]. This finding was in consistent with the results mentioned that liver fibrosis severity and subsequent cirrhosis were correlated with high AST levels [19].

The results of platelet count showed significant difference between GI & G III and between GII & GIII but not between GI & GII, so platelets decreased significantly in severe fibrosis or cirrhosis and these results were in agreement with the previous results [20]. Thrombocytopenia may be related to the bone marrow supprression due to HCV infection as direct viral replication and/or in association with immune complexes deposition in bone marrow [21], and the decreased production of diseased liver to thrombopiotin. Decreased platelet count was the earliest indicator of cirrhosis [22].

The current study showed a significant positive correlation between fibrosis stage and grade of inflammation. These findings were in accordance with previous reports [23]. This could be attributed to that the necroinflammatory process implicated in fibrogenesis process and activation of stellate cells around the necroinflammatory lesions. Thus, severe degrees of inflammatory activity can predict worsening of hepatic fibrosis [24].

As regard to direct serum fibrogenesis markers, which reflect extracellular matrix turnover: MMP-2 & TIMP-1were used to assess stage of fibrosis, instead of invasive liver biopsy. Our MMP-2 results showed significant difference between GI & GIII and between GII & GIII, but insignificant difference between GI & GII. This means that serum MMP-2 was significantly higher in cirrhotic patients, significant positive correlation between serum MMP-2 and the stage

of fibrosis, and Ishak score of fibrosis. This was in accordance with the results of Abdel-Samea et al., [20]. This could suggest that MMP-2 reliably to differentiate between cirrhotic and non cirrhotic particularly when liver biopsy has obstacles.

TIMP-1 showed significant difference between GI & GIII, and between GII & GIII but no significant difference between GI & GII. This means that serum TIMP-1 was significantly higher in cirrhotic patients than that of chronic hepatitis patients in accordance with Badra et al., [25]. A significant correlation was noticed between serum TIMP-1 and the stage of fibrosis and this finding was in consistent with their results. It was mentioned, also that serum TIMP-1 values can detect fibrosis with comparable efficiency, which was correlated with histological activity [9, 26].

The efficacy of TIMP-1 in detection of stage of fibrosis could be attributed to that TIMP-1 is produced mainly by activated hepatic stellate cells, and kupffer cells [27]. It is well established that activation of hepatic stellate cells, is a key event in the pathophysiology of hepatic fibrosis, and is accompanied by induction of TIMP-1[28]. TIMP-1 has been suggested as a profibrogenic factor to promote liver fibrosis.

APRI score was reported to have correlations with the stages of histological fibrosis [29], in agreement with the present results .While Khairy et al, showed that APRI score had moderate degree of accuracy [10]. Ma et al, considered it as a tool with limited expense, widespread availability, a promising noninvasive alternative to liver biopsy for detecting hepatic fibrosis and treatment response in patients with chronic hepatitis C [30].

Although the outcome of non-invasive markers in different studies is not the same but multiplicity of markers can give more accuracy. The combined indicators of fibrosis: TIMP-1, MMP-2 and APRI score in the current study showed a higher sensitivity, specificity, and strong correlations, with histopathology staging of fibrosis and grading of liver inflammation. AUC also indicated excellent test.

CONCLUSION

Combined serum level of TIMP-1 and MMP-2 with APRI score, are noninvasive tests, simple, inexpensive, and capable of accurate reflection of hepatic inflammation and early fibrosis in patients with HCV-related hepatitis, and developing cirrhosis. So, they could replace liver biopsy in the future or potentially decrease the number of liver biopsies especially in presence of obstacles.

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Conflicts of interest: The authors declare no conflict of interest.

Ethical approval: The study was approved by the Ethical Committee of Tanta Faculty of Medicine and a written informed consent was taken from each participant that follows principles in the Declaration of Helsinki.

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Prevalence and Outcome of Bleeding Gastro-esophageal Varices in Medical Intensive Care Unit at Zagazig University Hospitals, Egypt

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Key words:prevalence; hematemsis; gastro-esophageal varices;

Background and study aim: Ruptured gastro-esophageal varices (GEV) are the most severe and frequent cause of gastrointestinal bleeding in cirrhotic patients, leading to death in 5% to 8% of patients during the first 48 hours. Recently, the 6-week mortality rate has fallen from 34% to 20% due to the development of effective treatment strategies. This study was conducted to find out the prevalence of GEV as a cause of upper GI bleeding, a common medical emergency, and to find out the effect of other co-morbidities and risk factors on the outcome.

Patients and Methods: This was an observational longitudinal prospective study. It included patients admitted to hematemesis subunit of medical ICU at Zagazig University Hospitals with endoscopic diagnosis of bleeding GEV in the period from May to September 2013. 448 patients were enrolled in this study. 374 patients of them were observed until stabilization and discharge (survived group) and 74 patients were observed throughout their stay in the medical ICU and unfortunately passed away (deceased group). All subjects of our study were subjected to complete history, full physical examination, routine investigations and upper GIT endoscopy.

Results: There was a significant positive correlation between hospital mortality and the age of the patients. The mean age of the deceased group was 59.44 ys ± 5.89 compared to 47.9 ys ± 9.93 of the survived group (p-value=0.036). Also the incidence of mortality in male sex was more than female sex (p-value= 0.005). The stage of liver disease according to Child-Pough class, MELD Score (model of end stage liver disease), the presence of complication of cirrhosis and abnormal liver functions (high serum bilirubin, elevated ALT, low serum albumin and prolonged INR) were the most important factors contributing to mortality in intensive care patients suffering from GEV bleeding (p-value= 0.002). The presence of co-morbid conditions (DM, COPD, renal failure), which were found in 20.3% of our patients, was associated with increased mortality (p-value=0.007). Severity of bleeding, delayed endoscopic intervension, hemodynamic instability (at the time of endoscopy), all were associated with increased risk of mortality. high APACHE II score (Acute Physiology and Chronic Health Evaluation) is associated with marked increase in mortality.

Conclusion: Patients with variceal bleeding comprised a great burden to our medical ICU being about 13.7% of all admitted patients. The short term ICU mortality of these patients is 16.5%. The multivariate regression analysis identified the APACHE II score, MELD score, severity of the bleeding attacks, time to endoscopic intervention. ALT level, advancing age, presence of co-morbidities and spontaneous bacterial peritonitis (SBP) were found to be independent risk factors. So, primary and secondary prevention as well as better adherence to current guidelines for management of such cases to minimize the mortality as much as possible are recommended.

INTRODUCTION

Ruptured gastroesophageal varices (GEV) are the most severe and frequent cause of gastrointestinal bleeding in cirrhotic patients leading to death in 5% to 8% of patients during the first 48 hours [1]. So, variceal bleeding (VB) is a medical emergency and its management should be undertaken in an intensive care setting by an experienced medical staff. High mortality rates were reported in older literature for patients with acute VB although there was a trend towards improved survival in those patients [2].

The current recommended haemostatic treatment of VB is to start a vasoactive drug on admission and associate endoscopic therapy at the time of diagnostic endoscopy [3].

Advances in pharmacological and endoscopic therapies have led to a decrease in mortality [4]. The 6-week mortality rate has fallen from 34% to 20% due to the development of effective treatment strategies [5]. Also, due to advances in medications and endoscopy, upper GI hemorrhage is now usually treated without surgery, Patients with upper GI hemorrhage often present with hematemsis, melena, or hematochezia if the hemorrhage is severe. The presentation of bleeding depends on the amount and location of hemorrhage [6].

The prevalence of variceal bleeding in a population of in-hospital patients is about 16%. [7]. Another study stated that, in cirrhotic patients without varices at first endoscopy, the prevalence of new varices is 5–10% [8]. Also according to Groszmann et al. [9] it was postulated that the varices are present in about 30–40% of compensated cirrhotic patients and in 60% of decompensated patients.

This study was planned for estimation of the prevalence of bleeding esophageal varices in medical intensive care unit with evaluation and analysis of clinical, biochemical and endoscopic features of involved patients. Also, assessment of prognostic scoring system in relation to patient outcome and study of the risk factors affecting the mortality in this group of patients.

PATIENTS AND METHODS

This study was carried out in the hematemesis subunit of medical intensive care unit of Internal Medicine Department, Faculty of Medicine, Zagazig University Hospitals in the period from May to September 2013.

The study was conducted on 448 subjects who were admitted because of bleeding due to ruptured GEV as diagnosed by Endoscopy. They were divided, according to survival, into 2 groups : *survived group,* which included 374 patients admitted and observed throughout their hospital stay and *deceased group,* which included 74 patients admitted and observed throughout their stay in the medical ICU and unfortunately passed away. The survived group included 245 males and 129 females with a mean age ±SD of 47.9 ±9.93 years. They were discharged after stabilization. The deceased group included 60 males and 14 females with a mean age ± SD of 59.44±5.89 years. This number was selected from a total number of 735 patients admitted to the ICU with a preliminary diagnosis of acute upper GIT bleeding.

Patients were excluded from the study if they were suffering from non variceal bleeding as diagnosed by upper GIT endoscopy, non endoscopized patients (dropped out cases), refusal of the patients or their relatives to participate in the study. All patients were submitted to full clinical assessment including history taking, physical examination, and the following investigations: complete blood picture, liver function tests, renal function tests, bleeding profile, random blood glucose level, arterial blood gases, ascitic fluid examination. Other investigations included : chest x-rays, abdominal ultrasonography and upper GIT endoscopy. The liver condition was assessed using Child- Pugh class (A, B and C) and MELD score (Model of End stage liver Disease) The MELD score was calculated using the original formula without including the cause of liver disease [10].

MELD score = (9.57 x (creatinine mg/dL) +3.78 x (bilirubin mg/dL) + 11.20 x (INR) +6.43)

Pre-endoscopic evaluation to identify those patients who may be at risk of further bleeding or dying and those who would require blood transfusion or intervention to stop bleeding either endoscopic or surgical, we used Blatchford score (Glasgow-Blatchford Score) . The score was developed using a combination of clinical and laboratory Parameters (presentation with syncope or melaena, evidence of hepatic or cardiac disease, pulse and blood pressure, hemoglobin and urea) [11].

The score is calculated using the table below:

Glasgow-Blatchford Score	
Admission risk marker	Score component value
Blood Urea	
≥6·5 <8·0	2
≥8·0 <10·0	3
≥10·0 <25·0	4
≥25	6
Haemoglobin (g/L) for men	
≥12.0 <13.0	1
≥10.0 <12.0	3
<10.0	6
Haemoglobin (g/L) for women	
≥10.0 <12.0	1
<10.0	6
Systolic blood pressure (mm Hg)	
100–109	1
90–99	2
<90	3
Other markers	
Pulse ≥100 (per min)	1
Presentation with melaena	1
Presentation with syncope	2
Hepatic disease	2
Cardiac failure	2

In the validation group, scores of 6 or more were associated with a greater than 50% risk of needing an intervention.

By using APACHE II scoring system [12], we evaluated the clinical state of our patients and assessed their mortality.

Statistical Analysis:

All data were coded, checked, entered and analyzed using SPSS software version 17.

RESULTS

Figure (1): Flow chart showing frequency distribution of the patients with upper gastrointestinal bleeding in medical ICU of Zagazig University Hospitals (period from May to September 2013).

817 patients were admitted to medical ICU of Zagazig University hospitals during study period with preliminary diagnosis of UGIB (24.9% of total admission). 448 (61%) of these patients (i.e. 13.7% of all ICU cases) confirmed to have variceal bleeding and this means the prevalence of variceal bleeding in medical ICU of Zagazig University Hospitals was 13.7%.

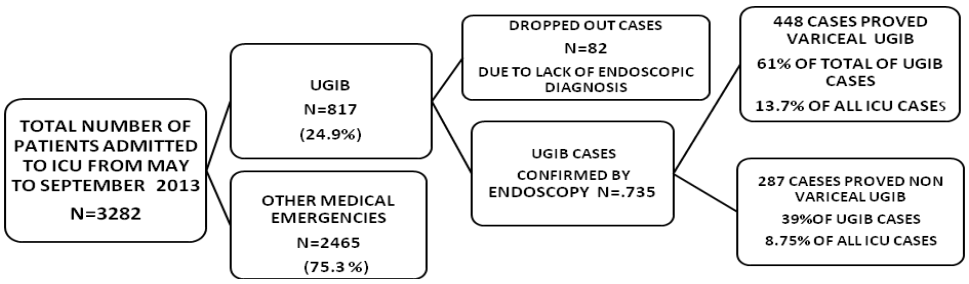

Table (1): Demographic and clinical characteristics of the study group (n=448).

	Number of the patients	Percentage%
Age (yrs) (X±S.D)	52.3±9.42	
Sex. M/F (%) - Male	305	68.1%
- Female	143	31.9%
Smoking - Smoker	104	23.2%
-Nonsmoker	344	76.8%
NSAID use -Used	110	24.6%
-Not used	338	75.4%
Number of attacks -1st.	86	19.2%
-2nd.	130	29.0%
-Recurrent	232	51.3%
Severity of the attack		
- Mild	135	30.1%
-Moderate	182	40.6%
-Sever.(H.D unstable)	131	29.2%
History of CLD		
-Known CLD.	378	84.4%
-1st discovered CLD	70	15.6%
Child class - Class A	22	4.9%
-Class B	188	42.0%
-Class C	238	53.1%
Comorbidity: - Diabetic	40	8.9%
- Heart failure	11	2.5%
- COPD	21	4.7%
- Renal failure.	19	4.2%
Complicated patient:		
SBP	118	26.3%
Hepatocellular carcinoma	81	18.1%
Ascites	386	86.1%
Bleeding at Endoscopy		
- Bleeder	105	23.4%
-Non-bleeder	343	76.6%
Vasopressor use: Used	107	23.7%
Not used	341	76.3%

*Demographic and clinical data of study population: 68.1% were males, 31.9%were females, 23.2% were smokers, 24.6% gave a history of recent NSAIDs intake, 19.2% presented for the 1st time, 29% presented for 2nd time while 51.3% gave a history of more than 2 attacks of bleeding. Regarding bleeding severity, mild, moderate and sever bleeding encountered in 30.1, 40.6% and 29.2% respectively. Regarding liver disease severity 4.9% were Child (A), 42% were Child (B), while 53.1% were Child (C). 20.3% had co-morbid disease association. These patients presented with complication as spontaneous bacterial peritonitis (SBP) in (26.3%), hepatocellular carcinoma (HCC) in 18.1% and ascites in (86.1%). During Endoscopy active bleeding was encountered in 23.4% of the cases.

Table (2): Univariate analysis of the data of the deceased group compared to survived group

	Deceased group (No = 74)		Survival group (No = 374)		t Value	χ^2	P Value
Age (yrs), (X±S.D)	59.44	5.89	47.9	9.93	2.947		0.036*
Sex. Male	60	80.2%	245	65.5%			
Female	14	17.8%	129	34.5%		0.83	0.005*
Smoker. Smoker	12	16.4%	92	24.6%			
Non-smoker	62	83.6%	282	75.4%		1.75	0.131
NSAIDS use User.	18	23.3%	126	22.4%			
Non user	56	76.7%	248	77.6%		0.13	0.811
Number of attacks							
1st. attack	2	2.7%	84	22.5%			
2nd. attack	22	28.8%	109	29.1%		18.62	0.003*
Recurrent	50	68.5%	181	48.4%			
Severity Mild	2	2.7%	132	35.3%			
Moderate	15	19.2%	168	44.9%		0.98	0.004*
Severe	57	78.1%	74	19.8%			
Child class Class A	0	0	22	5.9%			
Class B	5	5.5%	184	49.2%		10.79	0.029*
Class C	69	94.6%	168	44.9%			
Comorbidity: Diabetes	26	35.6%	14	3.7%			
Heart failure	5	6.8%	6	1.6%			
COPD	4	5.5%	17	4.5%		103.8	0.024*
Renal Failure	16	17.8%	6	1.6%			
Complications: SBP	28	38.4%	90	24.1%		3.33	0.026*
HCC	51	69.9%	30	8%		18.29	0.039*
Ascites	69	92%	314	84%		10.13	0.056*
Bleeding during edoscopy							
Bleeder	54	72.4%	85	22.7%		2.53	0.039*
Non- bleeder	20	27.6%	289	77.3%			
Endoscopy ≤ 8 hrs	10	13.7%	229	61.2%			
9-11 hrs	14	17.8%	71	19%			
12-23 hrs	23	31.5%	56	15%		9.23	0.031*
24-48 hrs	27	37%	18	4.8%			
Vasopressor use: Used	15	19.2%	92	24.6%			
Not used	59	80.8%	281	75.1%		1.63	0.54
Endoscopy Grade 2 OV	5	6.8%	147	39%			
Grade 3 OV	34	46.6%	121	32%			
GOV	14	19.2%	66	17%		27.42	0.042*
Isolated GV	20	27.4%	38	10%			
APACHE II	26.75	3.68	16.98	3.39	27.46		0.003*
Meld score.	29.3	6.98	13.44	5.24	18.42		0.004*
Alaninetransferase(ALT)	51.6	19.43	47.16	16.5	1.81		0.047*
Platelets count.	77.71	39.18	131.52	71.97	10.85		0.036*
Creatinine.	4.88	3.92	2.07	1.02	4.68		0.035*
Urea level	121	19	52	38	36.8		0.039*
Bilirubin	8.02	5.9	2.48	4.94	6.65		0.027*
INR	7.89	3.28	3.22	1.62	4.8		0.014*

(χ2) = chi-square value, (t-Value) = independent t-test value, (p value) =significance value when (p-value) <0.05 =significant difference, (p value) <0.01 = highly significant difference, (p-value) >0.05 = no significant difference.

Table (2): Univariate analysis of the data of the deceased group compared to survived group: Higher age, male sex, increasing number of bleeding attack, increasing severity of the attack, association with higher child class of liver disease, presence of other co-morbidity, or other cirrhotic complications, delayed endoscopic intervention, increased grade of esophageal varices, presence of gastric varices, higher child-pough, APACHE II, MELD scores and low platelets count are predictors of mortality.

Table (3): Backword stepwise regression analysis of the factor predicting survival among study group.

Variable	B value	St. Er	WALD	Sig.
APACHE II	3.50	1.63	4.16	0.023 *
MELD SCORE	1.35	0.04	12.97	0.034*
Severity of the attacks	2.54	3.66	5.24	0.036*
Time to endoscopy	2.25	0.13	3.75	0.041*
ALT	1.89	0.09	3.91	0.042*
AGE	1.76	0.12	3.79	0.046*
SBP	1.55	0.05	2.47	0.049*
SEX	3.25	775	0.00	1
Sr. creatinine	7.78	993	0.00	1
INR	0.14	29.62	0.00	1
PLAT. NO	0.04	10.23	0.00	1
Constant.	-28.2	6.39	19.56	-------

*** = P is significant**

Table (3): Backword stepwise regression analysis of the factor predicting survival among study group: The APACHE II score (p=0.023) and MELD SCORE (p =0.034), severity of the attacks (p= 0.036), time to Endoscopy (p= 0.041), ALT level (p= 0.042), advancing Age (p= 0.046),presence of Co-morbidities (p =0.048) and occurrence of spontaneous bacterial peritonitis (SBP) (p= 0.049) are independent risk factors for mortality in patients with bleeding varices.

DISCUSSION

High mortality rates have been reported in older literature for patients with acute variceal bleeding although there was a trend towards improved survival in recent years [13].

In our study we estimated the prevalence of upper gastrointestinal bleeding (UGIB) in medical ICU within the period from May to September 2013 and it was 13.7% of all cases admitted to medical ICU and 61% of patients admitted with UGIB in that period, 84.4% of them known previously to suffer from chronic liver disease(CLD), and the rest (15.6%) were diagnosed to be hepatic for the first time. The relatively high rate of variceal UGIB in our country may be attributed to high prevalence of chronic virus C hepatitis and bilharzial peri-portal fibrosis [14].

The high prevalence of variceal UGIB is considered one of the most serious health problems affecting our patients and a serious burden on our hospitals.

The reported prevalence of esophageal varices in patient with chronic liver disease (CLD) is variable. Schepis et al. [15], in a comprehensive review, gave figures ranging between 24% and 80%, with a mean of about 60%. The prevalence of varices appears to be related to the degree of liver dysfunction. D'Amico et al. [16] found that the prevalence was 30% for compensated CLD patients and 60% for decompensated CLD patients. This was further supported by data from Primignani et al. [8] who revealed, in a population of compensated CLD (88% Child Class A, 12% Child Class B) patients, that the prevalence of varices was 16%. This low prevalence compared to our values may be due to the high percentage of class A patients in this study. Because of this variability, and because of the relative inaccuracy of non-invasive tests, it has been recommended that all patients with cirrhosis should be evaluated by endoscopy to ascertain the presence of portal hypertension [17].

We tried to demonstrate the relationship between early in-hospital mortality and many factors and to calculate the risk of their presence in the involved patients.

Our results showed that the mortality rate of variceal bleeding (VB) was 16.5%. Alserag and Everhart [18] reported that variceal bleeding mortality was 14.5%. Kind et al. [19] reported that mortality of VB was 19.5%. In another study done by Lewis et al. [20] the mortality of VB ranged from 28 to 63%. Early mortality was 20% in a study done by Carbonell et al. [21].

The decreasing mortality in patients with variceal bleeding can be explained by the early endoscopic intervention, better resuscitation and better management of comorbid conditions.

In this study, there was a significant positive correlation between hospital mortality and the age of the patients. Age is also a predictor of death identified in studies done by Sempere et al. [22] and D'Amico et al. [23]. The age related mortality is probably related to the presence of three factors in the older population: a longer duration of liver disease, a greater chance to have associated comorbidities, and the difficulty of liver transplantation in the elderly.

In our study, sex had an impact on mortality. The incidence of mortality in male sex was more than female sex (80% vs 18% respectively). Male sex, according to Gines et al. [24] indicated poorer prognosis in cirrhotic patients, independent of the stage of the underlying liver disease, age or co-morbidity. Rockall et al. [1] clarified the positive correlation between mortality and each of the patient's age and male sex

Our study also revealed that there was significant positive correlation between mortality and Child-Pugh score. The risk of mortality increased when going from (A) to (C). This was in agreement with Patch and Dagher [25] and D'Amico et al. [26] who showed that 6 weeks mortality seemed to be more related to the stage of the liver disease than to any other factors. Also, Garcia-Taso et al. [27], stated that the stage of liver disease is the most important factor contributing to mortality in intensive care unit patients suffering from upper GIT bleeding.

We found that the mean Child-Pugh score in deceased patients was (11.95 ± 1.12), which was higher than survived patients' mean score (9.43± 2.24),

Our results revealed that co-morbid conditions (diabetes, COPD, renal failure and heart failure) were found in 20.3% of our patients and their presence increased the mortality compared to those who had not any comorbidity. This fact was proved by Marmo et al. [28] who had suggested that chronic comorbidities played a critical role in predicting health-care outcomes and mortality in variceal upper GIT bleeding.

In accordance with our results, Afessa et al. [29] had concluded that renal disease and coagulopathy constituted risk factors in their study. In a similar study, Blatchford et al. [11] demonstrated that renal failure and liver failure had been reported as a risk factors variceal rebleeding and mortality.

In this study, the presence of SBP and HCC, were independent predictors of mortality in the patients with variceal UGIB in agreement with Loomba, et al. [30].

Our results proved that the severity of bleeding episode was significantly positively correlated with the mortality. This was in agreement with a study conducted by Rockall et al. [1], who found that hemorrhage that needed surgery and re-bleeding were associated with a higher risk of death.

This work confirmed that early resuscitation and early endoscopy significantly reduced the risk of mortality. This was in agreement with Bosch et al. [3] and Garcia-Tsao et al. [27] who recommend that endoscopic intervention must be

performed within 12 h of admission in patients with acute VB to improve survival.

In agreement with previous results by Pompili et al. [31] we found that the mean ± S.D of ALT level in survived group (47.16 ± 16.5) was lower than that of the deceased group (51.6 ±19.43). The elevation of the ALT level at the time of acute variceal bleeding indicated an ongoing liver injury, possibly leading to deterioration of the functional hepatic mass and therefore, predicting subsequent mortality. D'Amico et al. [23] reported that the impact of ALT level on the prognosis of variceal hemorrhage is controversial.

The MELD score, initially developed to assess prognosis following TIPS placement, is now accepted as a useful prognostic model for patients with advanced cirrhosis [32].

Our study showed significant increase of bilirubin, creatinine, international normalizing ratio (INR) of the deceased group than the survived groups, As included in MELD score the serum Creatinine level, Bilirubin and INR. markedly affect the mortality in accordance to Malinchoc et al. [10].

The mean ± S.D of MELD score in deceased patients was higher compared to survived patients (29.3±6.98 vs 13.4±5.24). This means that when the MELD score is high, more mortality is expected. These results was concordant with that found by Amitrano et al. [33] who stated that a high MELD score is a predictor of early in-hospital mortality in patients with cirrhosis and variceal hemorrhage.

Our results were in agreement with the study of Fernández-Esparrach et al. [34] who stated that the MELD score is better prognostic indicator as it allows the assessment of renal dysfunction.

We demonstrated that low level of hemoglobin as well as blood pressure and the need for red blood cell transfusion were identified as risk factors for death in upper GI bleeding. This was in accordance with Blatchford et al. [11].

In this work high APACHE II score was associated with marked increase in the risk of the mortality. There was no great difference between our results and Butt et al. [35] who reported that mean ± S.D of APACHE II score for deceased and survivors (26.7 ± 6.38), (16.9 ± 3.39) respectively .

The regression analysis of our patients data demonstrated that the APACHE II score, MELD scale, severity of the attacks, time to endoscopy, ALT level, advanced age, comorbidities and SBP were the most independent factors affecting the early mortality of patients admitted with variceal UGIB.

The limitation of the study included lacking of long term follow up of these patients after discharge from medical ICU, missing of comment on other confounding factors like use of antibiotics during ICU stay and verification of the role of other important comorbidities like association with schistosomasis and *Helicobacter pylori.*

RECOMMENDATION

Being a great problem in our health care system, bleeding esophageal varices require a well formulated protocols for primary and secondary prevention as well as better adherence to current guidelines for management of such cases to minimize the mortality as much as possible. long term follow up studies are required to identify the significantly important prognostic factors.

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Immunoprophylaxis of Compulsory Hepatitis B Vaccination in Sharkia, Egypt

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Background and study aim: Hepatitis B vaccination has been included in routine infant's immunization in Egypt since 1991. Although it could provide high levels of sero-protection, the duration of that protection remains largely unknown. The aim of this study was to evaluate the initial immunoprophylaxis response to compulsory hepatitis B vaccination and its long term protection in Egyptian children.

Patients and methods: Antibodies to hepatitis B surface antigen (anti-HBs) was assessed in 200 apparently healthy Egyptian children.They were all vaccinated with hepatitis B vaccine in infancy through the compulsory vaccination program. They were classified into two groups, each contained 100 children. The 1st group included infants at least 2 months after completion of the 3 doses of the vaccine (≥8 months old). While the 2nd group included children aged 6-12 years old. Sera were obtained from all children and were tested for anti-HBs titer, HBsAg and antibodies to hepatitis B core antigen (anti-HBc) by ELISA. Children who were discovered to have non-protective titers (≤10 mIU/mL) received a booster dose of the vaccine and were re-evaluated within 2-4 weeks. Different factors associated with poor immune response were analyzed.

Results: The initial responders rate of 83% was detected in group I which was significantly higher than that in group ΙΙ of only 52%. Moreover, mean titer of Anti-Hbs was significantly decreased with age,(48.9 ±15.8 mIU/ml in group I vs 12.6 ± 5.2 mIU/ml in group II). Also, within the 2nd group, a negative correlation between Anti-HBs mean level and age in years was displayed. No significant difference could be detected in the percentage of infected children between both groups (4% in group I vs.6% in group II). Sixty two percent of the boosted children showed a significant increase in their mean anti-HBs titer. Male gender and rural residence were associated with poor vaccine response in both groups.

Conclusion: As maternal screening is not feasible in our country, Hep-B vaccine birth dose is a necessity to prevent perinatal infection. Most of infants vaccinated with compulsory Hep-B vaccine retained their immune protection years after vaccination either by high anti-HBs titer or efficient memory T cells. However, large scale and long term follow up study is still needed before clearly answering the question about the need for booster dosing and its proper timing.

INTRODUCTION

Hepatitis B virus (HBV) infection is a serious public health problem worldwide and a major cause of chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC) [1]. At least two billion people, or one third of the world's population, have been infected with HBV and an estimated 1 million people die each year from acute and chronic squeals secondary to HBV infection [2]. In addition, more than 400 million people, or 6% of the world's population, are chronic carriers of HBV. Approximately 4.5 million new cases of HBV infection occur worldwide annually, and one fourth of these cases progress to liver disease [3].

There is a marked difference in the geographic distribution of carrier rates, ranging from 10–20% in South-East Asia and Sub-Saharan Africa to less than 1% in Northern America and most of Europe [1, 4].In areas with low endemicity, most HBV infections are acquired via horizontal transmission among adolescents and young adults. Conversely, in areas of

high endemicity, the most common route of transmission is perinatal and the infection is often acquired during preschool years. The risk of becoming a HBV carrier is 90% in cases of perinatal infection, 25 -30%for infected infants and children under 5 years of age, and less than 10% for infected adults [2, 4]. More than 25% of infants and older children who acquire HBV infection will eventually develop HBV-related cirrhosis and/or HCC. Adults who have had chronic HBV infection since childhood develop HCC at a rate of 5% per decade, which is100 to 300 times the rate among uninfected persons [1, 4].

In the early nineties, the prevalence of hepatitis B virus (HBV) carriage in Egypt was reported as high as 20%.Considering that, most HBV infections in Egypt occurred vertically, universal infant vaccination is considered to be the key towards elimination of HBV infection [5].

The first HB vaccine, derived from human carriers' plasma, was approved for use in the United States in 1981. In 1991, the WHO recommended that all countries implement a policy of universal HB vaccination by 1997 [6]. Most countries have incorporated universal HBV vaccination into their national infant immunization programs. The estimated global coverage rate of infant HB vaccination increased from less than 1% in 1990 to 30% in 2000, and from nearly 50% in 2004 to 69% in 2008 [7, 8]. This program reduced not only the rate of persistent infection and the total prevalence of HBV in the younger generation, but also the occurrence of childhood HCC and fulminant hepatitis [9].

The HB vaccination at (0, 1 and 2 months or at 0, 1 and 6 months) after birth has been compulsory for all neonates born in areas of high endemicity to prevent perinatal transmission. Infants born to HBsAg-positive mothers and mothers of unknown HBsAg status should receive the HB vaccine and hepatitis B immunoglobulin (HBIG) within 24 hours of birth. Five to twenty% of infants born to HBsAg-positive and HBeAg-negative mothers and seventy to ninety% of infants born to HBeAg-positive mothers will get infected if not given immunoprophylaxis [3]. A compulsory vaccination program against hepatitis B infection was started in Egypt in 1991 using a yeast recombinant DNA vaccine (10 µg), with a schedule of 2, 4 and 6 months of age [10]. Sero-protection is assured when hepatitis B surface antibody (anti-HBs) levels are ≥10 mIU/mL[11]. Hepatitis B infection has significantly declined in the past decade as a result of HB vaccination and the introduction of other public health measures, such as the use of universal precautions in medical settings and blood screening tests. Recently, Egypt is considered to be a region of intermediate prevalence for HBV infection with a reported infection rate of 2-8% [12].

The immunogenicity of hepatitis B vaccine has been a subject of intense research since HB vaccine was adopted in most areas of the world [13]. Although high levels of sero-protection rates provided by HB vaccine (both early plasma-derived and the current recombinant) have been adequately confirmed [14], recent studies have found that after neonatal immunization with HB vaccine, a large proportion of the children, especially adolescents, exhibited waning immunity. Such decreased protection poses the risk of breakthrough infections [15]. So, more is needed to be learned about the duration of protection and indications for booster dosing [16].

The aim of the present study is to assess immune response to hepatitis B vaccine among Egyptian children vaccinated under the compulsory vaccination program, determine factors affecting immunoprophylaxis, and evaluate the need for a birth dose and boosters.

PATIENTS AND METHODS

Participants: This prospective comparative study was performed at Pediatric and Tropical Medicine Departments, Zagazig University Hospitals, (between December 2011 and June 2013). Two hundred apparently healthy children, who came for follow up after minor illness, were enrolled in our research study. Parents were interviewed and informed about the idea of the study. Consent and two contact numbers were taken for recall and follow up as needed.

Inclusion Criteria: All participants fulfilled the following inclusion criteria: 1-.Fully vaccinated in infancy through compulsory vaccination program with three doses of recombinant hepatitis B vaccine as intramuscular injection on 2,4,6 months of age in conjunction with DPT and Polio vaccines. 2- Their gestational ages were ≥ 37 weeks and birth weights were ≥ 2kg. 3- No history of clinically apparent acute or chronic

hepatic illness either for the participants or their mothers.

Study Design: The children were classified into two groups according to age, **Group I:** included 100 infants at least two months after completion of the vaccine series (≥ 8 months). They were classified according to their anti-HBs titer into: ***responders*** (anti-HBs titer was > 10 mIU/mL), and ***non- responders*** (anti-HBs titer ≤ 10mIU/mL). **Group II:** included 100 children aged from 6-12 years old who received the same vaccination series as infants. They were classified according to their anti-HBs titer into: ***immune*** (who retained anti-HBs titer of > 10mIU/mL) and ***non-immune*** (anti-HBs titer was ≤ 10mIU/mL). Moreover, HBsAg was tested in all participating children. Non-immune children were boosted and re-tested after 2-4 weeks to evaluate their memory T cell function. ***Anamnestic reaction*** to HB vaccine booster was defined as: non immune subject who was yielding anti-HBs titer of ≥ 100 mIU/mL or at least four- folds rise in the anti-HBs titer within 4 weeks after booster immunization [16].

Methods: All participants were subjected to full medical history and thorough clinical examination, complete blood count, liver function tests, quantitative anti-Hbs and qualitative HBsAg & anti-HBc titers by enzyme linked immunosorbent assay (Immlite 2000) in accordance with the manufacturer's instructions. Anti-HBc positive participants, which is considered an indicator of infection, were referred to the hepatology unit for thorough clinical evaluation and detailed investigations of their HBV markers (HBs Ag, anti-HBc, anti-HBs, HBeAg and HBV-DNA by PCR). Moreover, their maternal (HBsAg, HBeAg as well as HBV-DNA viral load) status were also evaluated.

Statistical Methods: Our data were collected and presented using SPSS for Windows (ver.10). Descriptive values for continuous variables were given as the mean ± SD, while categorical variables were expressed as numbers and percentages. Student t test was used to compare different study groups, Spearman correlation test was performed for the correlation analysis. P value of < 0.05 was considered statistically significant.

RESULTS

To recruit 100 children in group I, 123 infants were interviewed with their parents. Twenty three infants were excluded from the study due to incompletion of vaccine series at the time of interview. The vaccine completion rate was 81%. Mean ages in group I were 8.9± 2.15 months ranged from 8-12 months. Moreover, 117 children were interviewed to enroll 100 children in group II, 17 children were excluded due to unknown or incomplete hepatitis B vaccine doses during infancy. Their vaccine completion rate was 85%. Mean ages in group II were 7.8± 1.9 years ranged from 6-12 years. Apart from age, no statistically significant differences in vaccine completion rates, gender, or residency were found between the two groups.

83% of participants in group I showed protective level of anti-HBs antibodies compared to only 52% of children in group II. This data confirms a statistically significant reduction in immune-protective rates with age (Figure 1). High statistically significant reduction in mean titers of anti-HBs occurred years after vaccination (group II), when compared to those in group I as shown in Table 1. Moreover, significant negative correlation was observed between anti-HBs quantitative level and different ages (in years) in group II (Figure 2).

No statistically significant difference in percentage of infection (expressed by ani-HBc positivity) was detected between the two studied groups (4% vs 6% with p value> 0.05).Also positive rates of HBsAg showed no statistically significant difference between the two groups (calculated as 3% and 2%) in group I and II respectively. We evaluated the hepatitis markers in the infected subpopulation of both groups, and investigated their maternal HBsAg, HBeAg & HBV-DNA status. It was observed that 3/4 of infected participants in group I were positive for HBs Ag, and 2/4 of their mothers were HBsAg +ve but –veHBeAg with low viral loads. While in group II, 2/6 of infected children were positive for HBsAg and had an associated positive maternal HBsAg but –veHBeAg with low viral loads also (data not shown).

After exclusion of the infected cases (2 boys and 2 girls in group I& 4 boys and 2 girls in group II), a significant difference in the rates of anti-HBs between males and females in group I with a better response to vaccine in females than males (92% Vs75%) was noted. Moreover, 60% of girls in group II retained their immune protective levels years after vaccination as infants versus only 41% of boys of the same group (Table 2).

To evaluate the effect of residency on the immune response, 96 children were analyzed in group I,in which 90% of those lived in cities compared to 80% from rural areas were responders to the vaccine.Furthermore,94 children were analyzed in group II where 53% from urban residencehad retained their immune protective level of anti-HBs compared to 48% from rural residence with no statistically significant difference between the two subgroups.

Out of the non-immune46 children in group II who did not show any evidence of infection (negative for both anti-HBc and HBs antigen), only 29 returned for booster vaccination. They were retested 2-4 weeks after a single vaccine dose with mean time 23±2 days. 62% of boosted children demonstrated significant increase in their mean anti-HBs antibody titers, almost 10 folds more than values before boosting (P <0.05).This anamnestic reaction is considered an indicator of efficient memory T cell protection against hepatitis B viral infection, 38% did not develop such response which renders them at risk for infection upon exposure or breakthrough infections (Table 3).

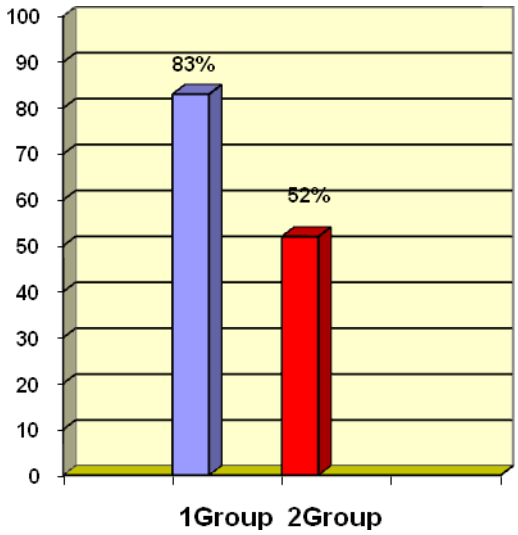

Fig. 1: Percentage of participants with protective levels of anti-HBs in studied groups

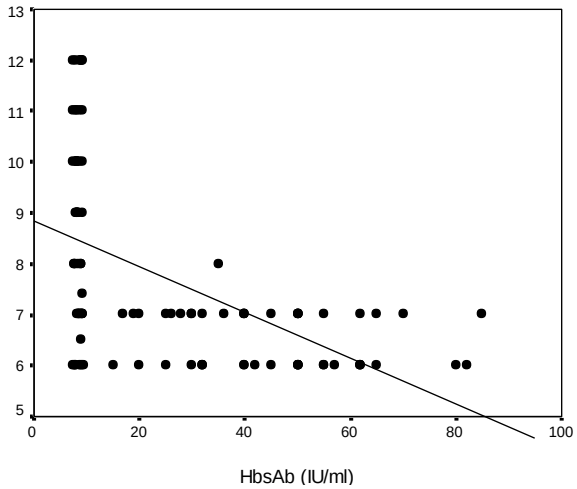

Fig. 2: Correlation between anti-HBs and different ages in group II

Table 1: Quantitative level of anti-HBs by ELISA in studied groups

Anti-HBs (mIU/ml)	Group I	Group II
Mean	48.9	12.6
S.D	15.8	5.2
Minimum	< 10	0 (undetectable)
Maximum	176	55
P value	< 0.001 H.S	

Table 2: Gender effect on positive rates for Anti-HBs in studied groups after exclusion of infected cases

Group / Gender	Group I (No: 96)					Group II (No: 94)				
	responders		non-responder		total	immune		non-immune		Total
	No.	%	No.	%	No.	No.	%	No.	%	44
Male	34	75	10	25	44	18	41	26	59	
Female	48	92	4	8	52	30	60	20	40	50
P	< 0.05					< 0.05				

Table 3: Results of boostering experiment on non-immune children in group II (No: 29)

Anti-Hbs	N	%	Mean	S.D
Responders [a]	18	62	154	18.3
Non- responders	11	38	32	2.5

DISCUSSION

The WHO recommended universal vaccination against HBV to ultimately eliminate HB infection; this recommendation had been progressively implemented in 168 countries having universal immunization programs by the end of 2006[17].Expanded program on immunization (EPI) is a component of the child survival project (CSP). In 1991, CSP/EPI developed a national plan to introduce national immunization for infants against hepatitis B virus infection which is endemic in Egypt. Licensed vaccine against HBV is administrated at 2, 4, 6 months of age coinciding with oral polio vaccine (OPV) and Diphtheria, Pertussis, Tetanus (DPT). It is given either separately or in a combination form as (DPT–Hep B vaccine [18]. The present study appreciates such schedule as the vaccine completion rates are high (82%-85%) in our groups with no significant difference at different age groups. No extra visits are needed to receive the HepB vaccine alone, same types of vaccines are given each visit, use of combination form (DPT-Hep B) vaccine will decrease the number of injection sites, number of needles and syringes used, and also the amount of space required for cold chain storage and transport [19] .All these advantages make this schedule the easiest, to be implemented and received with high completion rate. This schedule will prevent infections acquired during early childhood as well as those acquired later in life. Unfortunately, it will not prevent perinatal infections because it does not include a birth dose [19].

Two groups were studied to evaluate immunoprophylaxis yield of this schedule during childhood. The presence of protective levels of anti-HBs (>10 mIU/mL) is considered an indicator of the initial response to the vaccine in group I and also give a clue about the immune protection level years after vaccination in group II. The significant decrease in percentage and mean values of protective levels of Anti-HBs in childhood (group II) in comparison with infants

(group I) imply that, the majority of infants respond initially to the vaccine; but many of them lose their protective immune level during childhood. This result raises concerns about the durability of immune-prophylaxis in later childhood if the vaccine was given only in infancy.

Many follow-up studies of HB vaccine efficacy were reviewed and a wide fluctuation in their results was detected. *West and his team* screened children at 12 years of age who had received their HB vaccine in infancy and were low-risk for hepatitis B virus exposure. None of the children had anti-HBs < 10 mIU/ml with 100% retaining their immune response [20].While, Jafarzadeh and Montazerifar,evaluated the persistence of anti-HBs in healthy Iranian children receiving primary Hep B vaccination at 0, 1.5 &9 months of age. Only 47.9% had a protective level of anti-HBs at 10 years age [21].On 1999,El-Sawy and Mohamed evaluated the long term immunogenicity and efficacy of vaccination using the schedule of (2,4,6 months) in 180 children whose time lapse since last vaccination dose varied between one month and five years. High seroprotection rates (93.3%) were elicited. None of the participants had clinical hepatitis nor HBsAg positivity was detected in any participant but only one had positive results for anti-HBc test [22]. The longest follow up study, up to our knowledge, was that performed by Qian et al. who followed the long term immunogenicity and seroprotection in healthy individuals 1, 11 & 23 years after vaccination with either 10µgm or 20µgm doses of plasma derived HB vaccine. Around 50% of the participants still kept anti- HBs > 10mIU/mL at the age of 23 years, no clinically apparent cases of HB infection were observed throughout the entire study period [16].A major difference between the different studies was the timing of the initial vaccination dose, as some was given at birth while others 2 months later. The delay in initial vaccination series was associated with better persistence of anti-HBs. In agreement with our finding, a recent study by Agladioglu and colleague concluded that the scheme started at the end of the second month of life yielded a significantly higher immunogenicity than that started at birth [23]. This finding was explained by Hou et al., on the bases of insufficient maturation of the immune system in neonates and the possibility that maternally derived antibodies could interfere with active immunization response [24].

Mean titers of Anti-HBs antibody described at different literatures were 77 mIU/mL in Oon and co- workers and 880 mIU/mL by Goldjarb and colleagues [25, 26].Our results displayed much lower mean titer values (49 mIU/mL). This diminished immune response could be attributed to the short time gap between 2^{nd}& 3^{rd}doses of our schedule. Middleman and colleagues suggested that increased time between the 1^{st}& 2^{nd} as well as between the 2^{nd}& 3^{rd} doses was positively correlated with increased levels of anti-HBs mean titers [27].A number of authors reported in their studies that the concentration of anti-HBs after the third injection was dependent on the interval between the second and third doses with significantly higher levels if the duration was longer [28, 29]. Wilson and Nokes in their article set forth a mathematical model of hepatitis B antibody kinetics suggesting that immune memory depends not only on the response directly to antigen stimulation, but also on other cells that continue to clone even after the antigen is out of circulation, the accumulation may continue for months after the initial priming of the immune system. Thus, the longer, the time period after priming that the clonal expansion has to continue and the larger the boosted response till another dose could be [30]. This theory reasonably explains our low anti- HBs titers.

Despite the obvious conclusion that HB vaccine induced protective antibody levels were gradually fading by age, the positive rates for anti-HBc and HBsAg in our study were not significantly increased at different ages.4 % versus 6% for anti-HBc and 3% versus 2% for HBsAg in groups 1 and group II respectively. Similar results were demonstrated in a report on long-term protection in a population of *Alaska Natives* where none of the children at age of 10 years who had received the HB vaccine at birth was HbsAg + ve despite fading of their anti-HBs with age, compared to 16% of participants of those > 10 years old who were not vaccinated [31]. This could be explained by the persistence of immunologic memory. When those children were exposed to natural infection, they could quickly re-develop enough protective antibodies to combat active infection and chronic carriage [17].

Our booster experiment results confirmed the previous recommendation that administration of booster doses should not be based only on the level of anti-HBs but also on the measure of memory T cells function [32]. In the present study, 62% of children with negative anti-HBs who were challenged by booster vaccination developed an anamnestic response with significant increase in their anti-HBs mean titer to 154 ± 18.3 which is highly protective against infection. In another study, the vast majority of participants (82%) developed a rapid and robust anamnestic response after a booster dose at year 23 after the primary vaccination series [16].

However, in this study 38% of the boosted children did not experience such reaction within the same duration. Petersen and co-workers in their booster experiment found that 25% of their low-risk vaccinees, long-term immunologic memory to hepatitis B virus may be lost [33]. Despite of that, booster doses of HB vaccine are not currently recommended [29, 34]. Depending on the fact that, even with declining anti-HBs protective level with time, immune memory remains intact in most individuals. But with loosing of such memory protection, children are at greater risk of acquiring infection upon exposure to HBV infection as they approach adolescence and booster dose for that subpopulation is a demand! Could the long incubation period of 4-8 weeks for hepatitis B virus allow time for immune cells to prevent acute illness or chronic carriage among previous anamnestic memory loser subgroup?, a question which cannot be answered properly, except after large number and long-term follow-up studies for those sub-group into adolescence and early adulthood to detect any evidence of clinically significant break-through infection.

Some countries have chosen not to implement immunization at birth, but instead screen pregnant women for HBsAg. Hep vaccine birth dose and HB immunoglobulin were given only to infants born to HBsAg positive mothers [35].

Four of children in group I, were anti HBc + ve, of them 3/4 were +ve HBs Ag, 2/4 had associated +ve maternal HBsAg as well, indicating that perinatal transmission is a possibility. Lack of regular antenatal care facilities in our community, where maternal HBsAg status cannot be determined, makes first dose at birth for all infants a mandate to prevent perinatal transmission. The rationale for this suggestion is supported by the very high probability of developing chronic carrier state if the infection is acquired perinatally [36]. 20-90% incidence of chronic carrier occurs if infection was acquired < 5 years, perinatal infection is an important source during this period [37].Same recommendation was recently advised depending on the fact that, as maternal screening is costly and not usually feasible in developing countries so vaccination at birth is still a necessity [16].

It is important to determine predictors of non-responsiveness to hepatitis B vaccine and also to evaluate risk factors of losing the sero-protection status with age. The percentages of responsiveness in females were higher than males in both study groups. Similar results were observed in adult volunteers [38]. Dentico and colleagues also reported that male gender was considered one of the risk factors associated with non-response to HB vaccine [39]. In contrast, Jafarzadeh and Montazerifar reported equivalent seroprotection rates and mean titers of Anti-Hbs in both genders [21]. A trend toward higher responders' rate and higher sero-protection rate were observed among urban residents when compared to rural residents. These results can be explained by the common practice that cities usually have increased number of health care centers, better quality control, better supervision, more trained personnel and more supply of equipment for cold chain storage and transport. All previous advantages guaranteed perfect vaccine implementation and so subsequent better results even for the same vaccine brand given to the same population.

RECOMMENDATIONS

The hepatitis B vaccine of 0, 2, 9 months schedule is suggested to be followed in our country. As this regimen will prevent perinatal infection, through its birth dose for all live births; stimulate higher anti-Hbs response by wider time gap between 2nd, 3rd doses. While still coincide with already present vaccines' schedules but different vaccines are to be given at 2nd (DPT, polio, Hep.B) and 3rd (hepatitis B, measles) doses.

No need for booster doses until the end of childhood and early adolescence as the majority of vaccinees are protected either by keeping their sero-protective level at this age or through developing higher levels of anti-HBs

(anamnestic response) if exposed to the virus Ag through their immunologic memory stimulation. However, large–scale and longer follow–up study to evaluate memory anamnestic loser's outcome is needed to safely answer the question; do we need booster or absolutely not?

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Conflicts of interest: The authors declare no conflict of interest.

Ethical approval: The Study was approved by the Ethical Committee of Faculty of Medicine , Zagazig Universty. Informed consents were obtained from the parents.

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Hemodynamic Changes of Hepatic Veins as Predictors of Large Oesophageal Varices in Liver Cirrhotic Patients

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Key words: Liver cirrhosis- esophageal varices- Oesophagogastroduodenoscopy- Color Doppler Abdominal Ultrasound- hepatic vein.

Background and study aim: Oesophageal varices (OV) are morbid and mortal complications of portal hypertension. Oesophago-gastroduodenoscopy (OGD) is the main diagnostic yet semi- invasive tool; different non-invasive methods were developed for early prediction and assessment of OV yet not fully evaluated. This work aims at assessment of changes of hepatic venous wave patterns as early predictors of large OV in cirrhotic patients without history of variceal bleeding.

Patients and methods: A number of 50 previously diagnosed liver cirrhotic patients were subjected to detailed history taking. After exclusion criteria only 46 patients were included in the study. They were subjected to routine investigations, OGD, abdominal ultrasound (US) and Color Doppler studying of the hepatic veins (HV).

Results: Out of the total number (46 liver cirrhotic patients included in the study), the triphasic waves were detected in (26.1%), biphasic waves in (43.5%) and monophasic waves in (30.4 %). Small varices were detected in (65.2 %), while large varices were detected in (34.8 %).The sensitivity of loss of the triphasic waveform in detecting large varices was high (93.8 %), specificity was (36.7 %), the positive predictive value was (44.1 %) and the negative predictive value was high (91.7 %).

Conclusion: The loss of hepatic venous triphasic waveform - detected by Color Doppler Abdominal Ultrasound Study- is a weak predictor of large OV in liver cirrhotic patients without history of variceal bleeding.

INTRODUCTION

Oesophageal varices (OV) are one of the most awful complications of liver cirrhosis. Its incidence in cirrhotic patients ranges between 60- 80%. Upper gastrointestinal bleeding (UGB) - caused mainly by rupture of OV and to less extent by gastric varices - is disastrous implying a mortality rate of 17- 57% of these prone patients [1]. OV are the direct result of portal hypertension and their presence is usually correlated with the severity of liver disease. About 45% of patients with Child-Pugh A cirrhosis were found to have OV while this percentage jumps to 85% in those with Child- Pugh C [2]. The frequency of bleeding from large OV is 50-53% while that of small OV is 5-18% that can be attributed to the increased variceal wall tension in large OV [3,4].

Because of the dramatic destiny of OV, it is highly recommended to screen all liver cirrhotic patients for the presence of OV at the time of diagnosis and periodically by Oesophagogastroduodenoscopy (OGD). If OV were not detected in the first endoscopic screening, reevaluation after three years is recommended in compensated liver cirrhotic patients and annually in decompensated liver cirrhotic patients [5-7]. OGD is the gold standard diagnostic method of OV. However, many drawbacks were recorded against OGD including its invasiveness, risk of perforation, aspiration and bacteremia. Moreover, it is not accepted by many patients [8,9] and the cost of repeated screening stressed the need to develop non- invasive techniques for early prediction of recent, developing and large OV to reduce the frequency of

OGD so that it can be preserved for highly suspected cirrhotic patients [10,11]. Many clinical, radiological and chemical predictor methods were advocated either separately or in combinations such as platelet count, studying the spleen with portal vein diameter and Child-Paugh class [12-15].

Multiple Doppler indices were suggested to assess the severity of portal hypertension in cirrhotic patients including the study of hepatic venous waveform changes [16-19]. The normal hepatic venous waveform pattern is triphasic (two negative waves and one positive wave) according to variations in the central venous pressure of the cardiac cycle, but it changes to the biphasic and monophasic pattern in cirrhotic patients (Fig. 1). These changes are attributed to the progressive loss of hepatic compliance. Monophasic waves were found to correlate with the severity of liver disease (high scores of Child-Pugh classification) and poor survival rate [20-23].

This work aims at evaluating the changes of hepatic venous waveform as early predictors of large OV in liver cirrhotic patients without history of variceal bleeding.

PATIENTS AND METHODS

This is a collaborated work between Tropical Medicine, General Surgery and Radiology Departments, Faculty of Medicine, Zagazig University.

Inclusion criteria: The already diagnosed liver cirrhotic patients attending to Tropical outpatient clinic or admitted in the ward of Tropical Medicine Department, Zagazig University Hospitals were included in the study. The diagnosis of liver cirrhosis was already established by clinical approach, laboratory investigations, routine abdominal ultrasound, endoscopic data and liver biopsy.

Exclusion criteria: Patients with history of variceal bleeding, hepatocellular carcinoma, portal vein thrombosis, endoscopic variceal ligation or sclerotherapy were excluded from the study. Patients with cardiac, respiratory or renal diseases or those under treatment with drugs affecting portal haemodynamics (eg. propranolol) were also excluded.

All included patients were subjected to thorough history taking, clinical examination, routine laboratory investigations, routine abdominal US and Child- Pugh classification. Then they were subjected to:

***Oesophagogastroduodenoscopy (OGD):** OV were graded according to the guidelines of the American Association for the Study of Liver Diseases (AASLD) considering them small if less than 5 mm diameter and large if more than 5 mm diameter [24].

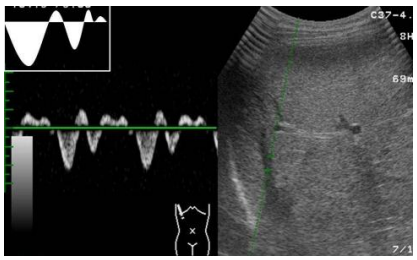
***Abdominal ultrasound examination:** It was done according to the following steps.

1. After 6-8 hours fasting, all patients were subjected to routine B- mode abdominal ultrasound (US) examination of the liver assessing the size, echogenicity, border irregularity and exclusion of focal lesions.
2. Doppler ultrasound using the 3.5 MHz convex probe (GE, Logic III US, expert, USA). The portal vein first assessed for exclusion of thrombosis then the probe was placed in the right intercostal spaces for tracing of the hepatic veins. Color Doppler was used to identify the pattern of blood flow in the hepatic veins.
3. Spectral analysis of the hepatic venous waveform pattern was obtained from the right hepatic vein 3 - 6 cm from its junction with inferior vena cava [25]. The middle hepatic vein was used for spectral analysis in some cases instead of tracing of the right hepatic vein.
4. Doppler study of waveforms of hepatic veins was recorded for at least 5-10 seconds during quiet breathing or at the end-inspiratory point with breath holding if quiet breathing is not possible. Three records were usually taken for each patient.
5. Color Doppler flow mapping was interpreted as a blue hepatic vein waveform indicating flow away from the US probe and a red hepatic vein waveform indicating flow toward the US probe.
6. Hepatic venous waveforms were classified into:

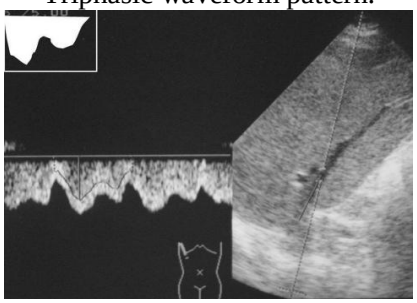
- Triphasic (normal) waves with reversed flow in at least one phase. It is considered triphasic if recognized in at least one of three records.
- Biphasic waves without reversed flow and with or without diminished phasic oscillation.

It is considered biphasic if recognized in at least one of three records.

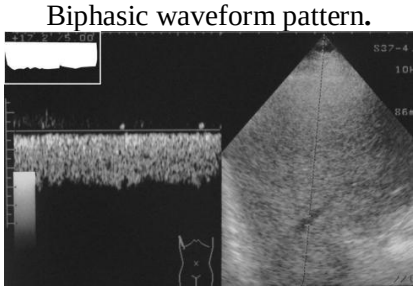
- Monophasic or flat pattern with or without fluttering. It was considered if the three records are monophasic.



Triphasic waveform pattern.



Biphasic waveform pattern.



Monophasic waveform pattern.

Figure (1): Hepatic venous waveform pattern

Statistical Analysis:

The relation between US wave and OV Size and some factors (age, sex, duration of illness, severity of liver disease) were done using the Pearson chi square test. The role of lost triphasic pattern in predicting large varices was determined by sensitivity, specificity, positive and negative predictive values and Kappa (K) measure of agreement.

$$\text{Positive predictive value} = \frac{\text{True positive}}{\text{True positive} + \text{false positive}}$$

$$\text{Negative predictive value} = \frac{\text{True negativee}}{\text{True negative} + \text{false negative}}$$

Probability equal or less than 0.05 is considered significant. Data analysis was done using Statistical package for social sciences (*SPSS, version* 15.0; Chicago, IL, USA).

Ethical approval:

Informed consent was taken from each patient. The research protocol was duly approved by the ethical committee of Zagazig University Hospitals.

RESULTS

A total number of 50 patients were suggested for the study. After exclusion criteria, 46 patients were included in the study. Males were 24patients (52.2%), while females were 22 patients (47.8%). Their ages ranged between 19 and 62 years (35.17±13.9). According to Child-Pugh classification, Child-Pugh class A included 24 patients (52.2%), Child- Pugh class B included 10 patients (21.7%) and Child- Pugh class C included 12 patients (26.1%). The duration of illness was less than 4 years in 17 patients (37%), 4-8 years in 17 patients (37%) and more than 8 years in 12 patients (26%). Patients with small OV were 30 patients (65.2%) while those with large OV were 16(34.8%). Patients with monophasic waves were 14 (30.4%), biphasic waves were 20 (43.5%) and those with triphasic waves were 12 (26.1%) (Table 1).

Table (2) showed that large OV were mainly represented among patient age group 25- 50 years while small OV were mainly represented among patients less than 25 years. Small OV were more prevalent than large OV among all age groups. However the differences were non-significant.

Large OV were mainly represented among patients with duration of illness more than 8 years, while small OV were mainly represented among patients with duration of illness less than 4 years. Large OV were more prevalent than small OV among patients with duration of illness more than 8 years, while small OV were more prevalent than large OV among patients with duration of illness 4-8 years or less than 4 years. The differences were significant.

Large OV were more prevalent among Child-Pugh class C patients, while small OV were more prevalent among Child- Pugh class A patients. Large OV were more prevalent among Child-Pugh class B and C patients, while small OV were more prevalent among Child-Pugh

class A patients. The differences were highly significant.

There was a non – significant difference regarding the size of OV between males and females.

Table (3) showed that triphasic waves were the most prevalent pattern among Child-Pugh class A patients, while biphasic waves were the most prevalent pattern among Child- Pugh class B and C patients. The differences were significant.

Biphasic waves were the most prevalent pattern among males, while triphasic waves were the most prevalent pattern among females. However the differences were non- significant.

Monophasic waves were the least prevalent pattern among patients younger than 25 years, while biphasic waves were the most prevalent pattern among patients 25- 50 years. Monophasic and biphasic waves were equally presented in patients older than 50 years, while triphasic waves were not encountered among this age group. All the differences were non- significant.

Biphasic waves were the most prevalent pattern among patients with duration of illness less than 4 years, while monophasic waves were the least prevalent pattern among patients with duration of illness 4- 8 years and biphasic waves were the most prevalent pattern among patients with duration of illness more than 8 years and no triphasic waves were encountered among this group. However, the differences were non-significant.

Biphasic waves were the most prevalent pattern among patients with small OV, while monophasic waves were the least prevalent pattern among patients with large OV and Biphasic and triphasic waves were equally presented in this group. All the differences were non-significant.

Table (4) shows that the sensitivity of monophasic waves to detect large OV was 42.9 %, while specificity was 57.9 %. The positive predictive value (PPV) was 85.7 %, while the negative predictive value (NPV) was 91.7 %.

The sensitivity of biphasic waves to detect large OV was 90 %, while the specificity was 50%. The PPV was 45%, while the NPV was 91.7 %.

The sensitivity of loss of triphasic waves (presence of monophasic or biphasic waves) to detect large OV was 93.8 %, while the specificity was 36.7 %. The PPV was 44.1 %, while the NPV was 91.7 %.

The overall result was the non- significant agreement between the results of OGD findings and the results of Doppler Abdominal US.

Table (1): Distribution of age, sex, duration of liver cirrhosis, Child- Pugh classification, OV size and Doppler US waveform among studied patients.

	Total No of patients (46)	
	No	%
Sex		
Male	24	52.2
Female	22	47.8
Age/year		
<25	17	37.0
25- 50	19	41.3
50+	10	21.7
$\bar{X}$±SD (Range)	35.17±13.9 (19-62)	
Child- Pugh classification		
A	24	52.2
B	10	21.7
C	12	26.11
Duration of liver cirrhosis/years		
< 4	17	37.0
4- 8	17	37.0
8+	12	26.0
OV size		
Small	30	65.2
Large	16	34.8
Doppler US waveform		
Monophsic	14	30.4
Biphasic	20	43.5
Triphasic	12	26.1

Table (2): Relation between age, sex, duration of liver cirrhosis, child- Pugh classification and OV size.

Total = 46 patients	Large		Small		X^2	P-value
	No (16)	(34.8%)	No (30)	(65.2%)		
Age/years						
<25	5	29.4	12	70.6		
25- 50	8	42.1	11	57.9	0.76	0.68
50+	3	30.0	7	70.0		
Duration of liver cirrhosis /years						
<4	3	17.6	14	82.4		
4- 8	5	29.4	12	70.6	7.79	0.02*
8+	8	66.7	4	33.3		
Child- Pugh classification						
A	2	8.3	22	91.7		
B	6	60.0	4	40.0	15.58	0.000**
C	8	66.7	4	33.3		
Sex						
Male	8	33.3	16	66.7		
Female	8	36.4	14	63.6		

*significant ** highly significant

Table (3): Relation between hepatic venous waveform detected by Doppler US and some factors including age, sex, Child- Pugh classification, duration of liver cirrhosis and OV size.

Total No = 46 patients.	Mono		Bi		Tri		X^2	P
	No 14	(30.4%)	No 20	(43.5 %)	No 12	(26.1 %)		
Child- Pugh classification								**
A	7	29.2	5	20.8	12	50		
B	4	40.0	6	60.0	0	0.0	17.63	0.001
C	3	25.0	9	75	0	0.0		
Sex								
Male	9	37.5	13	54.2	2	8.3	8.2	0.1
Female	5	22.7	7	31.8	10	45.5		
Age								
<25	5	29.4	6	35.3	6	35.3		
25-50	4	21.1	9	47.4	6	31.6	5.62	0.22
50+	5	50.0	5	50.0	0	0.0		
Duration of liver cirrhosis /years								
<4	4	23.5	7	41.2	6	35.3		
4- 8	5	29.4	6	35.3	6	35.3	5.89	0.21
8+	5	41.7	7	58.3	0	0.0		
OV-size								
Small	6	37.5	9	56.3	1	6.3	5.02	0.08
Large	8	26.7	11	36.7	11	36.7		

** highly significant

Table (4): Validity of US hepatic venous waveform for the diagnosis of large OV.

Total no=46 / Waveform	True +ve	True –ve	Sensitivity	specificity	PPV	NPV	Kappa	P
Monophasic alone	6	11	42.9 %	57.9 %	85.7	91.7	0.33	0.04 *
Biphasic alone	9	11	90.0 %	50.0 %	45.0	91.7	0.31	0.03 *
Combined Monophasic-Biphasic.	15	11	93.8 %	36.7 %	44.1	91.7	0.24	0.02 *

*significant

DISCUSSION

OV bleeding in liver cirrhotic patients is a critical medical emergency associated with a high mortality rate [26]. Large OV is an additional risk factor that predicts impending variceal bleeding [27]. Non- invasive prediction of large OV is a stressing need to allow early medical or OGD interference in patients with impending variceal bleeding [28].

Our study showed that Large OV were significantly associated with longer duration of liver cirrhosis while small OV were significantly associated with shorter duration of liver cirrhosis. This can be explained by the time availability for the development of portal hypertension and the formation of large OV. These results agree with those of Palmer [29] and Cales et. al., [30] who found that longer duration of liver cirrhosis was significantly, associated with the occurrence of large oesophageal varices.

Our study showed that large OV were more prevalent than small OV among Child-Pugh class B&C patients while small OV were more prevalent than large OV among Child-Pugh class A patients and the differences were highly significant. This means that large OV were more prevalent among patients with more severe liver disease while small OV were more prevalent among patients with less severe liver disease. This can be explained by the progressive increase in variceal wall tension with increased severity of liver disease and the eventual increase in portal hypertension. Our results agree with

those of Zaman et. al., [31] and Cherian et. al., [28] who found that advanced Child-Pugh class and low platelet count were associated with the presence of large OV.

Our study showed that hepatic venous triphasic waves were more encountered than other waves among Child- Pugh class A patients while biphasic waves were the most encountered among Child- Pugh class B&C patients. The differences were highly significant. This means that there was a relation between loss of hepatic venous triphasic waves and the severity of liver disease. This can be explained by the progressive decrease of hepatic compliance with the eventual increased severity of liver disease. This result agrees with that of Bhutto et. al., who found a significant relation between hepatic venous waveform pressure changes and the severity of hepatic dysfunction [32].

Our study showed that hepatic venous biphasic waves were the most encountered than other waves among patients with small OV, while monophasic waves were the least encountered among patients with large OV, however the differences were insignificant. The negative predictive values of biphasic and / or monophasic hepatic venous waveforms to diagnose large OV were very high that denote a poor agreement between loss of hepatic venous triphasic waves and the size of OV and that the loss of hepatic venous triphasic waves is a weak predictor of large OV. This result agrees with that of Bhutto et. al., [32] and Shabestari et. al., [33] who found an insignificant relation between hepatic venous waveform changes and the grading of oesophageal varices.

On the other hand, these results do not agree with those of Gorka et. al., [34] in a Saudi Arabian study, and Josepf et. al., [25] in an Indian study, who found that the loss of hepatic venous triphasic waves is a highly sensitive predictor of large OV. In the same direction Baik et. al., [35] - in their South Korean study- found that the assessment of Doppler US hepatic venous waveform is a useful non-noninvasive predictor of the severity of portal hypertension. This controversy can be attributed to many environmental, pathological and nutritional differences that can affect the pathogenesis of portal hypertension and hepatic venous waveform changes. The different inclusion and exclusion criteria could add to the different results such as studying patients with recent variceal bleeding while our study included only cirrhotic patients without previous history of variceal bleeding.

CONCLUSION

Loss of hepatic venous triphasic waves - detected by Color Doppler Ultrasound- is a weak predictor for the diagnosis of large OV in liver cirrhotic patients without history of variceal bleeding.

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Frequency of Cholestatic Liver Diseases in Zagazig University Hospitals, with Special Emphasize on Extrahepatic Causes

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Key words: extra-hepatic cholestasis; cholidocholithiasis; cancer head of pancreas, Ultrasonography; ERCP

Background and study aim: Extrahepatic cholestasis results from the mechanical obstruction of large bile ducts outside the liver or within the porta hepatis. The common etiologies of cholestasis have been reported to vary from one center to another. Our aim was to assess the frequency of cholestatic disorders in patients admitted to our department and try to identify the underlying extrahepatic causes aiming at improving the quality of management offered to this patients group.

Patients and Methods: In the present study, 506 cases admitted to our department were included. Sixty one of them met our inclusion criteria, of high serum alkaline phosphatase (ALP≥1.5 times the upper limit of normal), high gamma glutamyl transferase (GGT≥3 times the upper limit of normal) and ultrasonogrpahic (US) features of extrahepatic cholestasis (dilated intra and/or extra-hepatic ducts) of whatever etiology. The selected patients underwent the following: Full history taking and thorough physical examination, complete blood picture, liver and kidney function tests and abdominal CT. Also, ERCP was performed whenever needed and the tumor markers, alpha feto-protein & (CA 19-9) were assessed in selected cases "when mass lesions were detected".

Results: The frequency of cholestatic liver diseases presented with elevated both ALP & GGT as described above, was %19.7 (100 of 506 patients).While the frequency of extrahepatic cholestasis was 12.1% with females accounting for 54.1% and males were 45.9% with mean ages ± SD of 51.1 ± 11.7 years. Benign causes of surgical obstructive jaundice were more frequent than malignant ones (57.4% VS 42.6%). The most common cause was choledocholithiasis in 25/61 (40.9%), followed by cancer head of pancreas 9/61 (14.8%), peri-ampullary carcinoma 8/61 (13.11%), cholangiocarcinoma 5/61 (8.2%) and pancreatitis 4/61 (6.6%). Dark urine, clay stool and itching were more common in patients with malignant obstructive jaundice. There was a highly positive agreement between ALP and GGT with P <0.01.The sensitivity of U/S, CT and ERCP in detection of CBD stones were 76%, 88% and 100% respectively. While in detection of cancer head of pancreas, it was 66.7%, 100% and 100% respectively. The predictive values of US compared to ERCP in detection of CBD stones was 100% positive and 85.7% negative, while the predictive value of US compared to ERCP in detection of cancer head of pancreas was 100% positive and 94.5% negative. The predictive values of CT compared to ERCP in detection of CBD stones was 100% positive and 92.3% negative, while the predictive value of CT compared to ERCP in detection of cancer head of pancreas was 100% positive.

Conclusion: We concluded that, the frequency of cholestatic liver diseases in our department was %19.7. Among them, extrahepatic causes had a frequency of 12.1% with choledocholithiasis as the commonest benign etiology, while cancer head of pancreas was the most frequently met with malignancy. ERCP is considered the gold standard modality in diagnosis and management of extrahepatic cholestasis.

INTRODUCTION

Cholestasis is an impairment of bile formation and/or bile flow which may clinically present with fatigue, pruritus and, in its most overt form, jaundice [1]. Cholestatic jaundice is often accompanied by a broad spectrum of laboratory, clinical and histological abnormalities. Early biochemical markers in asymptomatic patients include elevated serum alkaline phosphatase (ALP) and gamma glutamyl transpeptidase (GGT) followed by conjugated hyper-bilirubinemia at more advanced stages [2].

Cholestatic liver diseases are characterized by accumulation of hepatotoxic substances, mitochondrial dysfunction and impairment of liver antioxidant defenses. The storage of hydrophobic bile acids has been incriminated as the main cause of hepatotoxicity with alteration of some important cell functions, such as the mitochondrial energy production leading to development of oxidative damage [3].

Moreover, the absence of bile salts from the intestine can produce malabsorption, leading to steatorrhea and deficiencies of fat-soluble vitamins (particularly A,D and K). Vitamin K deficiency leads to prolonged prothrombin time. In long-standing cholestasis, concomitant vitamin D and Ca malabsorption can cause osteoporosis or osteomalacia [4].

Also in obstructive jaundice, increased intestinal permeability has been postulated to be a key factor contributing to bacterial and endotoxins translocation to mesenteric lymph nodes, portal circulation and the liver. A suppressed clearance capacity of Kupffer cells, the main hepatic macrophage population, attributed to accumulation of bile acids into the liver, permits the "spillover" of endotoxins from portal into systemic circulation, with consecutive release of pro-inflammatory cytokines, potentially leading to the development of the so called "gut derived sepsis" [3,22].

Nevertheless, the first critical step in approach to patients with cholestatic liver diseases, is to differentiate intra- and extra-hepatic cholestasis, bearing in mind that several intra-hepatic causes of cholestatic jaundice can mimic extra-hepatic obstruction to varying degree [1].

Jaundice due to biliary obstruction may be caused by a heterogeneous group of diseases that include both benign and malignant conditions. Benign causes include choledocholithiasis, primary sclerosing cholangitis, Mirrizi syndrome, post-operative billiary stricture, post inflammatory stricture, pancreatitis, choledochal cyst, recurrent pyogenic cholangitis, parasitic diseases, duodenal diverticulosis and AIDS cholangiopathy [5].

While malignant causes include cancer head of pancreas, carcinoma of the gall bladder, cholangiocarcinoma, carcinoma of the duodenum, ampullary tumors, hepatocellular carcinoma, lymphoma and metastatic tumors [6].

Extrahepatic cholestasis may be additionally subdivided into intraductal and extraductal. Intraductal obstruction is caused by neoplasms, stone disease, biliary stricture, parasites, primary sclerosing cholangitis (PSC), AIDS-related cholangiopathy, and biliary tuberculosis. While extraductal obstruction is caused by external compression of the biliary ducts as that secondary to neoplasms, pancreatitis, or cystic duct stones with subsequent gallbladder distension [7].

In hepatic parenchymal diseases (e.g. cirrhosis), fibrosis may prevent the intrahepatic bile ducts dilatation, resulting in dilation confined to the extrahepatic bile ducts. The question then is whether the derangement of liver functions is related to the hepatic or extrahepatic etiology [8]. The common cause of obstructive jaundice have been reported to vary from one center to another.

Our aim was to assess the frequency of cholestatic disorders in patients admitted to our department and try to identify the underlying extrahepatic causes aiming at improving the quality of management offered to this patients group.

PATIENTSAND METHODS

The present cross sectional study was conducted in Tropical Medicine Department, Zagazig university hospitals during the period from May 2012 to March 2013.

The study was conducted on 506 cases admitted serially to our department and accepted to participate in the present work. They were all subjected to:

1- Serum Bilirubin (total & direct), ALT, AST and ALP levels were assayed by (colorimetric assay RXL, Semiens, Germany).

2- Serum gamma glutamyl transferase level: (by colorimetric assay, Cobas, Roche, Germany). The inclusion criteria were high serum alkaline phosphatase level (≥1.5 times the upper limit of normal), high gamma glutamyl transferase (≥3 times the upper limit of normal) according to EASL [9].

3- Pelvi abdominal ultrasound by Ezzaoti-Mylab 20. ultrasonographic features of extrahepatic cholestasis included (dilated extra and/or intrahepatic ducts) of whatever cause.

All selected patients underwent the following:

- Full history taking and through physical examination.

- Assessment of body mass index (BMI) was conducted using the following formula:

$$\mathrm{BMI} = \frac{\mathrm{mass(kg)}}{(\mathrm{height(m)})^2}$$

Body mass index (BMI) is defined as the individual's body mass divided by the square of their height with the value universally being given in units of kg/m^2.

- Complete blood count, using sympx K x 21 cell counter (Roche diagnostics Manheim, Germany).
- Tumor markers: cancer antigen (CA 19-9), normal levels <37 U/ml. [10] & Alpha feto-protein (AFP): normal range 10-20 ng/ml, while a level 400 ng/ml is considered diagnostic for HCC in the presence of suggestive ultrasonography [11].
- Abdominal CT: Triphasic CT scanning of the liver was performed with CTi/Pro GE medical system and Toshiba X-Vision single slicer CT scanner.
- ECRP whenever needed for diagnosis and/or management of extrahepatic cholestasis.

Statistically analysis:
Data were checked, entered and analyzed using Epi-Info (2000) for data processing and statistics.

Descriptive statistics:
Data were expressed as numbers and percentages for qualitative variables and mean ± standard deviation for quantitative one.

Validity of a screening test:
Sensitivity: is the ability of the test to detect true positive cases =

$$\frac{\text{True positive}}{\text{True positive} + \text{False negative}}$$

Specificity: is the ability of the test to detect true negative cases =

$$\frac{\text{True negative}}{\text{True negative} + \text{False positive}}$$

Positive predictive value: is the probability of disease in a patient with positive test result =

$$\frac{\text{True positive}}{\text{True positive} + \text{False positive}}$$

Negative predictive value: is the probability of not having the disease in a subject with negative test result =

$$\frac{\text{True negative}}{\text{True negative} + \text{False negative}}$$

Accuracy: is the proportion of all test results both positive and negative that are correct =

$$\frac{\text{True positive} + \text{True negative}}{\text{True positive} + \text{True negative} + \text{False positive} + \text{False negative}}$$

RESULTS

The frequency of cholestatic liver diseases was 19.7% (100 of 506 patients).While the extrahepatic cholestatic causes were detected in 61out of 506 studied cases representing 12.1% being more in middle aged patients and more in females than males as shown in table (1).

Table (1): Demographic data of cases with extrahepatic cholestasis.

	Sex		Age	
	Male	Female	Range	Mean ± SD
NO	28	33	25 – 72	51.1 ± 11.7
%	45.9	54.1		
Total NO = 61				

The majority of patients in our study had benign causes for their obstructive jaundice (57.4%) with choledocholithiasis as the most common cause comprising 40.9 % of cases. While malignant causes represented 42.6% of cases with cancer head of pancreas as the commonest malignancy detected, as shown table (2).

Table (2): Frequency of causes of extrahepatic cholestasis.

Etiology	NO	%
CBD stone	25	40.9%
Cancer head of pancreas	9	14.8%
Periampullary carcinoma	8	13.11%
Cholangiocarcinoma	5	8.2%
Acute pancreatitis	4	6.6%
Porta hepatis Lymphadenopathy	3	4.9%
Cholangitis	2	3.3%
Hepatocellular carcinoma	2	3.3%
Duodenal diverticulum	1	1.6%
Iatrogenic ligation of CBD	1	1.6%
Pancreatic pseudocyst	1	1.6%
Total	61	100%

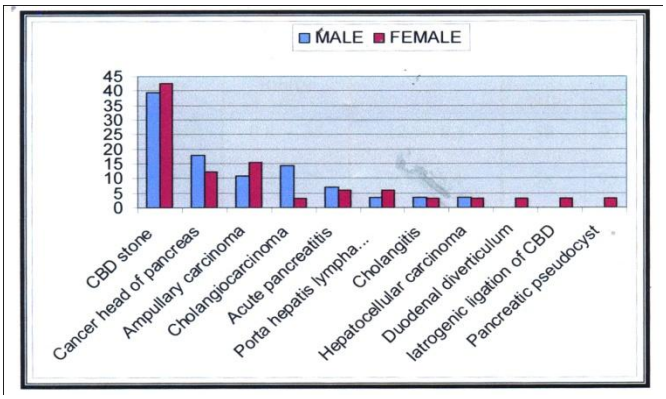


Fig. (1): Relation between etiology of extraheaptic cholestasis and sex

The percentage of malignant extrahepatic cholestasis was higher in males (50%) than females (36.4%) as seen in Figure (1). Most of the patients with benign obstructive jaundice were younger in age as the percentage of benign causes was the highest (85.7%) in the 20-40 years age group , while malignant causes were in elderly as the percentage of malignant causes was the highest (66.7%) in the age group of >60 years Table (3).

Table (3): Relation between malignant and non malignant causes of extrahepatic cholestasis and age

Age group		Malignant	Non malignant	Total	X2	P
20 -40 (adult)	**NO**	2	12	14	7.12	0.007
	%	14.3%	85.7%			
41-60 (middle)	**NO**	16	19	35	0.76	0.38
	%	45.7%	54.3%			
> 60 (elderly)	**NO**	8	4	12	18.87	0.001
	%	66.7%	33.3%			
Total		26	35	61		

Serum total and direct bilirubin levels were elevated in 95.1 % of cases with mean values of 12.5 and 9.3 respectively. There was a significant agreement between ALP and GGT, with P value <0.01, while serum transaminases were only mildly elevated in 34.4% of cases. Regarding the clinical manifestations, 58 cases (95.1%) presented with jaundice. The percentage of dark urine, itching and clay stool in malignant cases were 92.3%, 73.1% and 53.8% respectively,

compared to 82.8%, 62.8% and 48.6% respectively, in non malignant conditions. Charcot's triad was more common in benign conditions with a percentage of (40%) compared to (23.1%) in malignant cases.

Table (4): Frequency of clinical presentations of cases with extrahepatic cholestasis

Clinical presentation	NO	%
Jaundice	58	95.1%
Dark urine	53	86.9%
Itching	41	67.2
Vomiting	37	60.7%
Clay stool	31	50.8%
Weight loss	25	41%
Hepatomegally	22	36.1%
Charcot triad	20	32.8%
Scratch marks	16	26.2%
Bleeding manifestation	10	16.4%
Palpable abdominal mass	5	8.1%
Total	**61**	

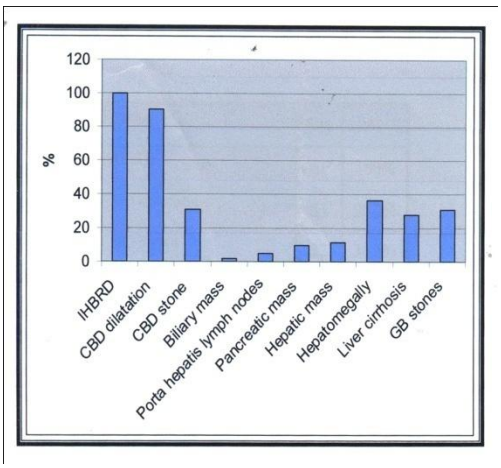


Fig. (2): Frequency of ultrasonographic features of extrahepatic cholestasis

According to imaging modalities ultrasound (U/S) was done to all cases. It picked dilated intrahepatic channels in 100% of patients of extrahepatic cholestasis and dilated CBD in 90.2% of them. CBD stones were detected in 31.1% while masses were detected in only 27.8% and most of the times, it was in the head of pancreas Figure (2). Abdominal computed tomography (CT) was done to all cases to confirm the diagnosis of (U/S) and/or to establish the cause of obstruction when U/S couldn't detect it. It picked dilated intrahepatic channels in 100% & dilated CBD in 90.2%, while CBD stones were detected in 36.1%.

Masses were detected in 41% of cases Figure (3). Endoscopic retrograde cholangi-pancreatography (ERCP) was done to the cases which were not diagnosed by US or CT or as a therapeutic tool. It could detect all the 25 cases with CBD stones representing 40.9% of all cases with extra-hepatic cholestasis. Cholangiocarcinoma was detected in 5 cases, in which 3 of them showed hilar CBD malignant stricture "Klatskin tumor" and 2 cases showed distal CBD stricture. Porta-hepatis lymphadenopathy and HCC were demonstrated as either hilar or distal malignant CBD stricture (Figure 4).

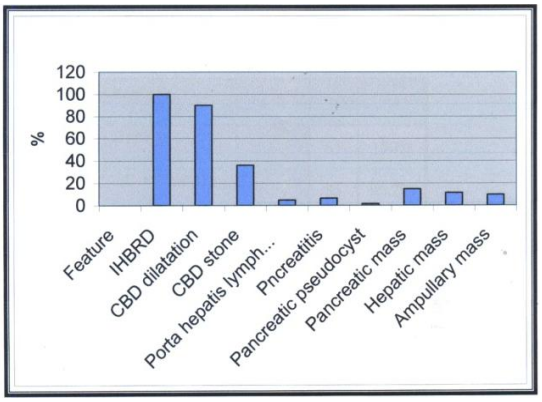


Fig. (4): Frequency of CT features of extrahepatic cholestasis

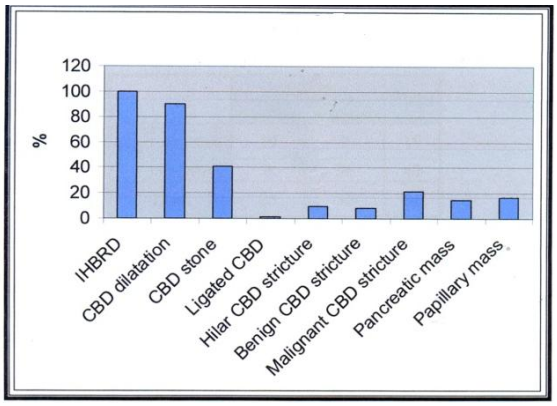


Fig. (5): ERCP features of cases with extrahepatic cholestasis

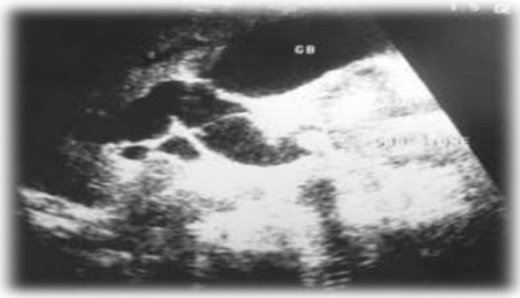
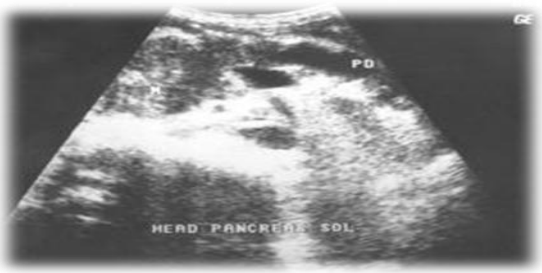
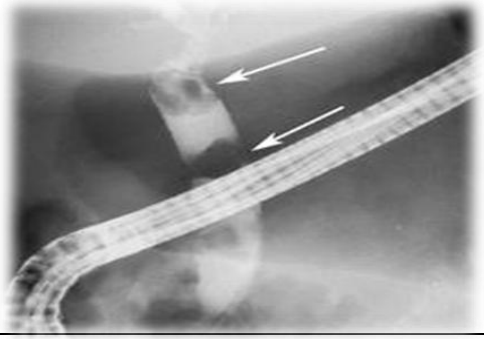
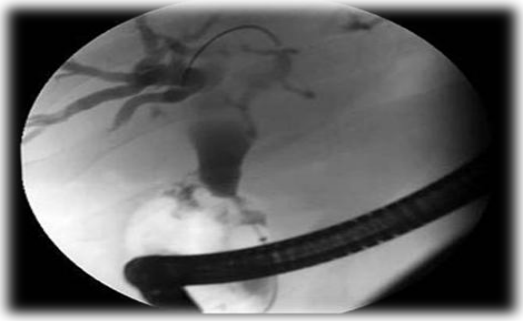
US picture of distal end CBD stone	**US picture of pancreatic head mass**
	
ERCP of CBD stone	**ERCP showing an Irregular stricture of CBD caused by pancreatic head mass**
	

Table (5): Relation between BMI and sex in cases with GB stones

		BMI											
		>25				25 – 30				> 30			
Sex		Male		Female		Male		Female		Male		Female	
		No	%	No	%	No	%	No	%	No	%	No	%
GB stone	-ve	1	100	-	0	20	87	14	63.6	3	60	4	44.4
	+ve	-	0	1	100	3	13	8	36.4	2	40	5	55.6
X2		-				3.311				0.311			
P		-				0.069				0.577			

As shown in (Table 5) there was an increased percentage of females with BMI more than 30 in patients with GB stones, though it was non significant as P value was > 0.05. Regarding the group with BMI < 25, there were only 2 patients (which was not sufficient for statistical analysis).

The sensitivity of U/S, CT and ERCP in detection of CBD stones was 76%, 88% and 100% respectively, whereas their sensitivity in detection of cancer head of pancreas was 66.7%, 100% and 100% respectively. While the predictive values of US compared to ERCP in detection of CBD stones was 100% positive and 85.7% negative, and the predictive value of US compared to ERCP in detection of cancer head of pancreas was 100% positive and 94.5% negative. The predictive values of CT compared to ERCP in detection of CBD stones was 100% positive and 92.3% negative, while the predictive value of CT compared to ERCP in detection of cancer head of pancreas was 100% positive.

DISCUSSION

Among the many functions of the liver, production of bile is the most distinctive and liver specific. Adults produce approximately 500 ml of bile per day which is an aqueous solution containing bile salts, cholesterol proteins, bilrubin conjugates and others [5].

Cholestasis is caused by obstruction within the liver (intrahepatic) or outside the liver (extra-hepatic). Intrahepatic cholestasis is characterized by widespread blockage of small ducts or by disorders, such as hepatitis, that impair the body's ability to eliminate bile. Extra-hepatic cholestasis results from the mechanical obstruction to large bile ducts outside the liver or within the porta hepatis [6].

This cross sectional study was performed to assess the frequency of cholestatic liver diseases and identify the frequency of extrahepatic causes as a health problem in our department. It was found that 61 out of 506 cases admitted to our tropical department had extraheaptic cholestasis representing 12.1%. The high frequency of extraheaptic cholestasis in our study could be attributed to performing this study on a defined population, with high prevalence of chronic liver diseases and cirrhosis which increased the risk of gall bladder and common bile duct stones as noted by Marrelli [7] who studied the prevalence of calcular cholecystitis in Egyptian patients with chronic liver disease and found that chronic hepatitis C virus (HCV) infection is considered an important risk factor for the development of gallstone disease in those patients.

The lower incidence of obstructive jaundice in other studies as that reported by Khalili and Wilson [8] who reported its prevalence to be only 5 cases per 1000 people could be due to differences in the study group, as they assessed their incidence through a large scale study conducted on the general population. Also, the increased incidence of hepatocellular carcinoma in cases admitted to our department, could have contributed to the increased frequency in our study. The extra- hepatic cholestasis was more common among middle aged patients with a mean age of 51 years and a range of 25-72 years. This is in agreement with Bektas [9] who found almost similar results.

The majority of our patients had benign obstructive jaundice (57.4%) while malignant causes comprised (42.6%) which was in agreement with many other authors[10,11,12,13] who documented that the percentage of benign to malignant causes were about 60% - 40%.

Choledocholithiasis was the most common cause among various causes of extrahepatic cholestasis in our study with a percentage of 40.9%. Similar results were demonstrated by Khurram et al. [14] who stated that choledocholithiasis caused 35% of cases, being also the most common etiology of obstructive jaundice in their study. Moreover, in our study, patients with BMI >30 showed increased percentage of GB stones (55.6%), while in those with BMI (25-30), the percentage was (36.4%). Obesity and high dietary cholesterol could lead to increased biliary cholesterol secretion and decreased bile acid synthesis and pool which assist in cholesterol gall stone formation [21].

In our work extrahepatic cholestasis was more common in females (54.1%), which was usually benign in nature (60.6%) and common bile duct stone (CBD) obstruction as the most common cause in them (42.2%). This may be due to the higher prevalence of cholelithiasis in females. Women are at greater risk of developing gall bladder cholesterol stones, which could be attributed to their estrogen levels. Many studies as Everson [17] have shown that, female steroid hormones can significantly alter hepatobiliary physiology. As the gall bladder volume increases during pregnancy or by the prolonged use of contraceptive pills and its emptying is delayed as well. Moreover, the biliary cholesterol content was increased and the bile acid metabolism was altered by the previous conditions.

Other benign causes in our study were acute pancreatitis (6.6%), cholangitis (3.3%), duodenal diverticulum, pancreatic pseudocyst and iatrogenic common bile duct (CBD) ligation with a percentage of (1.6%) for each of them. This was not in agreement with Absi et al[18] who found Ascariasis and Hydatid disease of the billiary tract to be important benign causes of extra-hepatic cholestasis, this difference could be attributed to the fact that their study was conducted in Saudi Arabia where high prevalence of these parasitic infections is present.

The percentage of pancreatitis and its complications as causes of extra-hepatic cholestasis, were higher in other studies done in USA and Europe by Granger and Remick, and Banks [19,20]. This could be explained by the high alcohol consumption in these countries predisposing to chronic pancreatitis.

The percentage of malignant extrahepatic cholestasis was higher in males (50%) than females (36.4%), being mainly cancer head of pancreas with reported incidence of 17.9%. This is in agreement with Kiran and Pokola [21] who also found malignant obstructive jaundice to be more in males than females. Cancer head of pancreas had higher incidence with more mortality in males than females. This may be due to increased frequency of tobacco use in males which is considered a risk factor for that malignancy [22]. Similar observations were obtained by Syed et al.[10] and Khurram et al. [14] who found that the commonest causes of benign and malignant obstructive jaundice were cholidocholithiasis and cancer head of pancreas respectively .

Other malignant causes in our study included, ampullary carcinoma (13.11%), cholangiocarcinoma (8.2%), porta hepatis lymphadenopathy (4.9%) and hepatocellular carcinoma (3.3%). The results of Zarin et al.[23], was found to be similar to ours regarding malignant causes other than cancer head of pancreas.

Regarding the clinical manifestations, 58 cases (95.1%) presented with jaundice. The other three cases were one patient diagnosed as ampullary carcinoma which is usually manifested by intermittent jaundice. Way [24] postulated that, the intermittent jaundice demonstrated in patients with ampullary carcinoma is attributed to tumor necrosis allowing passage of bile with transient relief of symptoms. While, the other two cases were manifested mainly with itching and upper abdominal pain. They were both diagnosed as CBD stone induced obstruction. Flores et al [25] mentioned that, CBD stones can lead to intermittent jaundice due to the bell and valve action induced by the stone at the lower end of CBD leading to partial and intermittent obstruction.

The percentage of dark urine, itching and clay stool in malignant cases were 92.3%, 73.1% and 53.8% respectively, compared to 82.8%, 62.8% and 48.6% respectively, in non malignant conditions. Charcot’s triad was more common in benign conditions with a percentage of (40%) compared to (23.1%) in malignant cases. Sharma and Ahuja [26] said that dark urine and clay stool were more common in malignant obstructive jaundice while Charcot's triad was a more frequent presentation in benign conditions.

In selected cases in our study with extrahepatic cholestasis, the frequency of gall bladder (GB) stones was higher among cirrhotic patients (52.9%) than non cirrhotics (22.7%) with a

significant relation between them. The pathophysiologic mechanisms responsible for induction of GB stones in cirrhotic patients may be related to altered bile pigments secretion, increased estrogen levels and/or abnormal gallbladder motility in cirrhosis[27]. The abnormal bile secretion in patients with liver cirrhosis may also be due to diminished liver reserve, damaged bile ductules, increased GB wall thickness caused by hyperemia and edema as well as decreased contractility with impaired emptying which could further contribute to gall stone formation [28].

In our study, there was a positive agreement between both GGT and ALP. This indicates that both markers together should be used for confirmation of cholestasis. The same finding was previously confirmed by [29].

According to imaging modalities ultrasound (U/S) was done to all cases. It picked dilated intrahepatic channels in 100% of patients of extrahepatic cholestasis and dilated CBD in 90.2% of them. CBD stones were detected in 31.1% while masses were detected in only 27.8% and most of the times, it was in the head of pancreas. The diagnostic accuracy of ultrasound was also studied by Akhtar and Mufti [30] and was close to our results as it was shown to be 85%.

Abdominal computed tomography (CT) was done to all cases to confirm the diagnosis of (U/S) and/or to establish the cause of obstruction when U/S couldn't detect it. It picked dilated intrahepatic channels in 100% & dilated CBD in 90.2%, while CBD stones were detected in 36.1%. Masses were detected in 41%. It was superior to ultrasound in detection and localization of masses. It could detect 6 out of 8 cases with ampullary masses and all the 9 cases with pancreatic masses compared to zero and 6 cases respectively detected by ultrasound. CT could also detect the 4 cases with acute pancreatitis while U/S couldn't detect any of them. The higher efficacy of CT in diagnosis and staging of tumors causing obstructive jaundice has also been reported by Pasanen, et al. [31].

Endoscopic retrograde cholangiopancreato-graphy (ERCP) was done to the cases which were not diagnosed by US or CT or as a therapeutic tool. It could detect all the 25 cases with CBD stones representing 40.9% of all cases with extrahepatic cholestasis. Cholangiocarcinoma was detected in 5 cases, in which 3 of them showed hilar CBD malignant stricture "Klatskin tumor" and 2 cases showed distal CBD stricture. Porta-hepatis lymphadenopathy and HCC were demonstrated as either hilar or distal malignant CBD stricture.

The sensitivity of US, CT and ERCP in detection of the various causes of extrohepatic obstruction was 76%, 88% and 100% respectively. These findings are broadly in agreement with studies done at various other centers for CT [31] and ERCP [32].

We can conclude that, the frequency of cholestatic liver diseases was 19.7%, among them; extrahepatic causes comprised 12.1% in our department. Ultrasonography is considered the best primary non invasive diagnostic modality for extrahepatic cholestasis. While CT scans has more accuracy in detection of biliary and pancreatic masses and ERCP, is considered the gold standard modality in diagnosis and treatment of extrhepatic cholestasis with excellent accuracy in identification of the cause.

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Ethical approval: Informed consent was obtained from all participants, and the study was approved by the Ethical committee of Faculty of Medicine, Zagazig University.

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Video Case: Juvenile Polyposis Syndrome in 11- Years Old Girl

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A 11-years old Egyptian girl presented by bleeding per rectum. On endoscopic examination, multiple sigmoid colon polyps(>5) with mucosal prolapse were seen. Polypectomy was done in multiple sessions (this video shows the first session) . Microscopic examination confirmed the diagnosis of Juvenile Polyposis . Juvenile polyposis (JP) is an autosomal dominant hamartomatous polyposis syndrome where affected individuals are predisposed to colorectal and upper gastrointestinal cancer. Forty-five percent of JP patients have mutations or deletions involving the coding regions of SMAD4 and BMPR1A[1]. Juvenile polyps have a distinctive histology characterized by an abundance of edematous lamina propria with inflammatory cells and cystically dilated glands lined by cuboidal to columnar epithelium with reactive changes. Clinically, juvenile polyposis syndrome is defined by the presence of 5 or more juvenile polyps in the colorectum, juvenile polyps throughout the gastrointestinal tract or any number of juvenile polyps and a positive family history of juvenile polyposis[2].

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Image Case : Hydatid Disease of the Liver

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Hydatid Disease which is also referred to Echinococcosis or echinococcal disease, is a parasitic disease that affects both humans and other mammals, such as sheep, dogs, rodents and horses [1].

Hydatid disease remains a clinical problem worldwide, especially in areas where animal husbandry and subsistence farming form an integral part of community life [2]. The liver is the most commonly involved organ (52-77%) [3], but hydatid disease may affect any part of the body either as a primary or secondary event [2].

There are four forms of hydatid disease. *Echinococcus granulosus* (EG) is the most common and gives rise to cystic hydatid disease (CHD). *Echinococcus multilocularis* is uncommon and causes alveolar hydatid disease (AHD), which is far more aggressive and frequently mimics malignancy [4]. The rarest clinical form is *Echinococcus vogeli* or polycystic hydatid disease (PHD), with characteristics between CHD and AHD [5]. *Echinococcus shiquicus*, has been identified on the Tibetan plateau but to date no human infection has been described. CHD is a zoonosis infecting a variety of domestic and wild animals. There is no host specificity for the larval stage of EG, but the commonest intermediate hosts are sheep, cattle, buffalo, camels and pigs [6].

The developing hydatid cyst has three layers. The outer pericyst is composed of host fibroblasts, eosinophils, giant cells and modified hepatocytes. The middle laminated membrane is a cellular and impermeable to bacteria, and the innermost layer, the germinal layer or brood capsule, is translucent and is the origin of scolices and daughter cysts within the primary cyst [4]. The cyst usually contains crystal-clear fluid which is strongly antigenic and may cause anaphylaxis if released into the circulation of the host. Most cysts remain silent when small and present only when complications such as rupture into the biliary tree, bacterial superinfection or free intra-abdominal rupture occur. Owing to the lack of symptoms in the early stages, the actual accurate assessment of the growth rate of these cysts is difficult [2].

Several classifications of CHD exist. All were developed in endemic areas, and are important because they enable the most appropriate treatment option to be selected. The classifications are not comparable, however, which makes comparative analysis difficult. The two most widely used classifications are the morphological classifications proposed by Gharbi et al [7]. in 1981 (Table I) and Lewall and McCorkell[8] in 1985, which are based on pathology and natural history.

In 1997, the WHO Informal Working Group classification on Echinococcosis (WHO-IWGE) proposed a new standardized classification based on ultrasound images [8]. This classification is intended to follow the natural history of CHD and is divided into three groups. The first group are active, fertile cysts containing viable scolices, the second group are in a transitional stage owing to compromise either by host defense or chemotherapy, and the third group are inactive, having lost their fertility, and are degenerative [2].

Imaging modalities range from simple to complex and invasive. Plain radiographs of the abdomen and chest may reveal a thin rim of calcification delineating a cyst, or an elevated hemidiaphragm. Both signs are nonspecific. Ultrasound is readily available and cost effective. A cyst containing daughter cysts and hydatid sand (debris) are highly suggestive. Several studies have documented the excellent sensitivity (100%) of ultrasound. [9,10] A computed tomography (CT) scan of the abdomen gives better information concerning location, accessibility and possible complications. It is also helpful in identifying exogenous cysts, and the volume of the cyst can be estimated. CT is an important investigation when there is diagnostic uncertainty on ultrasound, when planning surgical intervention or when recurrent disease is diagnosed. Magnetic resonance imaging (MRI) adds little to CT scanning and is not cost effective. Endoscopic retrograde cholangiopancreatography (ERCP) remains an important tool in cases where rupture into the biliary tree has occurred, allowing both the diagnosis of major biliary communication and clearance of the common bile duct (CBD) prior to surgery or intervention [9,10]

We reported a 40 years old male with a chronic RUQ pain, an enlarged liver and a palpable mass. The patient was referred to the x-ray department for a longitudinal abdominal ultrasound. A cystic mass with floating membrane and posterior enhancement was reported indicating a Liver hydatid cyst. The Hydatid cyst was in the range of Gharbi's type II (Image 1).

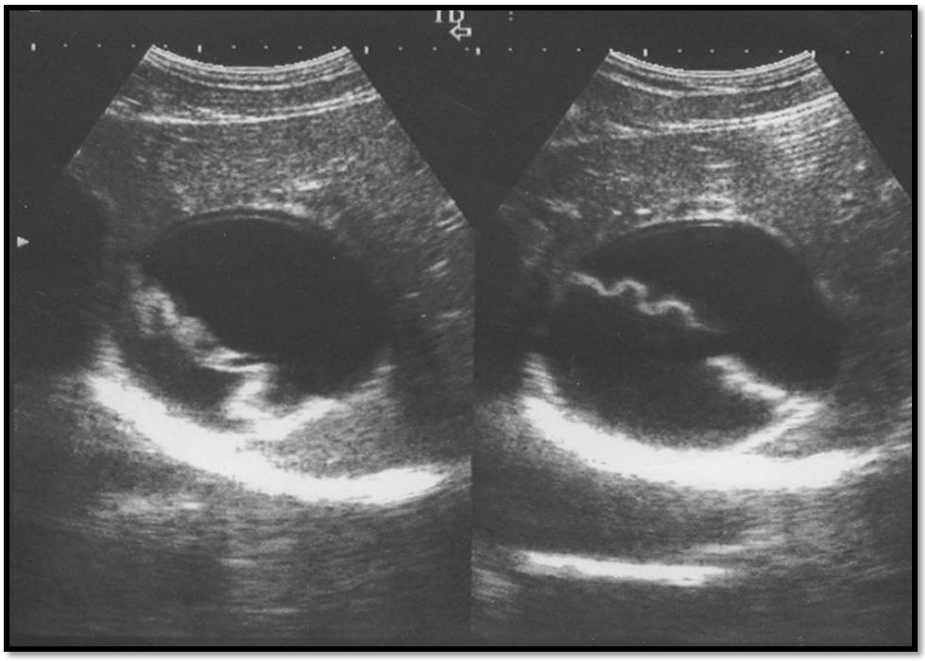

Image (1) : Gharbi type II: Fluid collection with a detached membrane

Table (1) : Gharbi classification of hydatid cysts

Type	Description
I	Pure fluid collection
II	Fluid collection with a detached membrane
III	Fluid collection with multiple septa and/or daughter cysts
IV	Hyperechoic with high internal echoes
V	Cyst with reflecting calcified thick wall

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ERCP : Is it Really Safe in Cirrhosis?

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Dear editor

Endoscopic-retrograde-cholangiopancreatography (ERCP) currently has a pivotal importance in management of a wide variety of hepato-pancreatico-biliary disorders. It is a major endoscopic technique that requires not only high volume centres but also highly qualified endoscopic teams [1]. Patients with liver cirrhosis are at increased risk for complications after surgery [2] and probably also after the major endoscopic techniques including the ERCP. That is why the paper published by El-Naggar et al., 2013 in the Afro-Egyptian Journal of Infectious and Endemic Diseases [3] seems interesting; the authors performed several invasive procedures in patients with Child A and B liver cirrhosis and the outcomes were unexpectedly excellent.

Several issues needs elaboration, firstly the randomization in this study was not clear, weather before or after initial cannulation attempts?. It is remarkable that there was no post-ERCP pancreatitis in group A i.e. patients in whom repeated attempts at standard cannulation failed before fistulotomy. It seems that a good number of patients seem to have proximal and distal bile duct strictures ; the etiologies of these bile duct strictures in patients with cirrhosis were not clear?. Did these patients have cholangitis, jaundice, or underlying malignancies? These may have an impact in this category of patients and would certainly change the outcomes. Lastly the authors did not include patients with Child C cirrhosis, in fact these patients with advanced deterioration of liver functions are not infrequently seen with indications for ERCP and a challenge frequently seen to or not perform ERCP in these patients. The literature is lacking for recommendations based on evidence for ERCP in such situations. Probably, in the future the authors or other investigators may conduct a study to delineate the situation in patients with Child C cirrhosis who are known to have coagulopathy, poor performance status, recurrent hepatic encephalopathy and frequent infectious complications.

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