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3- **Abbreviations:** Define abbreviations that are not standard in this field at their first occurrence in the article (even if mentioned in the abstract). Ensure consistency of abbreviations throughout the article

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5- **Global Impact Factor** http://globalimpactfactor.com/journals-list/?snap=A

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

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8- **Pubicon Science Index:** http://www.pubicon.org/APUIIR.aspx?cmd=Afro-Egyptian%20Journal%20of%20Infectious%20and%20Endemic%20Diseases

Contents

Impact of Hepatic Steatosis on Response to Antiviral Therapy in Egyptian Patients with Chronic Hepatitis C

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Key words:
Hepatic steatosis, Chronic hepatitis C virus

Background and study aim: Hepatic steatosis in hepatitis C virus (HCV) infected patients has been shown to enhance the progression of liver fibrosis and decrease the response to antiviral therapy. The current study is designed to investigate the impact of hepatic steatosis on the outcome of pegylated interferon and ribavirin combination therapy in patients with chronic hepatitis C genotype 4.

Patients and Methods: A total number of 200 patients were selected from 270 patients who were referred to HCV Treatment Unit of New Mansoura General Hospital from February 2012 to August 2013 after taking an informed consent. They were 129 males and 71 females, their ages ranged from 25 to 55 years (mean value, 35.5±15.2). They had proven chronic hepatitis C virus based on history of exposure, clinical manifestations, positive anti-HCV antibody, positive HCV viremia, and liver biopsy findings suggestive of chronic hepatitis C.

Results: *Group I*: included 100 patients (70 men and 30 women; mean age of 42.9±12 years) without liver steatosis. *Group II*: included 100 patients (59 men and 41 women; mean age of 45.23±11 years) with liver steatosis. In terms of steatosis grading using the NAS and METAVIR scoring systems, 50% had no staetosis while 8.5% had mild staetosis, 18.5% had moderate steatosis and 23% had severe steatosis. Body mass index of patients receiving interferon is significant between both groups. Hepatomegaly shows significant values between both groups. Platelets count, ALT, AST, S.Cholesterol & S.Triglycerides levels has statistically significant differences between group I (non steatotic) and group II (steatotic). There is statistically significant difference between both groups on necro-inflamatory activity grades. High statistical significance difference between grading of steatosis and Necro-inflammation. Statistical significance difference between grading of steatosis and fibrosis stages. Statistical significance difference between both groups at SVR and Steatosis has a negative effect on SVR by comparison to non steatotic group. High degree of hepatic steatosis has a negative impact on pagylated interferon and ribavirin therapy in chronic HCV genotype 4 minimizing sustained virologic response rates.

Conclusion: Our study confirms that hepatic steatosis correlates with BMI, S.cholesterol, S.triglycerides, fibrosis, necro-inflammatory stages and has a negative impact on response to antiviral therapy.

INTRODUCTION

The incidence of hepatitis C virus (HCV) infections is falling in some countries, however the burden of the disease in Egypt continues to rise. It has been estimated that, by 2030, HCV will cause substantially higher morbidity and mortality than HIV. Chronic Hepatitis C (CHC) occurs in 70% to 80% of those who contract the virus, 20% of whom will progress to cirrhosis within 2-3 decades; a quarter of these will develop decompensated liver disease, hepato-cellular carcinoma (HCC) and will need liver transplantation. A recent study has shown that HCV infected persons have three times higher death rates than those of age-matched general population [1].

In patients with chronic HCV infection, steatosis is attributable to a variable combination of the mechanisms considered to play a role in the pathogenesis of NAFLD; insulin resistance in the obese and in the lean subject along with a direct effect of HCV on hepatic lipid metabolism that leads to triglyceride accumulation through inhibition of export proteins that are required for very low density lipoprotein (VLDL) assembly and secretion [2].

Hepatic steatosis resulting from host metabolic factors or via direct viral effect has been associated with increased fibrosis, regardless of genotype. Worsening hepatic steatosis also is associated with increased periportal necrosis, hepatocyte apoptosis, and fibrosis progression [3].

Leptin also may mediate fibrogenesis. This satiety hormone has been shown to be profibrogenic and up-regulation of leptin signaling may lead to fibrosis progression. In CHC genotype 1 patients there appears to be a correlation between serum leptin levels, steatosis, and fibrosis [4].

Steatosis seems to reduce the likelihood of obtaining sustained virological response (SVR) from HCV medications at least in people with HCV non-3 type, the impact of steatosis on SVR in genotype 3 is less clear [5].

The risk of non-response to antiviral therapy was increased 2-fold if significant steatosis or steatohepatitis (SH) were present on biopsy. Furthermore, there is a significant difference in overall SVR between groups. It is noteworthy that the effect of steatosis/SH on SVR was greatest in the genotype 2 or 3 patients [6].

It is become clear that there is direct viral mechanisms involved in the development of steatosis in people infected with HCV genotype 3, although in genotype other than 3 other co-factors such as high BMI, heavy alcohol intake, elevated blood lipids, glucose intolerance and diabetes greatly promotes the development of steatosis[5].

Besides obesity, type 2 diabetes mellitus (DM) and hypertriglyceridemia have also been associated with hepatic steatosis in patients with non-alcoholic fatty liver disease [7].

Aim of the work:

The current study is designed to investigate the impact of hepatic steatosis on the outcome of pegylated interferon and ribavirin combination therapy in patients with chronic hepatitis C.

PATIENTS AND METHODS

A total number of 200 patients were selected from 270 patients who were referred to HCV Treatment Unit of New Mansoura General Hospital from February 2012 to August 2013 after taking an informed consent. They were 129 males and 71 females, their ages were ranging from 25 to 55 years with mean value of 35.5±15.2 with proven chronic hepatitis C virus based on history of exposure, clinical manifestations, positive anti-HCV antibody, positive HCV vireamia, and liver biopsy findings suggestive of chronic hepatitis with documented chronic hepatitis C

According to the histopathological grade of steatosis, enrolled patients were classified into two groups:

Group I: Included 100 patients (70 men and 30 women; mean age of 42.9±12 years) without liver steatosis.

Group II: Included 100 patients (59 men and 41 women; mean age of 45.23±11 years) with liver steatosis.

Exclusion criteria:

Patients were excluded from the study if one or more of the following conditions were present:

1- Previous IFN therapy.
2- Evidence of other liver diseases including; hepatitis B, autoimmune hepatitis, alcoholic liver disease or drug induced hepatitis.
3- Decompensated liver disease with a history of variceal hemorrhage, ascites, or hepatic encephalopathy.
4- Concurrent cardiac or respiratory diseases or diabetes mellitus.
5- Patients with a leukocyte count lower than 4000/mm^3, neutropenia (<1500 cells/mm^3), a hemoglobin level lower than 12 g/dL for women and lower than 13 g/dL for men, thrombocytopenia (<90,000 cells/mm^3).
6- Creatinine concentration 1.5 times the upper limit of normal.
7- Neoplastic disease, unstable thyroid dysfunction, unstable psychiatric disorder or history of any organ transplantation.
8- Current therapy with immune modulatory agents or immunosuppressive within the last 6 months.

All the included patients were subjected to the following:

Full medical history, thorough clinical examination, laboratory investigation (CBC, ALT, AST, S. bilirubin, serum albumin, prothrombin time, INR,

HCV antibody, HBsAg, quantitative PCR before treatment and at weeks 12, 24, 48 and 24 weeks after end of treatment, s. cholesterol, s. triglycerides, s.TSH, fasting blood sugar, renal functions, s. Alkaline phosphatase, ANA, AFP and IHA for bilharziasis), imaging (abdominal ultrasonography) and liver biopsy.

Statistical analysis:

Data were collected, tabulated and statistically analyzed by computer using SPSS version 16. The following tests were used; arithmetic mean, standard deviation (SD), standard student "t test", Chi square Test (X^2), sensitivity, specificity, accuracy, positive predictive value, negative predictive value, linear correlation coefficient [r], Roc curve (Receiver operating characteristic curve) and significance of results (P value) .

RESULTS

Chronic HCV genotype 4 is associated with hepatic steatosis which is mostly metabolic associated with elevated BMI, triglycerides and cholesterol levels.

- Body mass index of patients has statistical significant between both groups as shown in Table (1).
- Hepatomegaly shows significant values between both groups.
- Platelets count, ALT, AST, s.cholesterol and s. triglycerides levels has statistically significant difference between group I (non steatotic) and group II (steatotic) group as shown in Table (2).
- Statistical significance between both groups on necroinflamatory activity grades as shown in Table (3).
- High statistical significance between grading of steatosis and necro-inflammation as shown in Table (4) and Figure (2).
- Statistical significance between grading of steatosis and fibrosis stages as shown in Table (4) and Figure (3).
- Statistical significance between both groups at SVR and steatosis has a negative effect on SVR by comparison to non steatotic group as shown in Table (5).
- High degree of hepatic steatosis has a negative impact on pagylated interferon and ribavirin therapy in chronic HCV genotype 4 minimizing sustained virologic response rates.

Table (1): Gender, age and BMI distribution among studied groups.

	Group I		Group II		X^2	P.Value
	N	%	N	%		
Male	70	70	59	59	2.642	> 0.05
Female	30	30	41	41		
Total	100		100			
	Mean	SD	Mean	SD		
BMI	27.6	2.19	29.54	1.2	t- test= 7.77	< 0.05
Age	42.9	12	45.23	11	t- test= 1.43	> 0.05

Table (2): Other laboratory findings before starting treatment in two groups.

	Group I	Group II	t- test	P.Value
AST(up to 40 U/ml) -Mean -Range	 55±13 25-70	 63±19 30-87	3.48	< 0.05*
ALT(up to 45 U/ml) -Mean -Range	 57±17 32-82	 66±21 40-103	3.33	< 0.05*
Serum Albumin (3.5-5gm/dl) -Mean -Range	 4.2±0.9 3.2-5	 4±1.1 3-5.2	1.41	> 0.05
Serum Bilirubin (0.5-1.2 mg/dl) -Mean -Range	 0.9±0.2 0.4-1.3	 1±0.49 0.5-1.6	1.89#	> 0.05
Fasting blood sugar(80-110mg/dl) -Mean -Range	 103±0.32 70-115	 110±0.36 80-142	1.45	> 0.05
Serum Creatinine (0.7-1.2 mg/dl) -Mean -Range	 0.9±0.3 0.5-1.4	 1±0.42 0.7-1.5	1.94	> 0.05
AFP(up to 10 ng/ml) -Mean -Range	 8±4.2 4-20	 9±3.2 5-31	1.89	> 0.05
TSH (0.3-5 ul/ml) -Mean -Range	 2.5±1.48 0.2-6.7	 2.9±1.39 0.3-8.1	1.97	> 0.05
HCV RNA(undetected level up to 15 -Mean -Range	 8,000000±141000 12500 – 15,000000	 7,650000±133000 11780 – 20,050000	18.06#	> 0.05
HBS Ag (negative)	negative	negative		
ANA (negative)	negative	negative		
Alkaline phosphatase (21-92 mg/dl) -Mean -Range	 42±12 18 - 85	 45±16 20 – 90	1.5	> 0.05
Cholesterol (up to 200 mg/dl) -Mean -Range	 160±43 90 - 195	 180±52 110 – 290	2.96#	< 0.05*
Triglycerides(up to 150 mg/dl) -Mean -Range	 110±52 80 - 155	 130±62 90 - 210	2.47#	< 0.05*

Mann Whitney test.

Table (3): Necro-inflammatory activity stages and fibrosis grades in both groups.

	Group I		Group II		X^2	*P.Value*
	No	%	No	%		
Activity						
-A0	12	12	5	5		
-A1	29	29	25	25	7.945	< 0.05*
-A2	38	38	33	33		
-A3	21	21	37	37		
Fibrosis						
-F0	5	5	4	4		
-F1	18	18	14	14		
-F2	29	29	23	23	4.980	> 0.05
-F3	27	27	24	24		
-F4	21	21	35	35		
Steatosis						
-Mild			17	17		
-Moderate			37	37		
-Severe			46	46		

Table (4): Grading of steatosis and necro-inflammatory with fibrosis stages in group II (steatosis group).

	Mild Steatosis		Moderate Steatosis		Sever Steatosis		X^2	*P.Value*
	No	%	No	%	No	%		
Activity								
-A0	4	23.5	1	2.7	0	0		
-A1	6	35.3	16	43.2	3	6.5	33.694	<0.001*
-A2	4	23.5	10	27	19	41.3		
-A3	3	17.6	10	27	24	52.2		
Fibrosis								
-F0	2	11.8	2	5.4	0	0		
-F1	6	35.3	5	13.5	3	6.5		
-F2	4	23.5	10	27	9	19.6	17.883	< 0.05*
-F3	3	17.6	9	24.3	12	26.1		
-F4	2	11.8	11	29.7	22	47.8		

Table (5): Virological response (VR) at 12, 24, 48 weeks and SVR in both groups.

	Group I		Group II		X^2	P.Value
	N	%	N	%		
PCR at 12 weeks						
-Responder	85	85	75	75	3.125	> 0.05
-Non responder	15	15	25	25		
PCR at 24 weeks						
-Responder	80	94.1	67	89.3	1.222	> 0.05
-Non responder	5	5.9	8	10.7		
PCR at 48 weeks						
-Responder	77	96.3	64	95.5	0.049	> 0.05
-Non responder	3	3.8	3	4.5		
SVR						
-Responder	71	92.2	51	79.7	4.699	< 0.05*
-Non responder	6	7.8	13	20.3		

* Significant P < 0.05

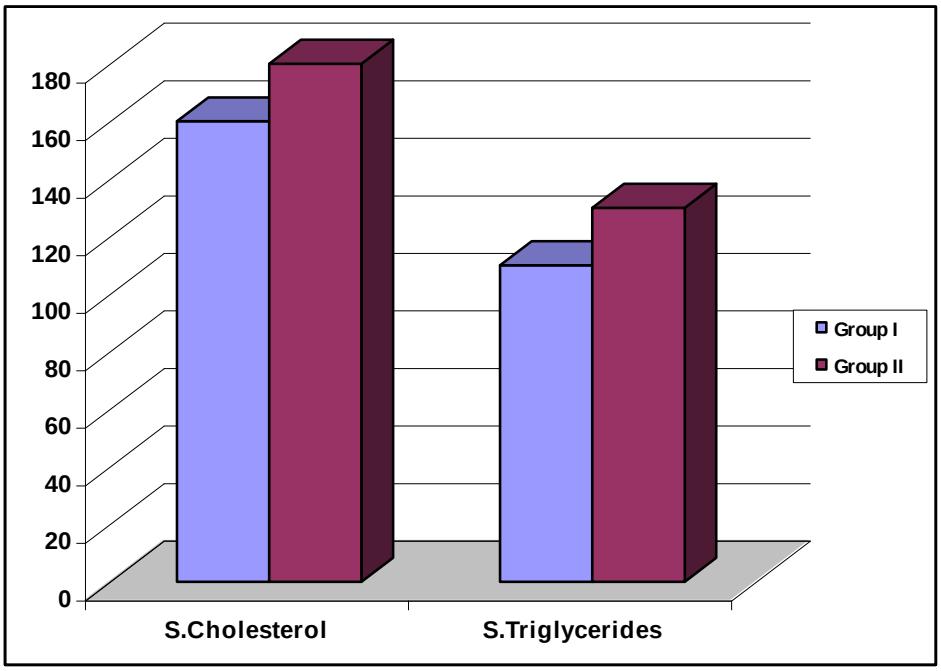

Figure (1): Cholesterol and triglycerides values in group I and group II.

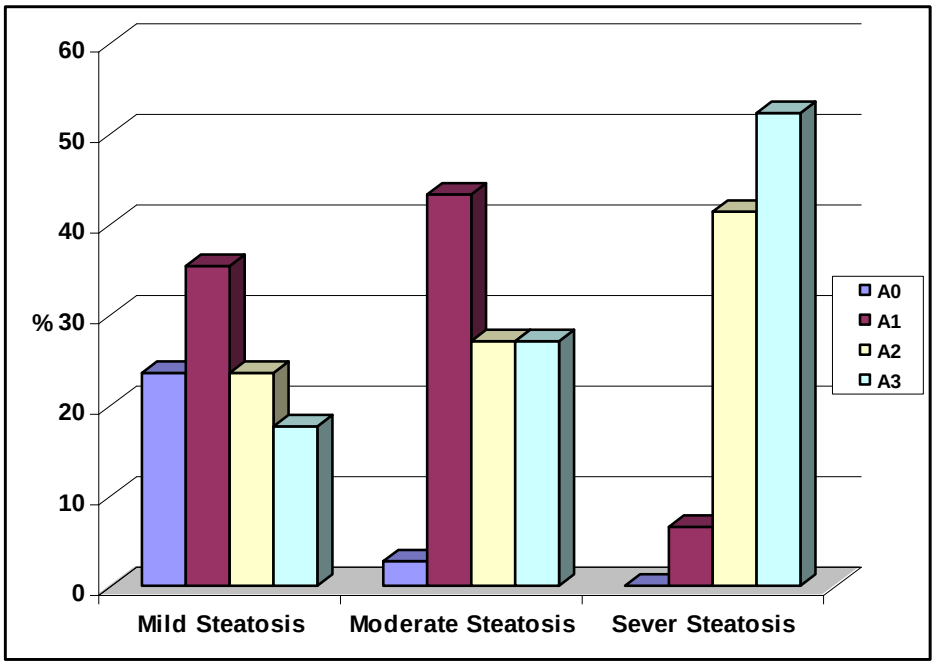

Figure (2): Necro–inflammatory stages in relation to degrees of steatosis.

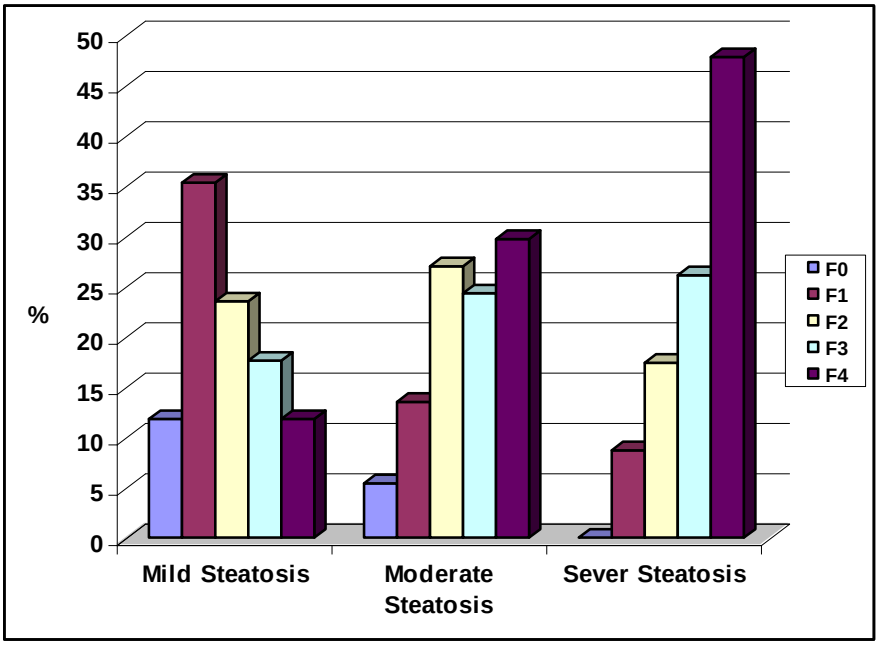

Figure (3): Fibrosis stages in relation to degrees of steatosis.

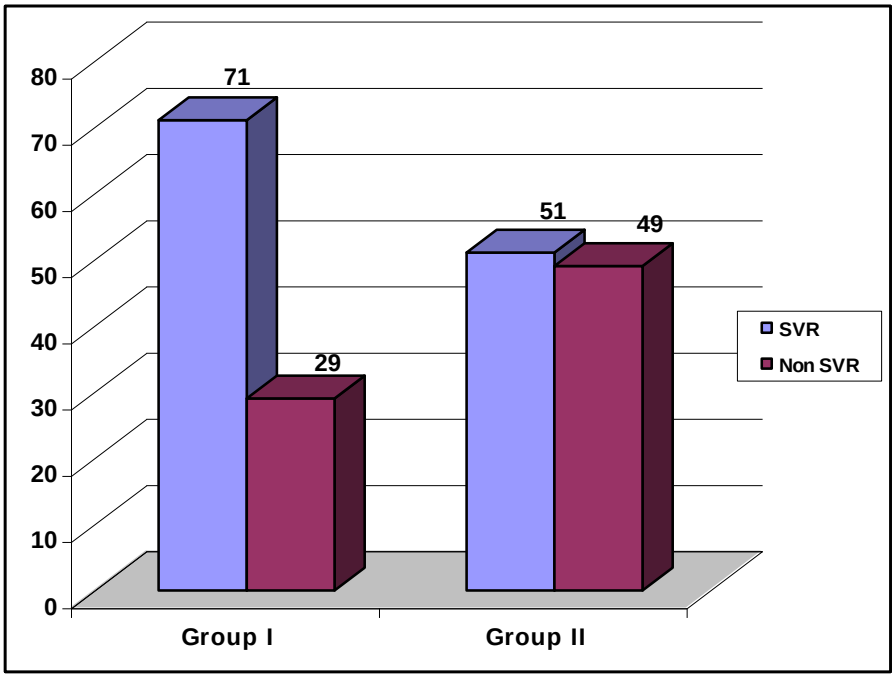

Figure (4): SVR and non SVR values in group I (non steatotic) and group II (steatotic).

DISCUSSION

In the current work, there were a significant corelation between BMI and steatosis so it means that steatosis in HCV genotype 4 is metabolic as shown in table (1) and this is in agreement with El-Zayadi et al. [8] who concluded that steatosis was independently associated with diabetes

mellitus as well as increased BMI. These findings support the proposed causal relation to metabolic factors rather than to cytopathic effect of HCV genotype 4.

Our results revealed statistical significance relation between levels of cholesterol and triglycerides and presence of steatosis as shown in Table (2) and Figure (1). Also our findings are in accordance with Sanyal et al. [9], who reported that the presence of steatosis in HCV genotype 4 infected patients was strongly associated with obesity, diabetes mellitus and abnormal lipid profile.

Our results revealed statistical significant relation concerning AST & ALT levels with patients of group I compared with those of group II ($P<0.05$) as shown in Table (2) and this is in agreement with Minerva [10] mentioned that liver enzymes levels are raised in case of steatosis.

In our series, there was no significant correlation between steatosis and viral load as shown in table (2) and this is an agreement with El-Zayadi et al. [8] who concluded that the presence of steatosis in 55.6%, 52.6%, 56.3% and 60% of genotype 4 patients with very low, low, moderate and severe viral load respectively. This may reflect the absence of association between viral load and steatosis in genotype 4 patients as the proportion of patients with steatosis did not show significant increase with the increase in viral load.

In the present study, there was a significant correlation between stage of fibrosis and steatosis level as shown in Table (4) and Figure (3) and this in accordance with Lonardo et al. [11] who revealed that steatosis is a definite cofactor of chronic hepatitis C which accelerates the progression to end-stage liver disease.

In our study there is a high significant correlation between steatosis and necro-inflamtion as shown in Table (4) and Figure (2) and this in accordance with Lonardo et al. [12] who declared that Presence of steatosis was also correlated with necro-inflammatory activity in nonalcoholic steatohepatitis as well as chronic hepatitis C.

Our study shows that steatosis has a negative effect on SVR by comparison to non steatotic group as shown in Table (5) and Figure (4) and this is in accordance with Fried et al. [13] who concluded that advanced hepatic fibrosis is a negative predictor of SVR to therapy Everson et al. [13].

Our study shows that there is a good correlation between direct fibrosis progression rate and histological activity because fibrosis might be a result of the necro-inflammatory activity as shown in Table (4,5) and this accordance with Mendes et al. [14] who found good correlation between direct fibrosis progression rate and histological activity because fibrosis might be a result of the necro-inflammatory activity.

CONCLUSION

There is strong association between the degree of liver steatosis and fibrosis staging. Although patients with high grading scores tended to have more steatosis; there is a statistical significant correlation between the degree of steatosis and the necro-inflammatory grade

Hepatic steatosis and obesity were predictors of poor response to pegylated IFN and ribavirin therapy in CHC genotype 4.So reduction of the weight before treatment is an important to improve sustained response rates. The evaluation of hepatic steatosis is not only useful for prediction of treatment outcome, but also important for investigation of new approaches toward overcoming the IFN resistance in patients with chronic hepatitis C.

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Study of the Correlation between *Helicobacter pylori* Infection and Hepatic Encephalopathy in Patients with Liver Cirrhosis

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H.pylori, Hepatic encephalopathy, ELISA, Liver cirrhosis

Background and study aim: Ammonia plays a major role in hepatic encephalopathy pathogenesis. Most of ammonia is known to be produced by the action of colonic bacteria which possess a urease enzyme activity. *H. pylori* which infects the stomach possesses a stronger urease activity which produce a large amount of ammonia that may precipitate hepatic encephalopathy (HE). The aim of the present study is to determine the correlation between *Helicobacter pylori* infection and HE in patients with liver cirrhosis.

Patients and Methods: One hundred patients (50 patients of liver cirrhosis with hepatic encephalopathy and 50 patients of liver cirrhosis without hepatic encephalopathy) were evaluated for presence of *H. pylori* by stool antigen test (ELISA method) and for blood ammonia level estimation.

Results: Pevalence of *H. pylori* infection in the study groups (patients of liver cirrhosis with and without hepatic encephalopathy) was 70% (liver cirrhosis with hepatic encephalopathy group (A) 80%, and liver cirrhosis without hepatic encephalopathy group (B) 60%). Mean blood ammonia levels were: 82.14± 47.9 mmol/l for group A (liver cirrhosis with hepatic encephalopathy) and 36.44± 17.9 mmol/l for group B (liver cirrhosis without hepatic encephalopathy). Prevalence of *H. pylori* and blood ammonia level were found significantly increasing with the severity and the degree of hepatic encephalopathy.

Conclusion: There is a significant association between *H. pylori* and hepatic encephalopathy in patients with liver cirrhosis. There may be a role of anti-*H. pylori* therapy in patients of hepatic encephalopathy and should be investigated further.

INTRODUCTION

Hepatic encephalopathy (HE) is a dangerous complication affecting patients with liver cirrhosis.The reversible nature of this neuropsychiatric syndrome with its widespread cerebral changes suggests a metabolic mechanism.The brain is exposed to several neuroactive toxins, in particular ammonia, due to failed hepatic clearance or the abnormal peripheral mechanisms of the cirrhotic [1]. So ammonia plays a key role in HE and even the more recent theories that involve other agents also incorporate an increased ammonia level as an exacerbation factor [2]. Although kidney and muscle may liberate ammonia, most is of gut origin produced by action of bacterial flora on dietary protein and on epithelial and bacterial debris. Normally ammonia is extracted by the liver where it is used for synthesis of urea. In cirrhotics however, large quantities of ammonia reach the systemic circulation because of portal systemic shunting and impaired urea synthesis [3]. Bacterial urease enzyme contributes significantly to absorbed ammonia as a result of daily hydrolysis of 15-30% of total body urea [4]. Although this activity is usually attributed to faecal bacteria, the stomach, which possesses strong activity when infected with *Helicobacter pylori*, is considered as an alternative site. *H. pylori* are known to produce copious amounts of ammonia due to its strong urease activity many times greater than that of urease positive enterobacteria.

Urea readily diffuses from blood into the gastric lumen where, in the presence of *H.pylori* is hydrolyzed to ammonia leading to a rise in arterial levels in 50% of normal subjects and in virtually all cirrhotics [5]. It has been shown that the ammonia concentrations in portal and venous blood significantly increased after the instillation of 1 ml 10^7 colony forming units (CFU)/ml of *H. Pylori* in the stomach of cirrhotic rats, suggesting that the ammonia produced by *H. pylori* has a role in the pathogenesis of hyperammonaemia when this organism is widely distributed and is present in large numbers in the stomach, particularly in the presence of liver cirrhosis [6]. Blood ammonia levels were found to be higher in cirrhotic patients with *H. pylori* infection than in uninfected patients in some studies [7].

This finding was supported by some therapeutic trials on the effect of *H. pylori* eradication therapy on hyperammonaemia in patients with liver cirrhosis [8].

PATIENTS AND METHODS

The present study was conducted on one hundred patients (50 patients with liver cirrhosis and hepatic encephalopathy and 50 patients with liver cirrhosis without hepatic encephalopathy).

They were choosen after taking written consent from out-patient clinic and in-patient department in Helwan Fever Hospital during the period from November 2013 to June 2014.

The patients were divided into two groups:

Group (A): Hepatic encephalopathy group comprised of 50 patients (30 males and 20 females) with age range from 44 to 69 years with a mean age 56.24 ± 8.91

Group (B): Non- hepatic encephalopathy group containing 50 patients (28 males and 22 females) with age range from 39 to 64 years with a mean age 45.56 ± 11.72.

All patients (with and without hepatic encephalopathy) were subjected to the following:

- Full history taking.
- Clinical examination with special stress on presence of stigmata of liver cirrhosis.
- Abdominal ultrasound.
- Laboratory investigations:
  - Liver biochemical profile: namely total bilirubin, direct bilirubin, alanine transaminase (ALT), aspartate transaminase (AST), albumin, prothrombin time and concentration **[9]**.
  - Arterial blood ammonia estimation **[10]**.
  - Stool antigen test based on ELISA technique (enzyme linked immunosorbent assay) to detect *H.pylori* antigens [11].

Statistical analysis:

All patients/ data were tabulated and processed by SPSS 12.0 statistical package for Windows xp.

Descriptive data:

Descriptive statistics were calculated in the form of: Mean, Standard deviation (±SD), minimum and maximum and frequency (No-%).

Analytical statistics:

- Quantitative variables were expressed by mean ± standard deviation (SD) and analyzed by ANOVA test.
- Spearman correlation was used to correlate different grades of HE with laboratory variables.
- Qualitative variables were expressed by number and percent and analysed by Chi-square test.
- In all test, P value was considered significant when less than 0.05.

RESULTS

Results of the present study can be summarized in the following:

- Manifestations (symptoms and signs) of liver cirrhosis were present in the two groups of the study.
- All grades of hepatic encephalopathy were represented in hepatic encephalopathy group (A).
- As regarding symptoms halitosis and heart burn were significantly higher in hepatic encephalopathy group (A) than non-hepatic encephalopathy group (B).
- On the other hand, there was no significant difference between the studied groups as regard dyspepsia, abdominal distension, nausea, vomiting, abdominal pain and dysphagia.
- As regarding signs there is no significant difference between the studied groups.
- Ultrasonographic features of cirrhosis were present in the two studied groups with no significant difference between them.
- The mean values of serum T. Bilirubin in group (A) were significantly higher than that of group (B).
- There were no significant difference between ALT mean values in the studied groups.

- There were no significant differences between AST mean values in the studied groups.
- There were no significant differences between albumin mean values in the studied groups.
- There were no significant differences between prothrombin concentration mean values in the studied groups.
- Prevalence of *H. pylori* infection was significantly higher in group (A) than in group (B).
- Prevalence of *H. pylori* infection was significantly increasing with increasing the grade and severity of hepatic encephalopathy in group (A).
- The mean ammonia levels were significantly higher in group (A) than group (B).
- The mean ammonia levels were significantly rising with increasing the grade and severity of hepatic encephalopathy in group (A).

Table (1): Hepatic encephalopathy grading in group A (HE group) of the study

HE Grade	HE group	
	N	%
Grade I	14	28
Grade II	14	28
Grade III	12	24
Grade IV	10	20
Total	50	100

Analysis of the studied hepatic encephalopathy group revealed a 14(28%) of the patients were grade I hepatic encephalopathy, 14(28%) of the patients were grade II hepatic encephalopathy, 12(24%) of the patients were grade III hepatic encephalopathy and 10(20%) of the patients were grade IV hepatic encephalopathy.

Table (2): Clinical picture: (Symptoms) of the studied groups

Item	HE group(A) N=50	%	Non-HE group (B) N=50	%	Total N	%	P. Value
Dyspepsia	30	60%	26	52%	56	56%	>0.05 NS
Halitosis	22	44%	17	34%	39	39%	**<0.05 S**
Abdominal distension	40	80%	40	80%	80	80%	>0.05 NS
Nausea	9	18%	11	22%	20	20%	>0.05 NS
Vomiting	8	16%	9	18%	17	17%	>0.05 NS
Abdominal pain	10	20%	8	16%	18	18%	>0.05 NS
Heart burn	18	36%	12	24%	30	30%	**<0.05 S**
Dysphagia	9	18%	7	14%	16	16%	>0.05 NS

NS =not significant. S = significant.

There is a significant difference between hepatic encephalopathy group and non-hepatic encephalopathy group as regarding (halitosis and heartburn) while no significant difference as regarding the other symptoms.

Table (3): Clinical picture: (Signs) of the studied groups

Item	HE group (A) N=50	%	Non-HE group (B) N=50	%	Total N	%	P.Value
Pallor	18	36%	16	32%	34	34%	>0.05 Not significant
Jaundice	30	60%	27	54%	57	57%	
Lower limb oedema	40	80%	35	70%	75	75%	
Splenomegaly	41	82%	37	74%	78	78%	
Ascites	33	66%	36	72%	69	69%	
Spider nevie	30	60%	32	64%	62	62%	
Palmer erytrhema	28	56%	22	44%	50	50%	
Ecchymotic patches	33	66%	31	62%	64	64%	
Clubbing	25	50%	25	50%	50	50%	

There is no significant difference between hepatic encephalopathy group and non-hepatic encephalopathy group as regarding signs (pallor, jaundice, lower limb oedema, splenomegaly, etc…)

Table (4): Abdominal ultrasound findings in the studied groups

Item	HE group (A) N=50	%	Non-HE group (B) N=50	%	Total N	%	P Value
Liver Size:							**>0.05 Not significant**
Average	9	18%	11	22%	20	20%	
Enlarged	5	10%	8	16%	13	13%	
Shrunken	36	72%	31	62%	67	67%	
Portal Vein thrombosis	7	14%	6	12%	13	13%	
Echopattern:							
Homogenous	0	0%	0	0%	0	0%	
Bright	27	54%	34	68%	61	61%	
Cirrhotic	50	100%	50	100%	100	100%	
Liver surface: Irregular	50	100%	50	100%	100	100%	
Spleen:							
Average	7	14%	12	24%	19	19%	
Enlarged	43	86%	38	76%	81	81%	
Ascites:	35	70%	38	76%	73	73%	

A non-significant difference was noticed between HE group and non-HE groupas regardabdominal ultrasound findings (Liver Size, echo pattern, surface, etc……..)

Table (5): Biochemical profile of the studied groups

Item	HE group(A) N=50	Non-HE group(B) N=50	P. value
Total bilirubin	2.3±1.3	1.6±0.9	**<0.05 (S)**
ALT	61±23.2	51.24±21.8	**>0.05 (NS)**
AST	59.4±19.2	50.3±18.2	
Albumin	2.45±0.6	3.86±0.35	
Prothrombin concentration	67.3% ±6	72.1%±3.8	

✓ NS =not significant. S = significant

- Total bilirubin was significantly higher in hepatic encephalopathy group than non HE group.
- ALT and AST were not significantly high in HE group as compared with non HE group
- Albumin was not significantly lower in HE group than non HE group
- Prothrombin concentration was not significantly lower in HE group than non HE group

Table (6): *H. pylori* stool antigen results in patients with liver cirrhosis with and without hepatic encephalopathy

Item	HE group (A)		Non-HE group (B)		Total		P value
	N=50	%	N=50	%	N	%	
H. pylori positive	40	80%	30	60%	70	70%	<0.05 Significant
H. pylori negative	10	20%	20	40%	30	30%	<0.05 Significant

Prevalence of *H. pylori* infection in the studied groups (patients of liver cirrhosis with and without hepatic encephalopathy) was 70%, (liver cirrhosis with hepatic encephalopathy was 80% and liver cirrhosis without hepatic encephalopathy was 60%) i.e. prevalence of *H. pylori* infection is significantly high in HE group than non HE group (P Value < 0.05)

Table (7): Correlation between *H.pylori* prevalance and hepatic encephalopathy grading.

Grading	Number	%	*H. pylori* +ve	%	P. value
Grade I	14	28%	9	64%	<0.05 Significant
Grade II	14	28%	11	8%	
Grade III	12	24%	11	91%	
Grade IV	10	20%	9	90%	
Total	50	100%	40	80%	

Prevalence of *H. pylori* was higher in grade III more than other grades i.e. 64% of grade I were positive, 78% of grade II were positive, 91% of grade III were positive and 90% of grade IV were positive.

Prevalence of *H. pylori* was found significantly increasing with the severity of hepatic encephalopathy (Graph 2).

Table (8): Results of ammonia level in patients of liver cirrhosis with and without hepatic encephalopathy

Item	HE group (A) N=50	Non-HE group (B) N=50	P value
Ammonia level mmol/l	82.14± 47.9	36.44± 17.9	<0.05 Significant

The normal range is 15-45moml/l, there is significant increase of blood ammonia level in hepatic encephalopathy group 82.14 ± 47.9 moml/l, as compared with non hepatic encephalopathy group 36.44± 17.9 as described in Table 9.

The blood ammonia level was significantly increased in HE group than non- HE group (P value was<0.05 Significant).

Table (9): Relation between blood ammonia level and different grades of hepatic encephalopathy.

Grading	Number	%	Ammonia level	P.value
Grade I	14	28%	80.40± 5.63	<0.05 Significant
Grade II	14	28%	84.35± 3.2	
Grade III	12	24%	87± 3.9	
Grade IV	10	20%	91± 2.8	

There is asignificantly rising blood ammonia level in hepatic encephalopathy group with increasing the grade of hepatic encephalopathy, It was 80.40± 5.63 moml/l in grade I hepatic encephalopathy patients, 84.35±3.2 moml/l in gradeII hepatic encephalopathy patients, 87±3.9moml/l in grade III hepatic encephalopathy and 91±2.8 moml/l in grade IV hepatic encephalopathy patients (Graph 3).

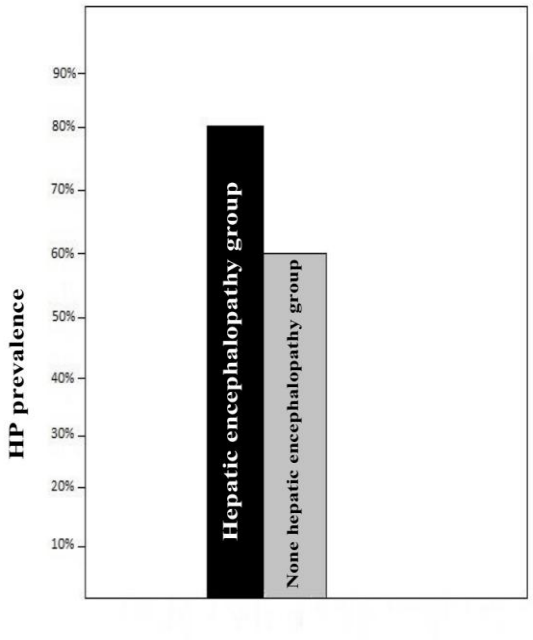

Figure (1): *H. pylori* prevalence in patients of liver cirrhosis with and without hepatic encephalopathy

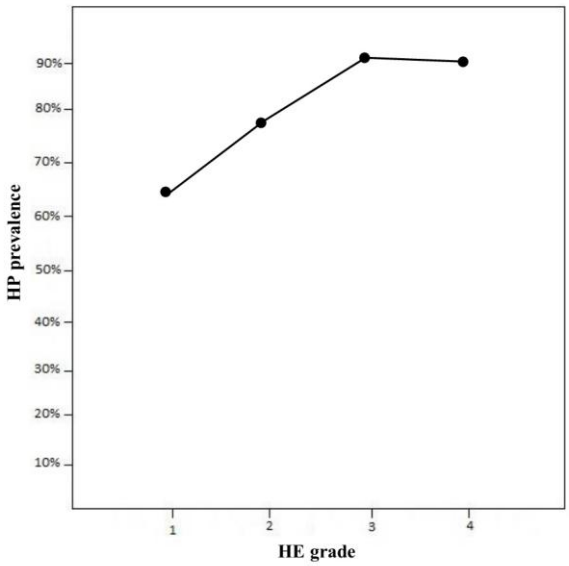

Figure (2): Correlation between H.pylori prevalence and hepatic encephalopathy grades

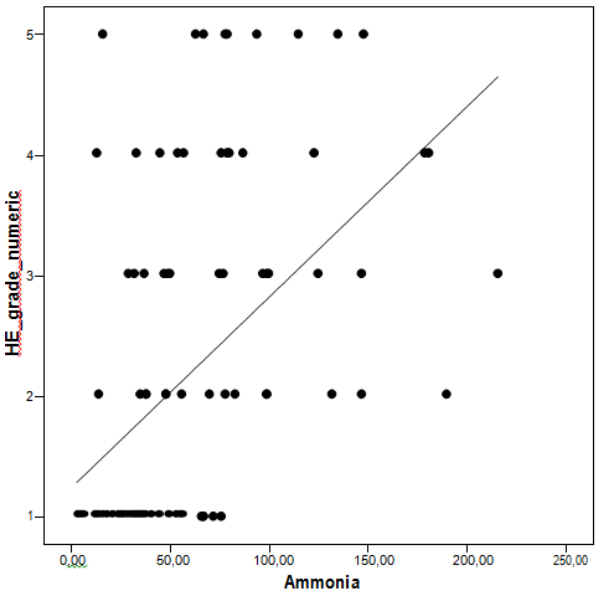

Figure (3): Correlation between hepatic encephalopathy grades and blood Ammonia level

DISCUSSION

Hepatic encephalopathy represents a reversible decrease in neurologic function linked to cirrhosis by several mechanisms; the explanation of which still remains to be fully elucidated [12].

One of the initial hypotheses advanced to explain hepatic encephalopathy pointed to a key role for nitrogenous by-products of protein breakdown absorbed from the colon and reversed into portal circulation. In the setting of portosystemic shunting, portal blood flow is diverted away from the liver into the vena cava, and hepatic encephalopathy could be due to the cytotoxic effect of ammonia, a key intermediatein nitrogen and protein metabolism produced by the normal intestinal bacterial flora [13].

In cirrhotic patients, *H. pylori* infection is associated with hepatic encephalopathy, especially in younger patients with decompensated liver disease [13].

Gastric *Helicobacter pylori* infection is believed to be associated with a higher risk of hepatic encephalopathy among patients with cirrhosis of liver [14].

H. pylori is known to possess a urease activity, and therefore, might be an important source for ammonia production. Hepatic encephalopathy, in certain settings, could deteriorate due to this enhanced amount of ammonia [15].

Infectious diseases are now being implicated frequently as the cause of diseases that have been considered previously to be of non-infectious aetiology. Infection by *H. pylori* is one of them [16].

H. pylori is also associated with coronary artery disease, cerebrovascular disease, growth retardation in children, and Raynaud's phenomenon [17].

The role of *H. pylori* as a cause of hyper-ammonaemia in patients with liver cirrhosis has still not been fully clarified [18].Few studies have investigated this issue and found that *H. pylori* infection was significantly more frequent in patients with encephalopathy than without [13].

In an interventional study, Zullo, et al. revealed a decrease in ammonia levels in patients treated by hydroxamic acid, an inhibitor of bacterial urease, while no changes were reported in the non-infected patients [19]. However, in a different situation, these were not confirmed by Vasconez et al. [16].

Siringo et al. reported a very high prevalence of *H. pylori* infection in cirrhotics, compared with blood donors ($p<0.0005$) in Italy [20].

In our study, we observed a significantly high prevalence of *H. pylori* among the hepatic patients with or without hepatic encephalopathy with higher prevalence in hepatic encephalopathy group (A) (80% positive), than non-hepatic encephalopathy group (B) (60% positive). Fan et al. found a higher seroprevalence of *H. pylori* in Chinese hepatic patients than in controls matched for age and socioeconomic status. The study was performed on 360 hepatic patients and 100 controls, the result was 72% seropositive in hepatic patients and 39% seropositive in control group [21].

In our study, there was a significantly higher prevalence of H. pylori and blood ammonia levels among the hepatic encephalopathy group (A) than non hepatic encephalopathy group (B). Prevalence of H. pylori was 80% positive in hepatic encephalopathy group (A) and 60% positive in non hepatic encephalopathy group (B), and mean blood ammonia levels were: 82.14 ± 47.9 mmol/l for group A (liver cirrhosis with hepatic encephalopathy), and 36.44 ± 17.9 mmol/l for group B (liver cirrhosis without hepatic encephalopathy). Our results were nearly similar to the results of Fan, in china which was 78% in hepatic encephalopathy group and 56% in hepatic patients without hepatic encephalopathy [21].

Sethar et al. reported that the frequency of *H. pylori* antibodies in patients presenting with porto-systemic encephalopathy due to liver disease is higher than control group .Seventy-six patients of porto-systemic encephalopathy due to liver diseases were selected . They were evaluated for hepatic encephalopathy grade, out of 76 patients who presented with porto-systemic encephalopathy, 59 (77.6%) had a positive H. pylori antibody test [22].

A study performed by Si J et al. in China to determine whether liver cirrhosis associated with *H. pylori* infection will induce increased serum ammonia. The mean levels of serum ammonia in the cirrhotic group with *H. pylori* infection were 142.2 +/- 13.35 mmol/L. They were increased significantly as compared with cirrhotic patients without *H. pylori* infection (37.23 +/- 7.04 mmol/L), so *H. pylori* infection can induce an

increase in serum ammonia in patients with liver dysfunction [23].

The results of our study are in agreement with their results, as there was a positive correlation between prevalence of *H. pylori* and hepatic encephalopathy. As well as prevalence of *H. pylori* and blood ammonia levels were found significantly increasing with the severity and degree of hepatic encephalopathy, which suggests that *H. pylori* infection may have a role in the pathogenesis of hepatic encephalopathy.

These findings do not correlate with the results of Guillermo et al. In their study they found H. pylori seropositivity in hepatic encephalopathy grade I (77.63%), grade II (78.13%), grade III (100%), grade IV (75%) [24].

In a study performed by Yang et al., three hundred and sixty-eight cirrhotic patients were enrolled. They observed that *H. pylori* infection aggravates the blood ammonia concentration and hepatic encephalopathy in cirrhotic patients. This study correlates with our study where there was a positive correlation between prevalence of *H. pylori* and hepatic encephalopathy [25].

A systemic review performed by Bang-Li Hu et al. surveyed twenty studies that explored the role of *H. pylori* in HE pathogenesis, eleven of which evidenced that the prevalence of *H. pylori* is higher in HE patients,while the evidence of nine studies failed to find that blood ammonia level was higher in *H. pylori* positive cirrhotic patients than in negative patients [26].

Chakrabarti et al. found no significant correlation between gastric juice ammonia concentrations and arterial ammonia levels in the study of 66 hepatic patients. The data suggest that liver impairment remains conclusive in ammonia disposal in patients with cirrhosis, whereas *H. pylori* infection does not seem to play a major role in the pathogenesis of hyperammonemia in these patients [27].

Also, in the study performed by Zullo et al. in Italy, who focused on the relationship between *H. pylori*, plasma ammonia levels, and intellectual function in cirrhotic patients. Forty-seven cirrhotics with latent or mild hepatic encephalopathy were enrolled in the study. This study failed to find a relationship between *H. pylori*, plasma ammonia levels, and psychometric testing scores in cirrhotic patients with latent or mild hepatic encephalopathy [28].

The reduction in blood ammonia levels after *H. pylori* eradication found in some studies has been attributed to a nonspecific effect of antibiotic therapy on ammonia producing gut flora rather than *H. pylori* eradication [29].

CONCLUSION

In conclusion; although colonic bacteria are considered to be the main source of ammonia and the major pathogenic factor for hepatic encephalopathy, *H. pylori* may be an important co-factor for pathogenicity of hepatic encephalopathy, based on being more prevalent in patients with chronic liver disease especially with hepatic encephalopathy.

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30.

Mean Platelet Volume as a Fibrosis Marker in Patients with Chronic Hepatitis C

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Key words:
MPV, chronic hepatitis C, fibrosis

Background and study aim: Liver biopsy limitations push us to search for new non invasive methods to detect liver fibrosis such as serum markers. The aim of this study is to evaluate mean platelet volume (MPV) as a fibrosis marker in patient with chronic hepatitis C.

Patients and methods: 150 patients diagnosed with chronic hepatitis C infection refereed to Tanta Fever Hospital in period from May 2013 to January 2014 and 20 healthy volunteers as a control were included. All of them were tested for Mean Platelet Volume (MPV) in comparison with who done liver biopsy as standard.

Results: Statistically significant differences in MPV and Platelet Count were seen in patients with chronic hepatitis C (CHC) compared to healthy controls (MPV: 8.95 ± 1.39fL vs. 7.57 ± 0.68 fl, P-value = 0.043; PC 226.03 ±68.36 vs. 188.9±46.49, P-value = 0.02)

Multi-variate Logistic regression analysis shows only 5 variables remained as independent risk factors for fibrosis progression: (MPV, Schistosomiasis, ALT, AST and Prothrombin time).

AST (OR 1.11, 95% CI 1.02 to 1.21), ALT (OR 0.92, 95% CI 0.86 to 0.99), PT (OR 2.11, 95% CI 1.15 to 3.88), and MPV (OR 2.28, 95% CI 1.22 to 4.25).

Cut-off values were calculated for diagnostic performance, and the cut-off value for MPV was 9.22 fl., sensitivity 75.5%, specificity 62%, PPV 40.3%, NPPV 93.4% and Accuracy rate 61.8%

Conclusion: We suggest that high MPV levels (especially those over 9.22 fl) may help to predict advanced fibrosis in patients with CHC .However, it should not be forgotten that MPV is not a specific marker for fibrosis, and the negative predictive rate seems more valuable to exclude a high fibrosis ratio in patients with CHC.

INTRODUCTION

Hepatitis C virus infection, with an estimated prevalence of more than 170 million people infected worldwide, is a major health problem[1] Prevalence rates reach up to 10-20% in parts of central Africa and Egypt[2] [3].

HCV infection and its complications represent major public health problem in Egypt, where 10%- 15% (about 9 million) of the general population is infected [4].

Monitoring of liver fibrosis progression is important in patients with chronic hepatitis C, not only because it prompts screening for HCC, but also those patients have the most urgent need for antiviral therapy[5].

Liver biopsy has been considered the gold standard and an in-dispensable reference method for therapeutic decisions regarding CHC, as treatment indication is based on histological findings including inflammatory grading and staging [6].

However, liver biopsy problems can limit its application as diagnostic procedure such as sampling errors and intra and inter observer variabilities [7] In addition; liver biopsy is an invasive and painful procedure, bleeding, biliary peritonitis, and pneumothorax and mortality range from 0.01% - 0.1%. In additional liver biopsy is contraindicated in the presence of coagulopathy, thrombocytopenia, and ascites [8]

These limitations push us to search for new non-invasive approaches such as serum markers of hepatic fibrosis examples are: AST/ALT Ratio, AST to Platelet Ratio Index (APRI score), Fibrotest and Actitest, PGA Index, Forns Index and Hepascore [9] and new imaging techniques (fibroscan) [10].

Platelet volume and its mean (MPV) is an indicator of platelet function, activity and aggregation capacity [11].

High Mean platelet volume levels (especially those over 8.4 fL) may help to predict advanced fibrosis in patients with chronic hepatitis C [12].

PATIENTS AND METHODS

This study was conducted on about 150 patients selected from 172 patients diagnosed with chronic hepatitis C infection who were refereed to Tanta Fever Hospital in period from May 2013 to January 2014. They were 87 males (58%) and 63 females (42%) and their age ranged from 18 years to 59 years with mean age of (41.61 ± 7.79) and 20 healthy volunteers as a control.

They were categorized into 2main groups: Group 1(patients group): 150 patients with CHC which subdivided according to liver biopsy METAVIR system into 4 subgroups: F0/F1: no fibrosis/portal fibrosis without septa (17 patients), F2: portal fibrosis with rare septa (82 patients), F3: numerous septa without cirrhosis (33 patients) and F4: cirrhosis (18 patients) .Group 2 (control group): 20 healthy volunteers as a control group. An informed consent was obtained before patients enter the study.

Chronic HCV infection was confirmed by detectable HCV-Ab by ELISA ≥ 6 months and serum HCV-RNA positivity by PCR.

With the following inclusion criteria: Age: 18-60 years, Proven HCV infection by HCV Ab and HCV RNA ≥6 months, compensated liver disease, BMI <30.

About 22 patients were excluded from the study because they had one or more of the following exclusion criteria: Co-infection with hepatitis B virus, other causes of liver disease, pregnancy for female, decompensated liver disease, diabetes mellitus, arthritis or any collagen disease, chest disease namely sarcoidosis and suppurative lung disease, liver transplantation, anticoagulant treatment and patients who had received specific antiviral therapy prior to study.

All patients will be subjected to the following: Full history taking, complete clinical examinations, laboratory tests (complete blood count, liver function tests, prothrombin time, renal function tests, schistosomal ab by ELISA, autoimmune markers (ANA), alpha-feto- protein and TSH), abdominal-pelvic ultrasound, HCV RNA by quantitative PCR, liver biopsy for histological examination and quantification of liver fibrosis and inflammation and measuring the mean platelet volume: is calculated by the following formula: MPV (FL)= [(platelet (%)/ Platelet count ($\mathbf{x}10^9$/L) or computerized calculation by complete blood counters (histogram) .

Statistical analysis

Data were collected, tabulated and statistically analyzed by computer using SPSS version 16. The following tests; arithmetic mean, standard deviation (SD), standard student "t test", Chi square Test (X^2), sensitivity, specificity, accuracy, positive predictive value ,negative predictive value, linear correlation coefficient (r) ,Roc curve (Receiver operating characteristic curve), significance of results (P value) to evaluate mean platelet volume as a fibrosis marker in patient with chronic hepatitis C.

RESULTS

Statistical comparison between the HCV patients GI (Patients group) and G II (Control group) shows no significant differences as regard age and the gender as shown in table (1). Statistical comparison between the HCV patients GI (Patients group) and G II (Control group) shows no significant differences as regard HB%,RBCS and WBCS, but it showed significant differences regarding MPV and Platelet count as shown in table (2) .Statistical comparison between the HCV patients GI (Patients group) and G II (Control group) shows no significant differences as regard total bilirubin, direct bilirubin, ALT, AST, alkaline phosphate and serum albumin but it showed significant differences regarding Prothrombin time as shown in table (3). Statistical comparison between the HCV patients GI (Patients group) and G II (Control group) shows no significant differences as regard creatinine and urea as shown in table (4). Statistical comparison between the HCV patients GI (Patients group) and G II (Control group) shows no significant differences as regarding random blood sugar (RBS), alpha feto protein (AFP), anti nuclear antibody titre (ANA) and thyroid

stimulating hormone (TSH) as shown in table (5). Statistical comparison between the HCV patients GI (Patients group) and G II (Control group) showed significant differences as regarding presence of the schistosomal antibodies as shown in table (6). Statistical comparison between the HCV patients GI (Patients group) and G II (Control group) showed significant differences as regarding ultrasound findings of the liver shown in table (7). Statistical analysis between HCV patients (group I) with different degrees of fibrosis (F0/F1-F2-F3-F4) regarding age, sex and PCR level of HCV RNA shows no significant differences between them., but show significant differences with MPV-platelets counts-schistosomiasis-ultra sounds findings- ALT- AST- prothrombin time as shown table (8). Uni-variate Logistic regression analysis shows association between different degrees of the fibrosis (F0/F1-F2-F3-F4) with MPV- platelets counts- schistosomiasis -ultra sounds findings- ALT- AST- prothrombin time. Multi-variate Logistic regression analysis shows only 5 variables remained as independent risk factors: (MPV, schistosomiasis, ALT, AST and prothrombin time.) as shown in table (9). As shown in the table, where 5 independent risk factors to of fibrosis are observed from all aspect of Area Under the Curve (AUC) , Cut Off Point (COP), sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy rate (AR). As shown in table (10) Comparison of Receiver Operator Characteristic Curves (ROC) for the diagnostic performance of ALT, AST, P.T and MPV in identifying fibrosis in chronic hepatitis C as shown in figure (1). A plot to obtain cut off value of MPV that displays sensitivity and specificity variation for each MPV value as shown in figure (2). A dot diagram that plots the distribution of CHC samples with different degrees of fibrosis (F0/F1-F2-F3-F4) around an 9.22 FL cut off value as shown in figure (3).

Table (1): Statistical comparison of the age and the gender between the studied groups

		GI (Patients group)		G II (Control group)		Total		X^2 test	P value
		No	%	No	%	No	%		
Age (years)	>40y	86	57.3	7	35	93	55	1.06	0.14 NS
	≤ 40y	64	42.7	13	65	77	45		
	Total	150	100	20	100	170	100		
Sex	Male	87	58	15	75.0	102	60	1.97	0.16 NS
	Female	63	42	5	25.0	68	40		
	Total	150	100	20	100	170	100		

NS, non significant

Table (2): Statistical comparison of the results of complete blood picture between studied groups (HB%-RBCS-WBCS-MPV- Platelets)

Variable	GI (Patients group)		G II (Control group)		Student t test	P value
	Mean	± SD	Mean	± SD		
HB% (N= 12-16 gm/dL)	14.096	1.73	13.23	1.62	2.112	0.076 NS
RBCs ($N = 4.5 - 6.5 \times 10^6$/mm)	5.19	0.65	5.2	0.88	0.05	0.96 NS
WBCs ($N = 4 - 11 \times 10^3$/mm)	6.9	1.81	6.91	1.47	0.011	0.99 NS
MPV(6.5- 11.5 Famtolitre (F.L) *femtolitre = 10^{-15}litres	8.95	1.39	7.57	0.68	4.38	0.043 S
Platelets count ($150 - 450 \times 10^3$/mm)	188.9	46.49	226.03	68.36	2.36	0.020 S

S, significant, NS, non significant

Table (3): Statistical comparison of the results of liver function tests between studied groups (T.BIL-D.BIL-ALT-AST- Alk. Ph-S.alb- P.T)

Variable	GI (Patients group)		G II (Control group)		Student t test	P value
	Mean	± SD	Mean	± SD		
Total bilirubin(mg/dl) (N=0.2-1.2mg/dl)	0.67	0.26	0.64	0.26	0.491	0.624 NS
Direct bilirubin(mg/dl) (N = upto 0.25 mg/dl)	0.222	0.11	0.226	0.14	0.127	0.899 NS
ALT (U/L) (up to 35)	56.59	46.8	42.8	12.75	1.31	0.048 NS
AST (U/L) (up to 41)	44.69	28.39	38.35	8.22	0.99	0.035 NS
Alkaline phosphate(U/L) (40-129 male /35-104 female)	102.49	58.13	129.1	85.74	1.81	0.073 NS
S. Albumin gm/dl (N=3.5-5.4)	4.36	0.36	4.33	0.39	0.397	0.692 NS
Prothrombin time (N=11:14 sec.)	12.77	0.60	12.83	0.55	0.43	0.037 S

S, significant, NS, non significant

Table (4): Statistical comparison of the results of renal function tests between studied groups (creatinine- urea)

Variable	GI (Patients group)		G II (Control group)		Student t test	P value
	Mean	± SD	Mean	± SD		
Creatinine (n=0.5-1.5) mg/dl	0.795	0.15	0.74	0.14	1.64	0.103 NS
Urea (n=15-45) mg/dl	24.54	5.5	22.0	4.14	1.43	0.155 NS

NS, non significant

Table (5): Statistical comparison of the results of (RBS-AFP-ANA-TSH) between studied groups

Variable	GI (Patients group)		G II (Control group)		Student t test	P value
	Mean	± SD	Mean	± SD		
Random blood sugar(RBS) (N= up to 140mg/dl)	101.21	37.3	103.2	27.21	0.231	0.818 NS
AlphaFetoProteins (AFP) (Up to 10 ng/ml)	5.22	9.76	3.18	4.16	1.79	0.074 NS
AntiNuclear Antibody titre(ANA) (Up to 14 u/ml)	8.58	2.49	7.96	2.17	1.07	0.285 NS
Thyroid Stimulating Hormone (N=0.27-4.2 uIU/ml)	2.67	10.85	1.52	0.77	1.41	0.16 NS

NS, non significant

Table (6): Statistical comparison of the results of Schistosomal antibodies between studied groups

Schistosomal antibodies	GI (Patients group)		G II (Control group)		Total		X^2 test	P value
	No	%	No	%	No	%		
Present	99	66%	9	45%	111	65.3	4.12	0.042 S
Absent	51	34%	11	55%	59	34.7		
Total	150	100	20	100	170	100		

S, significant

Table (7): Statistical comparison of the ultrasonographic findings of the liver between the studied groups

Ultra sound finding of liver	GI (Patients group)		G II (Control group)		Total		X^2 test	P value
	No	%	No	%	No	%		
Normal	53	35.3%	9	45%	61	35.90%	4.12	0.027 S
Fine periportal fibrosis	52	34.7%	8	40%	56	32.90%		
Coarse periportal fibrosis	22	14.7%	1	5%	24	14.10%		
Bright fatty liver	23	15.3%	2	10%	27	15.90%		
Total	150	100	20	100%	170	100%		

S, significant

Table (8): Statistical comparison between case groups regarding the different degrees of fibrosis (f0/f1-f2-f3-f4)

		F0-F1 N= 17	%	F2 N=82	%	F3 N=33	%	F4 N=18	%	X^2	P value
Age	>40y	13	76.47	43	52.43	22	66.66	10	55.55	1.325	0.362
	≤40y	4	23.52	39	47.56	11	33.33	8	44.44		
sex	Male	11	64.70	45	54.87	18	54.54	13	72.22	1.693	0.421
	Female	6	35.29	37	45.12	15	45.45	5	27.77		
MPV	> 8.5 FL	6	35.29	44	53.65	20	60.60	14	77.77	5.626	0.001
	≤ 8.5FL	11	64.70	38	46.34	13	39.39	4	22.22		
Plateets counts Normal (150-450) $\times 10^3 cmm$	Normal	15	88.23	76	92.6	20	60.60	10	55.55	2.325	0.014
	<150 $\times 10^3 cmm$	2	11.76	4	4.87	4	12.12	7	38.88		
	>450 $\times 10^3 cmm$	0	0	2	2.43	0	0	1	5.55		
Schistosomiasis	Positive	9	52.94	55	67.07	21	63.63	14	77.77	3.325	0.013
	Negative	8	47.05	27	32.92	12	36.36	4	22.22		
Ultra sounds finding	Normal	5	29.41	33	40.2	11	33.33	3	16.66	4.526	0.024
	Fine periportal fibrosis	7	41.17	28	34.14	15	45.45	5	27.77		
	Coarse periportal fibrosis	3	17.64	9	10.97	5	15.15	9	50		
	Bright fatty liver	2	11.76	12	14.63	3	9.09	1	5.55		
ALT	>39.5	9	52.94	51	62.19	22	66.66	11	61.11	3.258	0.022
	≤39.5	8	47.05	31	37.80	11	33.33	7	38.88		
AST	>38.5	8	47.05	49	59.7	23	69.69	8	44.44	4.619	0.035
	≤38.5	9	52.94	33	40.2	10	30.30	10	55.55		
Prothrombin time	>12.65	10	58.82	35	42.68	19	57.57	9	50	6.253	0.001
	≤12.65	17	100	47	57.31	14	42.42	9	50		
PCR OF HCV RNA	>MILLION	8	47.05	36	43.90	18	54.54	12	66.66	0.247	0.526
	≤MILLION	9	52.94	46	56.09	15	45.45	6	33.33		

Table (9): Correlation of variable data with degree of fibrosis and Logistic (Univariate and multivariate) regression analysis

Variables	Fibrosis degree (f0/f1-f2-f3-F4)		
	P- VALUE	Logistic regression analysis	
		Univariate OR* (95% CI**)	Multivariate OR (95 %CI)
Age	0.362 NS	1.02 (0.97-1.07)	--------
Sex	0.421 NS	1.75 (0.67-4.59)	--------
MPV	0.001 S	1.56 (1.07-2.27)	2.28 (1.22-4.25)
Platelets counts	0.014 S	0.98 (0.97-0.99)	------
Schistosomiasis	0.013 S	1.04 (0.99-1.08)	1.30 (1.15:2.20)
Ultra sounds finding	0.024 S	0.98 (0.93-1.03)	--------
ALT	0.022 S	1.02 (1.00-1.03)	0.92 (0.86-0.99)
AST	0.035 S	1.03 (1.01-1.05)	1.11 (1.02-1.21)
Prothrombin time	0.001 S	1.97 (1.28-3.04)	2.11 (1.15-3.88)
PCR OF HCV RNA	0.526 NS	0.58 (0.31-1.09)	--------

*OR: Odd Ratio **CI: Confidence interval

Table (10): Diagnostic measures of parameters in detection of liver fibrosis

Parameters	Diagnostic measures						
	Area Under Curve (AUC) %	Cut Off Point (COP) %	Sensitivity (Sen.) %	Specificity (Spec.) %	Positive Predicte Value (PPV) %	Negatie Predictive Value (NPV) %	Accuracy Rate (AR) %
ALT	0.547	39.5	59.3	75.0	40.9	87.1	68.2
AST	0.528	38.5	53.3	66.0	44.9	88.5	72.9
P.T	0.454	12.65	50.0	80.0	52.9	83.4	76.8
MPV	0.598	9.22	75.7	62.0	40.3	93.4	61.8

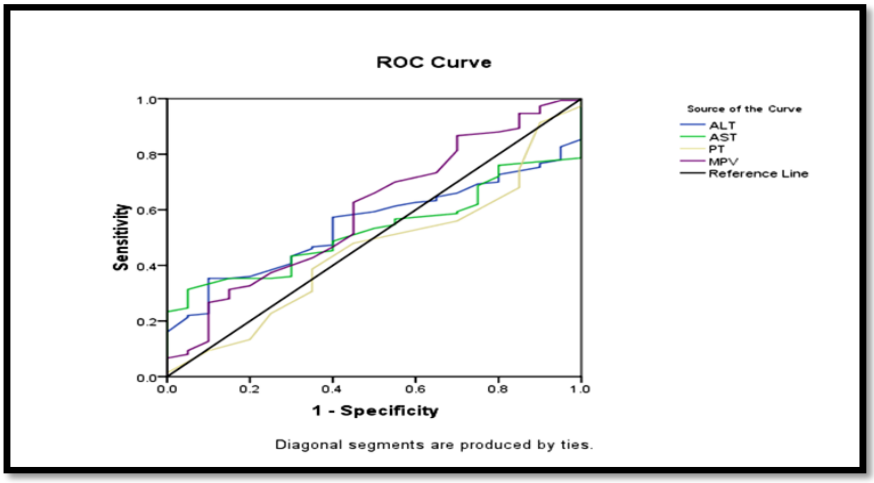

Figure (1): Comparison of Receiver Operator Characteristic curves (ROC) for the diagnostic performance of ALT, AST, P.T and MPV in identifying fibrosis in chronic hepatitis C.

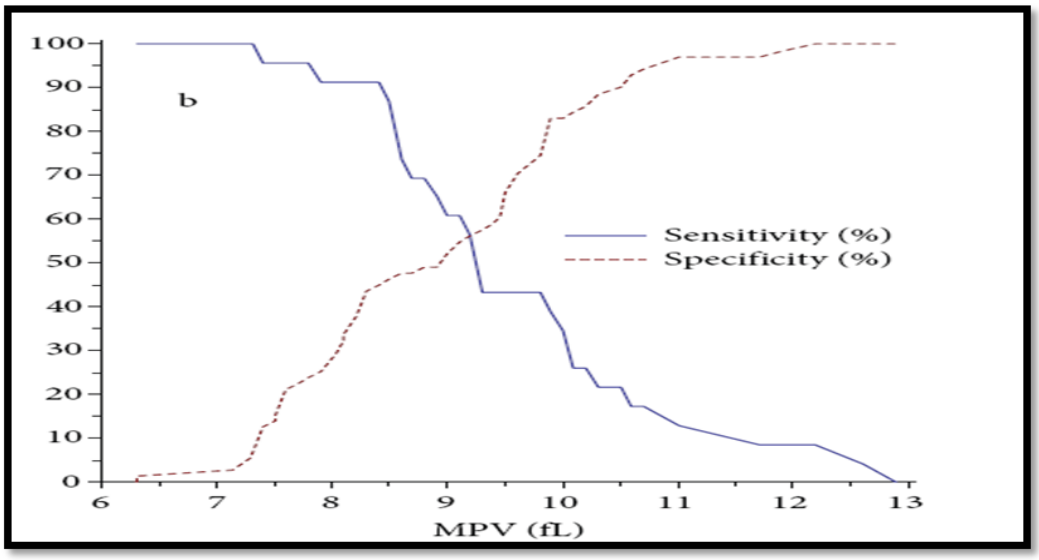

Figure (2): A plot to obtain cut off value of MPV that displays Sensitivity and specificity variation for each MPV value.

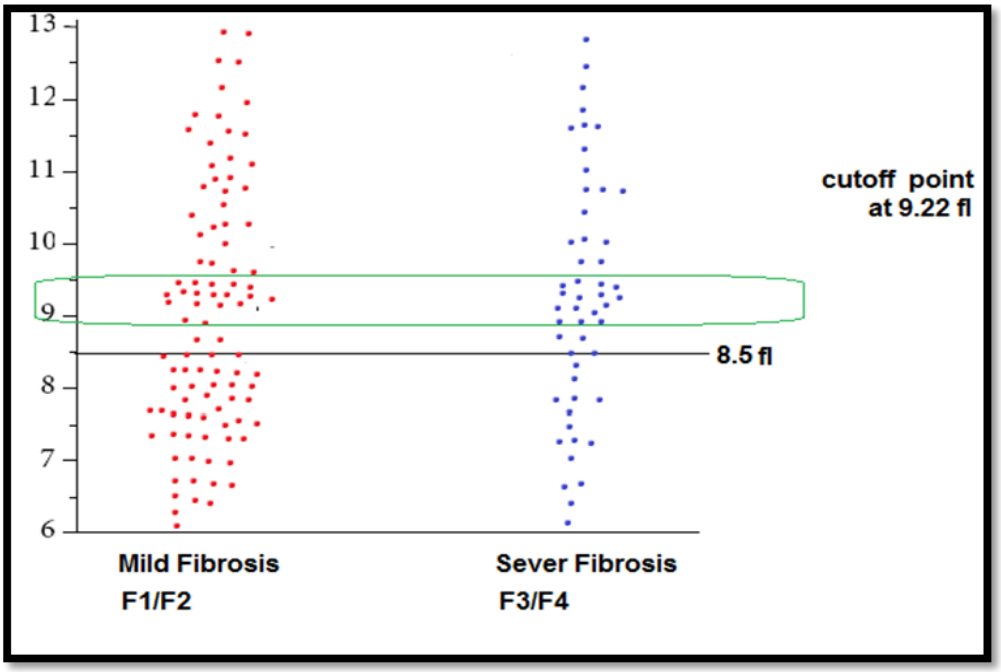

Figure (3): A dot diagram that plots the distribution of CHC samples with different degrees of fibrosis (F0/F1-F2-F3-F4) around an 9.22 FL cut off value.

DISCUSSION

Monitoring of liver fibrosis progression is important in patients with chronic hepatitis C, not only because it prompts screening for HCC, but also those patients have the most urgent need for antiviral therapy [5]

In this study, statistical analysis revealed no significant difference between the studied groups as regard age and sex and that disagrees with Poynard et al. [13] who found an increased rate of fibrosis if the age at infection was > 40 years and if sex was male and also with Ahmad et al. [14] who found that liver fibrosis stages increase with age increasing.

The present study showed non-significant differences between studied groups as regarding HB%-RBCS-WBCS-RBS where p-value >0.05 while it showed significant differences regarding platelets count (where p-value <0.05)

In this study, statistical comparison of studied groups showed no significant differences regarding total bilirubin, direct bilirubin and alkaline phosphate (where p-value >0.05), but showing significant differences regarding ALT and AST, (where p-value <0.05). This is in agreement with Ahmad et al. [14] who found that ALT and HB % were not significant, while AST levels were good to differentiate liver fibrosis stages, they also found that viral load, bilirubin, ALP, AST, serum albumin and platelet count were significantly associated with various fibrosis stages.They concluded that as the fibrosis increased to cirrhosis, bilirubin and serum ALP level also increased, while platelet count and serum albumin level gradually reduced so, construction of a new index for the prediction of fibrosis stage based on the relationship four-biochemical markers, ALP, bilirubin, albumin and platelet count, they developed a new fibrosis-cirrhosis index for the prediction of HCV disease progression from initial fibrosis stage to end stage cirrhosis., it can be represented as: FCI = (ALP × Bilirubin) / (Albumin × Platelet count) [14]

Peck-Radoslavljevic [15] showed that low platelet count (thrombocytopenia) is a valuable marker of advanced liver disease, but it may be related to many mechanisms : hypersplenism, myelo-suppression by HCV, decreased thrombopoetin production, autoimmune.

Chun et al. [16] Showed that severity of liver fibrosis was correlated significantly with a gradual increase in AST level as well as a decrease in platelet count, and that is called AST to platelet ratio index (APRI):

$$\text{APRI} = \frac{\text{AST level (/ULN)}}{\text{Platelet counts } (10^9/\text{L})} \times 100$$

Muzzi et al. **[17]** found that patients with fibrosis were older, had higher levels of fasting glucose, higher levels of fasting insulinemia, a higher HOMA score and had higher Metavir activity score and more steatosis than patients without fibrosis.

Gordon et al. [18] found that assay of AST levels had a stronger correlation than ALT with hepatic fibrosis.

Giannini et al. [19] found that the increase in AST levels is related to mitochondrial dysfunction and to reduced clearance of AST by hepatic sinusoidal cells. Reversal of AST/ALT was reported in patients who progress from chronic hepatitis to liver cirrhosis and the AST/ALT ratio of more than 1 had a good predictive value for advanced fibrosis.

Giannini et al. [19] found that an AST/ALT ratio had also a predictive value with ratio greater than 1.16 in identifying cirrhotic patients who died within 1 year follow up and had 81.3% sensitivity and 55.3% specificity.

Mustafa et al. [20] found that an inverse relationship between indirect bilirubin levels and advanced liver fibrosis caused by CHC genotype 1b.

Imbert-Bismut et al. [21] concluded that bilirubin may be used as marker of liver injury, while a change in ALP levels greater than 120 U/L can be indicative of advanced disease progression., These findings suggest that serum ALP and bilirubin may be used as serum markers to assess the disease progression and fibrosis stages in chronic HCV patients.

Murawaki et al. [22] and Lackner et al. [23] concluded that platelets not only predict fibrosis but also correlate with fibrotic stages .

Many studies supported that platelet count alone may be clinically valuable as a non-invasive serum marker for liver fibrosis and cirrhosis [24,25].

In this study statistical comparison between the studied groups showed no significant differences regarding serum Albumin, AFP, ANA and TSH (where p-value >0.005), but it showed significant differences regarding Prothrombin time (p-value

<0.05).This is in agreement with Croquet et al. [26] who noted that Prothrombin time (PT) as an index that reflects the synthesis capacity of the liver is one of the earliest indicators of liver cirrhosis and advanced fibrosis.

Hu et al. [27] showed that in patients with chronic hepatitis C , 23% of them had elevated serum AFP that is independently associated with stage III/IV hepatic fibrosis, elevated level of AST, and prolonged INR, where also serum AFP level of 15.0 µg/L was 22.8% sensitive and 94.5% specific for stage III/IV fibrosis.

In this study, statistical analysis revealed no significant differences between cases group and control group regarding serum creatinine and blood urea (where p-value > 0.05).Serra et al. [28] and Giannini et al. [29] found that Serum creatinine is increasingly being incorporated into prognostic models for patients with decompensated cirrhosis. In general, Creatinine and urea clearance are used to estimate glomerular filtration rate. using Creatinine based methods to estimate GFR in advanced liver disease patients is problematic for multiple reasons. Decline in hepatic functional capacity results in decreased creatine production and lower serum creatinine levels. Advanced liver disease patients are known to have less skeletal muscle mass, resulting in diminished creatine storage and less conversion of creatine to creatinine. All of these factors lead to a decreased serum creatinine level in advanced liver disease patients, making creatinine an unreliable factor in estimating GFR [30].

In this study statistical comparison between studied groups showed significant differences regarding schistosomiasis (p–value <0.05).Andrade [31] showed that schistosomasis invariably results in liver fibrosis of the host. This fibrosis may be represented by small focal areas of chronic inflammation and excess extracellular matrix deposited in periovular granulomas, distributed in variable numbers at the periphery of the portal vein system. This is the outcome of 90% of the infected population in endemic areas. Thus, host–parasite interactions in schistosomiasis help us to understand a number of important features of liver fibrosis: its initiation and regulation, the significance of accompanying vascular changes, the dynamics of fibrosis formation and regression with anti-parasitic treatment; host genetic and immunological contributions.

Kamal et al. [32] reported that HCV/schistosomiasis co-infected patients have more rapid progression of hepatic fibrosis than those with HCV mono-infection.

In contrast Ahmad et al. [33] showed that schistosomiasis co-infection with HCV and/or non-alcoholic steatohepatitis had no significant impact on fibrosis stage. Mahasen et al. [34] showed that positive schistosomal serology has no effect on fibrosis stage but it is significantly associated with failure of response to HCV treatment despite anti-schistosomal therapy.

Andrade [35] and Blanton et al. [36] showed that several clinical and pathological studies have shown that schistosomal hepatopathy is a reversible condition and that resolution of the schistosomiasis disease is accompanied by subsequent fibrosis resorption.

In this study statistical comparison between studied groups showed significant sensitivity of ultrasound (p–value <0.05).Chih-Ching et al. [37] concluded that routine clinical ultrasound is a not a sensitive predictor of early fibrosis in chronic viral hepatitis. Surface nodularity is the most sensitive sonographic feature for the detection of significant fibrosis and routine clinical ultrasound is the most useful for the detection of cirrhosis.

Bonekamp et al. [38] and Fontana and Lok [39] reported that Ultrasound is easily accessible in most health-care centers, making it the most commonly used imaging technique to evaluate chronic liver disease. Previous studies have demonstrated that ultrasound can predict liver cirrhosis or significant fibrosis.

Mathiesen et al. [40] and Nishiura et al. [41] concluded that the reasons for the low sensitivity and accuracy of ultrasound may be due to many factors. The pattern of fibrosis affects the extent of nodularity and echogenicity, and may account for the differences in the diagnostic performance seen between hepatitis B- and hepatitis C-related cirrhosis on ultrasound., However, the complex pattern of changes in chronic liver disease that is reflected in histopathology includes mixed features of steatosis, necrosis, and inflammation. These may affect the morphological appearance of the liver on ultrasound, rather than the presence of fibrous tissue alone.

Bonekamp et al. **[38]** demonstrated that wide range of ultrasound parameters and variable recommended algorithms reflect the limitations

of ultrasound, including operator dependency and limited accuracy in the staging of fibrosis. Currently, transient elastography (Fibroscan) and magnetic resonance elastography (MRE) provide the most reliable results in predicting fibrosis. However there is a need for larger longitudinal studies to define standardized diagnostic criteria for staging fibrosis with reproducible results before a noninvasive imaging technique can replace liver biopsy.

In this study statistical comparison between studied groups from aspect of degree of fibrosis and PCR level (HCV RNA) revealed no significant difference.This is in disagreement with Ahmad et al. [14] who showed that viral load was significant among fibrosis stages. It gradually increased in advanced fibrosis, and then suddenly dropped in cirrhosis.

In this study Statistical comparison between studied groups from aspect of degree of fibrosis and MPV showed significant difference (p-value <0.05) and positive correlation and cut off point at 9.22 fl, sensitivity (75.7%), specificity (62%) NPV (93.4%), PPV(40.3%) and AR (61.8%).This is in agreement with Karaman et al. [12] who found that MPV was significantly higher in patients with CHC when compared to control subjects. In contrast, PC was significantly lower in CHC patients. Portal hypertension and hyper-splenism in some of the subjects with advanced fibrosis may be the cause of this significant difference. And also suggest that high MPV levels (especially those over 8.4 fL) may help to predict advanced fibrosis in patients with CHC. However, it should not be forgotten that MPV is not a specific marker for fibrosis and a high NPR (Negative Predictive Rate) seems to be more important in helping to exclude a high fibrosis ratio in patients with CHC.

CONCLUSION

- Statistical analysis between HCV patients (group I) with different degrees of fibrosis (F0/F1-F2-F3-F4) regarding age ,sex and PCR level of HCV RNA showed no significant differences between them, but showed significant differences with MPV- Platelets counts- Schistosomiasis -Ultra sounds findings- ALT- AST- Prothrombin time.
- Uni-variate Logistic regression analysis showed association between different degrees of the fibrosis (F0/F1-F2-F3) with (MPV- Platelets counts-Schistosomiasis-Ultra sounds findings- ALT- AST- Prothrombin time). Multi-variate Logistic regression analysis shows only 5 variables remained as independent risk factors: (MPV, Schistosomiasis, ALT, AST and Prothrombin time).
- The results of this study suggest that high MPV level especially ≥9.22 fl as a cutoff point, may help to predict advanced fibrosis in patients with CHC. However, it should not be forgotten that MPV is not a specific marker for fibrosis where sensitivity (75.7%) and specificity (62%), PPV (40.3%) and AR (61.8%) and also high NPV (93.4%) seems to be more important in helping to exclude a high and fibrosis degree in patients with CHC.

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Monitoring Hepcidin Level in Chronic Hepatitis C Virus Patients during Therapy

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Hepcidin, HCV, Hepatitis, Interferon

Introduction and study aim : Egypt has the highest prevalence of hepatitis C in the world estimated about 15%. There are several host and viral factors that aid in predicting response to treatment, Hepcidine hormone is being investigated as one of these host factors.

The aim of the work is to assess the serum concentration of hepcidin in chronic hepatitis C patients and evaluate any possible association with the viral load during therapy.

Patients and methods: This study was carried on 35 chronic HCV patients on peg IFN/ Ribavirin therapy and 15 chronic HCV patients not on therapy as a control group.

Hepcidin hormone levels were measured in sera of patients before starting therapy (base line) then at 12 and 24 weeks during therapy. RT PCR was used to asses response to ongoing therapy.

Results: The level of hepcidin in all cases was low before starting therapy and it showed a significant increase during the course of therapy. This rise was detected earlier in responding cases. A negative correlation was found between baseline hepcidin level and baseline viral load of the responding cases.

Conclusion: Chronic HCV infection is associated with reduced level of serum hepcidin hormone. The reduced serum hepcidin in chronic HCV patients is fully reversible after IFN/RBV therapy. Initial rise in serum hepcidin concentration might have a potential for being used as one of the indicators of patient response to therapy.

INTRODUCTION

Hepatitis C virus (HCV) is a major health problem affecting 170 million people worldwide. The seroprevalence rate is about 1% in western countries and North America, 3-4% in some Mediterranean and Asian countries and up to 10-20% in parts of central Africa [1,2]. It is estimated that Egypt comes first worldwide in HCV prevalence, with incidence rates at 2.4 per 1,000, and an estimated average of 165,000 new incidents per year, according to the Centers for Disease Control and Prevention (CDC) [3].Infection follows a variable course; while it is often asymptomatic , some patients develop liver fibrosis and ultimately cirrhosis, which is apparent after many years[4]. Making HCV infection the leading cause of chronic hepatitis worldwide, where resolution of acute hepatitis is observed only in 15% and chronic infection develops in 85% of infected cases, progression to liver cirrhosis is seen in approximately 20% of patients after 10 years and to hepatocellular carcinoma (HCC) in a subset of them with a yearly incidence of 3% [5,6].The liver is a large, complex organ that is responsible for synthesizing and secretion of bile ,lipoproteins and plasma proteins, including clotting factors. Liver disease is often reflected by biochemical abnormalities of liver functions [7]. Out of the noninvasive markers of hepatic fibrosis that are recently being evaluated for their

ability to assess liver condition; is Hepcidin[8,9]. Hepcidin was first discovered in human blood ultrafiltrate and urine samples as a small bactericidal peptide (defensin and cathelicidin) and named liver–expressed antimicrobial peptide (LEAP–1) [8,9]. The name 'hepcidin' originates from the place of synthesis in hepatocytes (hep) and its antimicrobial activity (cidin). It has antibacterial (Escherichia coli, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus spp. group B) and antifungal activity (Candida albicans, Aspergillusniger, Aspergillusfumigatus) [10].

Recent work has also established the importance of the peptide hepcidin in iron homeostasis as a negative regulator of iron release into the system by duodenal enterocytes and reticuloendothelial macrophages [11, 12].

Hepcidin binds to the iron exporter ferroportin, which results in ferroportin internalization and degradation [13,14,15]. Explaining why iron accumulation in the liver is common in patients with chronic liver disease, as it is present in 10% to 36% of patients with chronic hepatitis C [16] and is even more common among patients with end-stage liver disease due to hepatitis C [17,18].

In addition to its antibacterial effect and its role in iron homeostasis, hepcidin was also found to be induced by inflammation [14], an effect believed to be dependent on cytokine production [15].

The discovery of hepcidin in 2000[19] not only opened the way to understand its antimicrobial and metabolic role but also raised the question of the use of hepcidin as a diagnostic and therapeutic tool in many diseases.

This study aimed to assess the serum concentration of hepcidin in chronic hepatitis C patients and evaluate any possible association with the disease activity during the first 24 weeks of pegylated IFN and Ribavirin therapy.

PATIENTS AND METHODS

This study was carried on 35 newly diagnosed chronic HCV patients enrolled in the National Control Strategy Program for Viral Hepatitis Treatment[20], after the first 12 weeks of therapy, five patients were still positive for HCV RNA and were excluded from the treatment program. So, only 30 patients continued in the treatment program. Also Fifteen chronic HCV patients not on IFN therapy were included in the study as controls to determine their serum hepcidin levels.

The study was approved by the Alexandria Faculty of Medicine Ethical committee and informed consent was obtained from patients before sampling.

All patients were diagnosed as chronic HCV through the presence of HCV antibodies in their sera for at least 6 months and presence of HCV RNA by quantitative real-time RT-PCR. Liver biopsy was done in order to assess the degree of liver fibrosis according to Metavir scoring system, and non of the included patients had liver cirrhosis, HBV, HDV nor HIV co-infections.

Patients received a combination therapy of: weekly dose of PEG-IFN-α2b (PEG-INTRON) administered subcutaneously at a dose (1.5 μg/ kg/week), and RBV administered orally at daily doses of 10.6 mg/kg/day (1000-1200 mg/day).

All patients were subjected to routine base line laboratory investigation including [21] :

1- Liver function test (ALT,AST,Serum Albumin).
2- Alkaline phosphatase.
3- Serum bilirubin.
4- Haemoglobin concentration.
5- Liver biopsy to assess degree of fibrosis.
6- Real Time PCR for serum HCV RNA level was done before PEG-IFN-α /RBV therapy and then at week 12 and week 24 during therapy using COBAS TaqMan HCV assay (TaqMan) (Roche Diagnostics). The COBAS AmpliPrep/COBAS TaqMan HCV test utilizes automated specimen preparation on the COBAS AmpliPrep Instrument then the processed specimen is added to the amplification mixture and transferred to the COBASTaqMan 48 Analyzer according to manufacturer instructions [22].
7- Detection of serum Hepcidin concentration before and during PEG-IFN-α / RBV therapy and at week 12 and week 24 of therapy for the 30 cases that achieved EVR and for the control group which was done at the beginning of the study and at the end of the six month which is the period of the study.

Hepcidin hormone measurement :

Blood samples were collected from patients by venipuncture at the beginning of therapy, then at 12 weeks and 24 weeks during therapy. Three ml blood were collected and allowed to clot for two hours at room temperature before centrifugation for 20 minutes at approximately 1000 xg[23,24] .

Then serum was removed and stored at -80°C to avoid loss of bioactivity or contamination.

Serum Hepcidin (HEPC) concentrations was measured using the quantitative sandwich enzyme immunoassay kit (USCN, Life Science Inc. Wuhan, China) according to manufacturer instructions.

The HEPC enzyme linked immunosorbent assay is quantitative sandwich immunoassay. The microtiter plate provided in this kit has been pre-coated with a monoclonal antibody specific for HEPC. Standards and samples were then added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for HEPC. Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After substrate solution was added, those wells that contain HEPC, exhibited a change in color proportional to hepcidin concentration in patient serum. The enzyme-substrate reaction was terminated and the color change was measured spectrophotometrically at a wavelength of 450nm +or- 10nm. The observed intensities of duplicate samples were averaged and compared to the curve derived from a serial dilution series of known hepcidin standards (0–4,000 pg/ml); observed intensities above the standard range were re-analyzed after further sample dilution.

Statistical analysis :

Data were fed to the computer using the Predictive Analytics Software (PASW Statistics 18). Qualitative data were described using number and percent. Quantitative data were described using median, minimum and maximum as well as mean and standard deviation.

The distributions of quantitative variables were tested for normality using Kolmogorov-Smirnov test, Shapiro-Wilk test. D'Agstino test was used if there was a conflict between the two previous tests. If it reveals normal data distribution, parametric tests was applied. If the data were abnormally distributed, non-parametric tests were used.

For abnormally distributed data, Mann-Whitney Test (for data distribution that was significantly deviated from normal) were used to analyze two independent population. If more than two population were analyzed Kruskal Wallis test to be used. Wilcoxon signed ranks test was used to compare between the different periods. Correlations between two quantitative variables were assessed using Spearman coefficient. Significance test results are quoted as two-tailed probabilities. Significance of the obtained results was judged at the 5% level.

RESULTS

The base line characteristics for the initially studied 35 cases (23 males and 12 females) are shown in table1,showing slightly elevated AST (SGOT) and alkaline phosphatase (ALP). While ALT (SGPT), albumin, bilirubin and Hb levels were within normal range.

As regards the baseline viral load for all 35 studied cases; twenty four (68.6%) cases were found to have low viral load (serum HCV RNA <600,000 IU⁄ml), two (5.7%) cases had a moderate viral load (serum HCV RNA between 600.000-800.000 IU⁄ml), and nine (25.7%) cases had a high viral load (serum HCV RNA >800,000 IU⁄ml).

The degree of liver fibrosis according to Metavir scoring system among the 35 studied cases was as follows; 16 (45.7 **%**) cases were F1, 14 (40**%**) cases were F2 and only 5 (14.3**%**) cases were F3. No statistically significant relation was found (P=0.318) between the degree of liver fibrosis and the viral load. Also no statistically significant relation was found between viral load and liver enzymes (ALT & AST) (P=0.367 & 0.587 respectively).

Follow up of cases during the first 24 weeks of therapy :

The 35 cases studied, were followed up by measuring their viral load initially, at 12 weeks then at 24 weeks during the course of therapy. Thirty (85.7%) cases out of the 35 showed an early virologic response EVR. While the remaining 5(14.3%) cases failed to respond to therapy (non responders) and were excluded from the treatment program and thus from the study.

Out of the 30 cases that achieved EVR and continued therapy, 24 (80 %) cases showed response to treatment and undetectable RNA at week 24 of ongoing therapy and continued therapy according to the national guidelines [20]. While the remaining 6 (20%) cases had detectable RNA and they were considered break through cases. So out of the 35 (100%) initially studied cases eleven (31.4%) (5 non responders and 6 break through cases) did not respond to therapy.

Serum Hepcidin measurement :
Serum hepcidin was measured for all the 30 cases that achieved EVR including responders (n=24) and break through cases (n=6) at baseline, 12 and 24 weeks during therapy. Where both groups showed rise in hepcidin level, with no statistically significant difference between both groups (p at base line= 0.938, p at 3 months= 0.775 and p at 6 months= 0.186) as shown in table (2).

Yet there was a statistically significant rise in the hepcidin level in responding cases from baseline to 12 weeks of therapy (P1=0.001) and from baseline to 24 weeks of therapy (P2<0.001) (Table 2, Fig. 1). While the break through cases had no statistically significant rise during the period from baseline to 12 weeks of therapy (P1= 0.058) which later showed a statistically significant rise from baseline to 24 weeks of therapy (P2= 0.046) and the rise from 12 to 24 weeks was insignificant for both groups (P3 for responders = 0.841, P3 for break through cases = 0.075) (Table 2, Fig. 2).

In other words this significant rise was demonstrated early in the responding cases at 12 weeks of therapy (P1= 0.001, P2 <0.001), while the break through cases showed hepcidin level significant rise only later on at 24 weeks of therapy (P1>0.05, P2=0.046)

Table (3) shows the baseline hepcidin level of both the 30 studied cases and the 15 control group of chronic HCV patients not under interferon therapy, with no statistically significant difference in serum hepcidin level detected between both groups (P=0.555), yet when the comparison was done again after 24 weeks of ongoing therapy, a statistically significant rise in hepcidin level in the study group occurred after receiving treatment (P<0.001).

Baseline viral load and baseline hepcidin concentration for the 30 EVR cases :
The 30 studied cases had variable hepcidin levels regardless of their viral load levels (Table 4) as there was no significant correlation between baseline viral load and baseline hepcidin levels (P= 0.650).

Yet with ongoing therapy a statistically significant negative correlation (P= 0.013*) between viral load and increased hepcidin level in the responding cases with therapy was found (Fig. 3). On the contrary for the breakthrough cases, although these patients were positive for HCV RNA, and still showed a rise in their hepcidin level, but this rise did not give a statistically significant correlation with the viral load (P= 0.156) (Fig. 4).

Table (1) : Routine laboratory investigations of the 35 studied cases

Test (normal range)	Min. – Max.	Mean ± SD	Median
ALT (7-40 IU/L)	10.0 – 82.0	34.66 ± 15.02	31.0
AST (5-35 IU/L)	8.0 – 118.0	47.46 ± 25.63	41.0
ALP (44-147 IU/L)	60.0 – 212.0	144.26 ± 37.46	147.0
Albumin (3.4-5.4 mg/dl)	3.20 – 5.0	4.37 ± 0.41	4.40
Billirubin (0.2-1.2 mg/dl)	0.50 – 2.10	0.85 ± 0.35	0.80
Hb (males: 13.8-17.2 g/dl) (females: 12-15 g/dl)	11.0 – 15.60	13.63 ± 1.17	13.70

Table (2) : Relation between responders and break through cases with hepcidin at different periods of therapy (measured by pg/ml)

Hepcidin base line	**Responders (n = 24)**	**Break through cases (n = 6)**	**Total (n = 30)**
Min. – Max.	5.0 – 257.50	7.0 – 234.0	5.0 – 257.50
Mean ± SD	105.94 ± 96.48	104.58 ± 99.30	105.67 ± 95.30
Median	73.50	87.50	75.0
P	0.938		
Hepcidin at 3m			
Min. – Max.	30.0 – 263.0	10.0 – 260.0	10.0 – 263.0
Mean ± SD	171.27 ± 74.68	151.17 ± 101.84	167.25 ± 79.23
Median	172.0	183.25	172.0
P	0.775		
Hepcidin at 6 m			
Min. – Max.	31.0 – 260.50	88.0 – 280.0	31.0 – 280.0
Mean ± SD	175.11 ± 68.0	213.58 ± 69.13	182.81 ± 68.82
Median	177.0	230.0	194.25
P	0.186		
p_1	0.001*	0.058	
p_2	<0.001*	0.046*	
p_3	0.841	0.075	

p_1: p value for Wilcoxon signed ranks test between Hepcidin base line and at 3m
p_2: p value for Wilcoxon signed ranks test Hepcidin base line and at 6 m
p_3: p value for Wilcoxon signed ranks test 3m and 6 m
*: Statistically significant at p≤0.05

Table (3): Comparison of hepcidin between control group and cases at baseline and after 24 weeks of ongoing therapy(measured by pg/ml)

Hepcidin	**Control (n= 15)**	**Cases (n= 30)**	**P**
At base line			
Min. – Max.	20.0 – 201.0	5.0 – 257.50	0.555
Mean ± SD	68.90 ± 43.21	105.67 ± 95.30	
Median	60.0	75.0	
At 6 months			
Min. – Max.	11.0 – 202.0	31.0 – 280.0	<0.001*
Mean ± SD	65.20 ± 45.91	182.81 ± 68.80	
Median	55.0	194.25	

P: p value for Mann Whitney test *: Statistically significant at p≤0.05

Table (4) : Relation between baseline viral load and baseline hepcidin(measured by pg/ml) concentration for 30 EVR cases

	Baseline Viral load			**P**
	Low (n = 21)	**Moderate (n = 1)**	**High (n = 8)**	
Hepcidin base line				
Min. – Max.	5.0 – 257.50	53.0 – 53.0	7.0 – 203.0	0.650
Mean ± SD	117.77 ± 102.89	53.0 ± -53.50	80.41 ± 76.32	
Median	75.0	53.0	63.50	

p: p value for Kruskal Wallis test

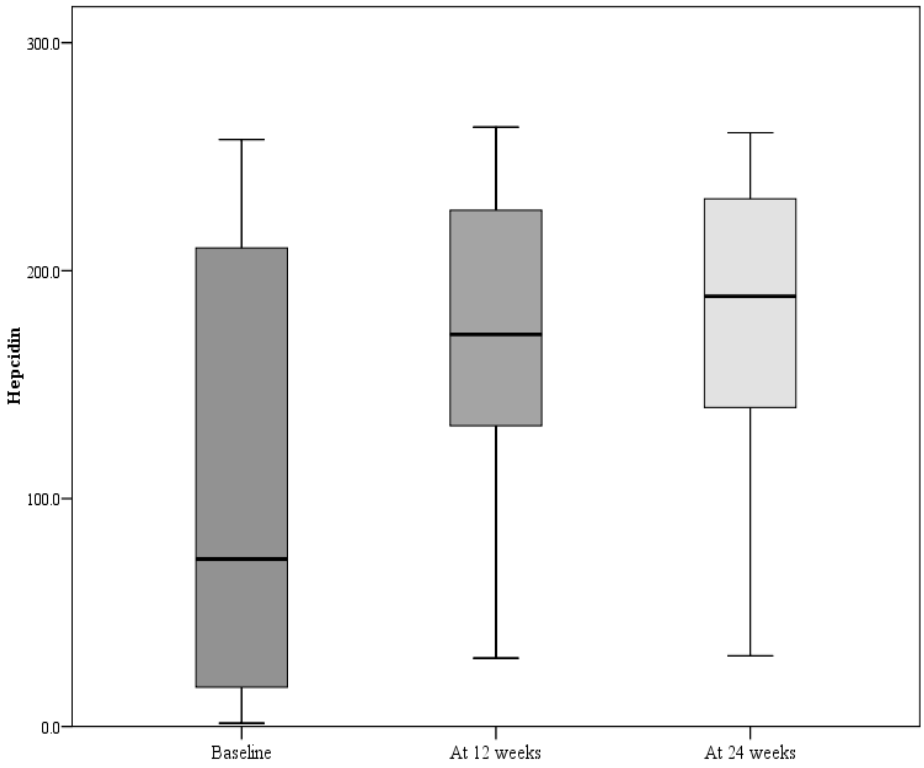

Fig. (1) : Hepcidin concentration of the 24 responding cases at different periods.

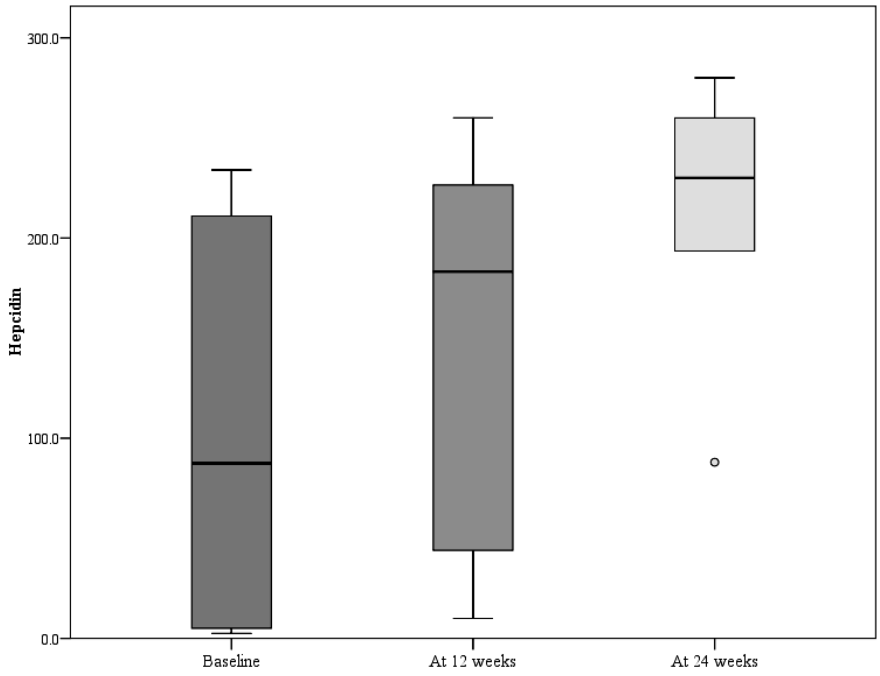

Fig. (2) : Hepcidin concentration for the 6 break through cases at different periods

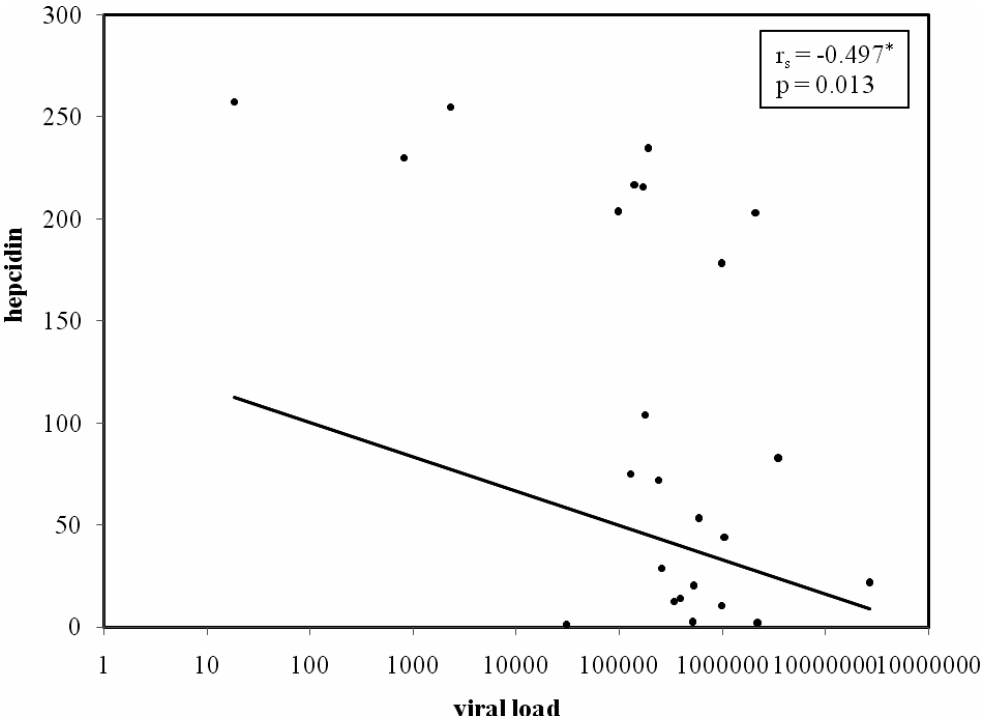

Fig. (3) : Correlation between viral load and hepcidin concentration of the 24 responding cases

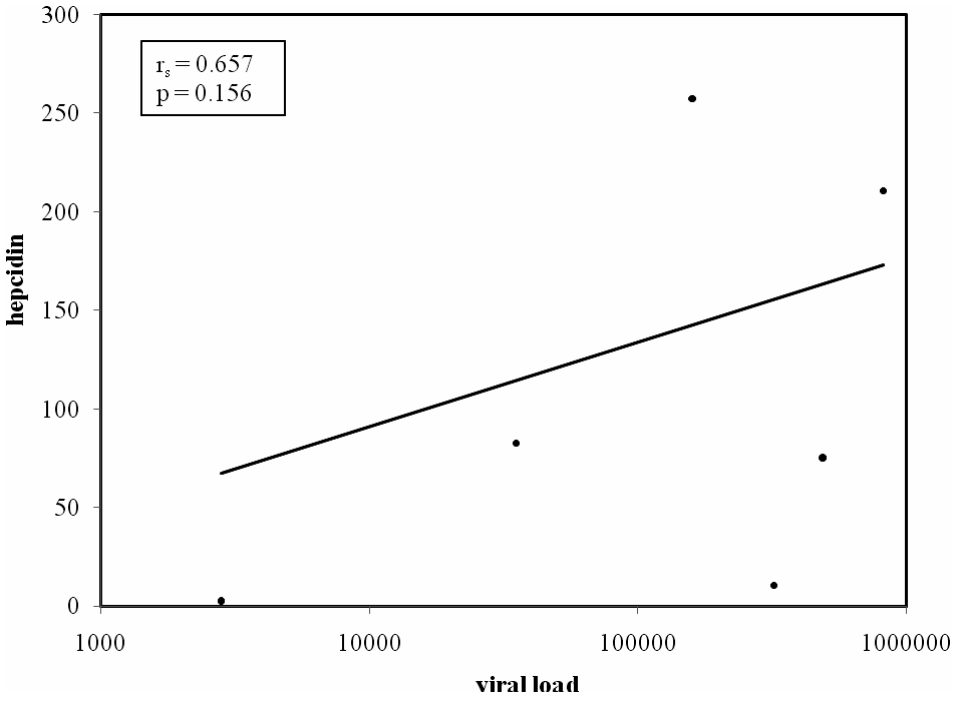

Fig. (4) : Correlation between viral load and hepcidin concentration of the 6 breakthrough cases

DISCUSSION

Regarding the sex distribution of the studied cases males were predominant, although patients' selection was fairly random. This is in agreement with the study conducted in the National Research Center, Cairo Egypt by Moataza H. Omran et al. [24] such observation is partly related to social risk factors for HCV transmission as drugs, schistosomiases and occupational exposure [26,27].

Real time RT-PCR was mandatory done for all patients as a prerequisite before starting PEG-IFN and RBV combination therapy with no significant correlation found between viral load and degree of liver fibrosis in the present study (P=0.318), as also reported by other several studies [28,29,30], where the serum HCV-RNA titer did not reflect the grades of liver necro-inflammatory activity and the stage of liver fibrosis, and this was attributed to the fact that the mechanism of liver damage caused by HCV is still not clear whether it's either due to direct damage by HCV or immune-mediated hepatic injury induced by HCV.

Also in the present study no statistically significant correlation between viral load and liver enzymes ALT and AST (P=0.367, 0.587 respectively). This was in concordance with Luay Ein [31] and Anand et al. [29] who observed no correlation between HCV viral load, and serum ALT and AST values.

In the present study the viral load of the 35 cases was assessed again at 12 weeks of therapy to evaluate EVR which is defined as undetectable or at least a 2-log decrease in HCV RNA, and the results showed that 30 (85.7%) patients achieved EVR, and the remaining 5 (14.3%) cases were non responders and thus were excluded from the treatment project.

In a study done by Gary et al. [32], it was detected that those patients who failed to achieve EVR after 12 weeks had no chance of reaching SVR, even if they completed the additional 9 months of treatment that was originally prescribed. Also they found that not all patients who had EVR ultimately achieved SVR. Where, SVR was also lower in patients who had a 2-log decrease in the HCV RNA level but remained PCR positive than in those who had undetectable HCV RNA by PCR after 12 weeks. Another factor that explains the failure of patients with EVR to reach SVR is the ability to adhere to the prescribed treatment regimen, as the reduction of PEG-IFN/RBV therapy or premature discontinuation in the first 12 weeks markedly reduces the chance of SVR.

Also in our study out of the 30 cases who achieved EVR and thus continued therapy, viral load was reassessed at 24 weeks during therapy and resulted in undetectable viral RNA in 24 (80%) cases, and detection of HCV RNA in the remaining 6 (20%) cases which were considered break through cases. So, out of the 35 initially studied cases, 11 (31.4%) (5 non responders and 6 break through cases) did not respond to therapy. This was matching with the reported treatment failure rate for genotype 4 (50%-21%) [33,34,35].

Hepcidin is a recently discovered iron regulatory hormone, it is synthesized predominantly in the liver. Synthesis of hepcidin is increased by iron overload and decreased by anemia and hypoxia. Moreover, it is also induced by infection and inflammation [36].

In our study hepcidin hormone concentration was measured for the 30 studied cases before starting therapy, at 12 weeks and at 24 weeks during ongoing therapy, and we found a significant rise in hepcidin level at different periods among all patients including the 24 (80%) responding cases and the 6 (20%) break through cases. This significant rise was demonstrated early in the responding cases at 12 weeks of therapy (P1= 0.001, P2 <0.001), while the break through cases showed hepcidin level significant rise only later on at 24 weeks of therapy (P1>0.05, P2=0.046)

Similar to our findings Ryan et al. [37] reached a conclusion that the hepcidin induction occurs following the initiation of PEG-IFN-a treatment for HCV associated with decrease in serum iron, and was greatest in those with the most significant decline in viral load as in the present study as it was found that chronic HCV represents a complex condition for hepcidin regulation. As inflammation and iron overload have a stimulatory effect on hepcidin expression, while HCV induced oxidative stress suppresses hepcidin expression [37].

In our study the correlation between the viral load and hepcidin level of the 6 (20%) break through cases before and at 24 weeks during the course of therapy showed that, although these patients turned positive in their RT-PCR assessment, they still showed a significant rise in their hepcidin level. However, there was no statistically significant relation between their viral load and hepcidin levels (P = 0.156).

On the other hand, hepcidin hormone concentration was measured for 15 chronic HCV patients not under therapy as control group. A correlation between the baseline hepcidin concentration of the 30 cases included in the study and the control group, was done with no significant difference detected in serum hepcidin levels between both groups (P= 0.555). While the comparison between hepcidin concentration in control group and responding cases at 24 weeks of ongoing therapy showed a significant rise of hepcidin level after receiving treatment (P<0.001).

Regarding the 24 (80%) cases that showed response to treatment, the correlation between their viral load and hepcidin levels before and at 24 weeks of therapy showed that the viral RNA disappeared after 24 weeks of therapy and the hepcidin concentration was significantly increased. There was a statistically significant negative relation between their viral load and hepcidin concentration (P= 0.013).

Recently, experimental evidence from cultured HCV infected cell lines as well as from HCV-infected mice suggested that hepcidin expression is down-regulated in HCV infection. Oxidative stress in the form of reactive oxygen species was responsible for the hepcidin suppression through increased histone deacetylase activity. Their findings support the in vitro evidence of HCV-induced hepcidin suppression, and suggest that HCV infection down-regulates serum hepcidin, while increasing inflammation and/or fibrosis tend to restore its levels. Also in vitro studies have suggested that weekly PEG-IFN-a administration led to hepcidin induction of benefit to HCV patients [37,38]. Thus if interferon free regimes are to be approved for future therapy studies can be planed to separate out the effect of interferon from directly acting anti-viral drugs on hepcidin.

This was in agreement with the study of Naoki Fujita et al. [39] that measured serum hepcidin before and after the completion of 48-week course of PFG-IFN plus ribavirin therapy in 27 chronic HCV patients. Twelve patients were assigned to SVR and the remaining 15 to non-SVR, and serum hepcidin was measured again 24 weeks after completing therapy. After the PEG-IFN plus ribavirin therapy, serum hepcidin levels were significantly increased in SVR patients ,but on the contrary to our study, hepcidin decreased significantly in non-SVR patients. From these results, relatively low hepcidin expression in chronic HCV patients seems to be directly related to HCV replication in the liver.

We studied the relation between hepcidin levels and degree of liver fibrosis and we noticed that there is no significant correlation between baseline hepcidin concentration of the 30 studied cases and degree of liver fibrosis (P= 0.407). The mean baseline hepcidin level for F1 cases was 126.31± 98.37, for F2 cases was 76.58 ± 83.62, and for F3 cases 137.38 ± 114.53

This was matching with the results of Aoki's reports [40], the histological grading were not significantly correlated with serum hepcidin in chronic HCV, mild and local inflammation which occurred in the liver of CHC may not induce hepcidin expression as P= 0.2359. This result was also supported by a study conducted by Usama et al. [41], which revealed that there was no significant correlation between serum prohepcidin which is a hepcidin precursor and hepatic fibrosis or inflammatory activity according to Metavir Scoring System in CHC patients.

In contrast, in 2010 the study of Tsochatzis et al. [38] in which the serum hepcidin levels were found to positively correlate with both necro-inflammation (r= 0.259, P= 0.011) and fibrosis (r= 0.214, P= 0.036). Moreover, serum hepcidin levels increased with increasing severity of fibrosis (P= 0.048).

We also studied the relation between liver enzymes (ALT, AST) and baseline hepcidin levels for the 30 studied cases and we found there was no significant correlation between hepcidin levels and liver enzymes ALT and AST (P= 0.332, 0.624 respectively).

In agreement with our results, Fujita et al. [39] found that there were no significant correlations between serum hepcidin levels and serum transaminase (AST and ALT). Similarly, Aoki et al. [40] demonstrated that among patients with hepatitis C, hepcidin mRNA expression in the liver did not correlate with aspartate aminotransferase and alanine aminotransferase. Also Elhamy et al. [42] has found no significant correlation between the expression of hepcidin mRNA and liver enzymes (ALT, AST).

In contrast, Tsochatzis et al. [38] concluded that in patients with chronic HCV, serum hepcidin correlated positively with aspartate amino-transferase (AST) and with alanine amino-transferase (ALT).

CONCLUSION

From the current study we can conclude that chronic HCV is associated with reduced level of serum hepcidin, however this reduced level is fully reversible with IFN/RBV therapy. Also the initial rise of serum hepcidin could be used as an indicator of patients response to therapy although we recommend a wider scale study (regarding patients numbers and duration) for a better understanding of the prognostic and monitoring role of hepcidin among chronic HCV patients on antiviral therapy.

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The Effect of Rabeprazole on Injection Sclerotherapy Complications after First Attack Bleeding Esophageal Varices

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oesophageal varices, sclerotherapy, proton pump inhibitors (PPI), rabeprazole

Background and study aim : Endoscopic variceal sclerotherapy (EVS) effectively controls bleeding esophageal varices (OV), however it has some adverse effects including sclerosant ulcers, chest pain, dysphagia and odynophagia**.** Gastric acid plays a central role in mediating and aggravating these complications. Proton pump inhibitors (PPI) are the most potent pharmacologic agents for inhibition of gastric acid secretion. Therefore, these agents are the logical candidates to combat the effects that gastric acid plays in post EVS complications. However, some authors still believe that there is no strong evidence to support their use. This study was designed to evaluate the effects of the use of PPI (rabeprazole) for 8 weeks after endoscopic sclerotherapy for first attack variceal bleeding on the prevention and treatment of complications after EVS. Moreover, we aim to assess the presence of any adverse effects for the use of this drug for this period in this specific patients group.

Patients and methods: One hundred patients with first attack variceal bleeding were included in the study. They were allocated randomly into a test group which received 20mg rabeprazole once daily oral dose following endoscopic sclerotherapy starting 6 hours after injection sclerotherapy and continued for 2 months and a control group which did not receive rabeprazole after sclerotherapy. For both groups, endoscopic, laboratory and clinical data were monitored every two weeks for a period 2 months.

Results: The test group had significantly lower frequency of all post sclerotherapy adverse symptoms, (dysphagia, odynophagia, heart burn, retrosternal and epigastric pain as well as dyspepsia) as well as lower overall rate of re-bleeding (14% vs 46% in the control group). There were no significant differences in the hematological parameters or endoscopic findings between test and control groups**.** Moreover, the use of the drug for two months was not associated with any significant infectious or non infectious complications including fever, hepatic encephalopathy, SBP, diarrhea and chest infection.

Conclusion: Rabeprazole use decreases post-sclerotherapy symptoms and decrease the rate of rebleeding after sclerotherapy without any increasing the complications related to acid supression.

INTRODUCTION

Portal hypertension is the leading cause of morbidity and mortality in liver cirrhosis. Complications of portal hypertension in cirrhotic patients include esophageal and gastric varices, portal hypertensive gastropathy, ascites, hepatorenal and hepatopulmonary syndromes as well as portopulmonary hypertension [1]. At the time of diagnosis about 60% of cirrhotic patients have esophageal varices of different grades. In patients without varices, the rate of developing esophageal varices is about 5% annually. Acute variceal bleeding is a medical emergency and a life threatening event with a mortality rate of about 25% [2].

Although 50% of all esophageal variceal bleeding episodes stop spontaneously, the rebleeding rate is high with about 50% of patients experiencing a second episode, usually within 2 weeks from the first episode. A second episode of bleeding puts the patient at a high mortality risk and is thus the reason for starting therapy as soon as possible [3]. Endoscopic sclerortherapy should be performed early after hospital admission, assuring that the patient is resuscitated and hemodynamically stable [4]. Endoscopic variceal sclerotherapy (EVS) effectively controls bleeding of esophageal varices (OV), however it has some adverse effects including post injection hemorrhage, chest pain, dysphagia and odynophagia [5].

Proton pumps are located on the cytoplasmic membrane of gastric parietal cells. They create an acidic environment in the gastric lumen through exchanging one hydrogen ion for one potassium ion via the hydrogen/potassium adenosine triphosphatase enzyme system (the H^+/K^+ ATPase pump) [6]. The proton pump is the terminal stage in gastric acid secretion, being directly responsible for secreting H^+ ions into the gastric lumen, making it an ideal target for inhibiting acid secretion [7]. Proton-pump inhibitors (PPIs) are a group of drugs whose main action is a pronounced and long-lasting reduction of gastric acid production. They act by irreversibly blocking the hydrogen/potassium adenosine triphosphatase enzyme system (the H^+/K^+ ATPase, or, more commonly, the gastric proton pump) of the gastric parietal cells [6]. PPIs thereby inhibit both basal and stimulated gastric acid secretion, independent of the nature of parietal cell stimulus. They act through blocking acid secretion from all three pathways (neuronal, paracrine and endocrine) simultaneously, so they are considered the most potent medications used to reduce gastric acid secretion [8].

Although all PPIs are effective in treatment of acid-related conditions, there are some differences in their clinical performance, regarding the degree and duration of gastric acid suppression [9]. Differences in PPIs hepatic metabolism may affect both efficacy and consistency, leading to small but significant variation in patient outcomes. PPI selection should therefore involve awareness of these relevant issues [10]. Acid suppressive therapy after EVS is advised as gastric acid may exacerbate post injection ulcers and delay healing [11]. Proton pump inhibitors (PPI) are the most potent pharmacologic agents for inhibition of gastric acid secretion. Therefore, these agents are the logical candidates to control the gastric acid injurious effects on post EVS complications [12]. However, some authors consider its use to be habit related and not evidence based [13].

Aim of the work :

This study aims to evaluate the effects of rabeprazole administration for 8 weeks after endoscopic sclerotherapy for first attack variceal bleeding on the prevention and treatment of complications after EVS. Moreover, we aim to assess the presence of any adverse effects as a result of the use of this drug for this period in this specific patients group.

PATIENTS AND METHODS

This prospective randomized clinical trial was conducted in the Intensive Care Unit (ICU), In-patient and Endoscopy Units of Tropical Medicine Department, Faculty of Medicine Zagazig University, during the period from September 2013 to July 2014.

Inclusion criteria :

1. Presence of liver cirrhosis, the diagnosis of cirrhosis was based on clinical, biochemical and ultrasonographic findings with Child-Pugh grading (group A and B were only included)
2. First attack of upper GIT bleeding, which was proven by upper GIT endoscopy to be coming from esophageal varices.
3. Signed informed consent.

Exclusion criteria :

1. Uncooperative patients and those unable to give written informed consent or couldn't return for routine follow up.
2. Endoscopically confirmed pre-existing esophageal ulcers.
3. Ongoing therapy with PPI.
4. Patients with other causes of upper GIT bleeding than esophageal varices.
5. Patients with Child-Pugh grade C.

Randomization :

Patients were randomly alternatively allocated into 2 groups :

- **Group I** : (test group) Patients with acute variceal bleeding who received 20mg rabeprazole once daily oral dose half an hour before breakfast following endoscopic sclerotherapy starting 6 hours after injection sclerotherapy for the varices and continued for 2 months.
- **Group II**: (control group) Patients with acute variceal bleeding who did not receive any PPIs following endoscopic variceal sclerotherapy during the same period, but received only a bland antacid for 48 hours after each endoscopic sclerotherapy.

All patients in the 2 groups were subjected to:

1. **Thorough medical history taking including:** age, gender, special habits of medical importance, history of other medical diseases, history of drug intake especially NSAID and anti-acid drugs, past attacks of haematemesis ,amount, colour, presence of melena and blood transfused, history of HCV or HBV

infection, previous or current history of hepatic encephalopathy.

2. **Thorough clinical examination including:**
   - General examination focusing on consciousness, vital signs, coloures, signs of liver cell failure & LL edema.
   - Local abdominal examination searching for signs of chronic liver disease and portal hypertension.

3. **laboratory investigations:**
   - Complete blood picture (CBC)
   - Liver function tests including: Total and direct serum bilirubin, serum albumin, Serum Aspartate amino Transferase (AST) and serum Alanine amino Transferase (ALT).
   - Prothrombin time (PT) and international normalized ratio (INR).
   - Kidney function tests including blood urea and serum creatinine.
   - Viral markers, anti HCV and HBs Ag using third generation ELISA kits.
   - Ascetic fluid sample, if possible, was obtained and analyzed physically, bio-chemically, cellularly and bacteriologically.

4. **Child-Pugh classification for all patients into:** A, B, and C class according the severity of cirrhosis **[14]:**

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

Points	Class	One year survival	Two year survival
5-6	A	100%	85%
7-9	B	81%	57%
10-15	C	45%	35%

5. **Abdominal ultrasonography:**
   All the patients were examined using TOSHIBA CAPASEE II device. They were examined according to the standard maneuvers. Liver, spleen, portal vein diameter, and presence of free fluid in the abdomen (ascites) were evaluated. The liver was evaluated and its size was noted. Cirrhotic appearance of the liver was shown by the coarse nodular appearance, increased echogenicity, its shrunken size and prominent caudate lobe **[15]**. Portal vein diameter was measured at a point of crossing the inferior vena cava. The spleen was evaluated for its length by measuring its bipolar diameter at the left mid-axillary line, it is considered enlarged if it was greater than 13 cm **[16]**.
6. **Upper gastrointestinal endoscopy:**
   Endoscopywas done using end flexible video-endoscope (PENTAX VIDEO unit of endoscopy). The patients were positioned on their left lateral position, with head supported on a small firm pillow to remain in a comfortable neutral position and a bite guard in their mouth. Sedation was received and the tip of endoscope was lubricated and checked for being functioning, regarding image quality, air and water, suction, and tip angulations. Then endoscope was introduced gently and under vision. The OV were shown as tortuous bluish cords running longitudinally within the esophagus and covered with mucosa.

The oesophageal varices were graded into 4 grades according to**Thakeb et al. [17]**.

Grade I: small straight cords of varices continued to lower 1/3of the esophagus.

Grade II: moderate sized clubbed varices with well-defined areas of normal mucosa between them, forming several distinct vertical cords and confined to lower third of esophagus.

Grade III: gross varices extending into the proximal half of the esophagus, which are so large and tortuous, that normal mucosa may not be visible in between unless the esophagus is fully distended with air.

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

Sclerotherapy was done for the oesophageal varices and total amount of sclerosant material and amount injected in each site were recorded.

Portal hypertensive gastropathy was classified according to the Italian endoscopic club classification validated in 1997 **[18]** :

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

Episodes of recurrent bleeding during the follow up period were recorded. The severity of recurrent bleeding was classified according to Cappell and friedel **[19]** as follows :

Physical signs	Mild	Moderate	Severe
Blood loss	<1 L	1-2 L	> or equal 2 L
Blood pressure	Normal	Normal –borderline low	Hypotensive
Orthostasis	No	Possible	Likely
Tachycardia	None- mild	Moderate	Severe
Skin	Warm-wellperfused	Diaphoretic	Cool- cold clamy
Respiratory rate	Normal	Normal- slightly decreased	Irregular
Urine output	Normal	Diminished	Poor
Sensorium	Alert- anxious	Anxious	Confused-drowsy

Follow up of the patients by daily morning temperature which was recorded by the patients in a sheet for early prediction of portal bacteraemia and bacterial peritonitis along with other infections as pneumonia and infective diarrhea.

Also the patients were also evaluated according to presence or absence of post-sclerotherapy symptoms including: epigastric pain, heart burn, retrosternal chest pain, dysphagia, dyspepsia, and odynophagea upon discharge and during the follow up visits every two weeks.

Patients used the non selective beta blocker (propranolol) for prevention of recurrent variceal bleeding, starting with 20 mg orally twice daily and increased to maximum tolerated dose or until heart rate reaches 55 beats per minute as recommended by the American association of the study of the liver disease [20].

Then follow up of the patients every 2 weeks and for 2 month by upper GIT endoscopy with commenting on the variceal condition as previous, PHG, bleeding and development of sclerosant ulcer. Also follow up every 2 weeks for 2 months by CBC, and follow up of the patients' adverse symptoms as previous.

Follow up of the patient's physical state for the likely development or improvement of ascites, lower limb edema, jaundice, and hepatic encephalopathy (HE). And follow up for development of diarrhea, chest infection, and abdominal pain and tenderness as indicators for SBP.

Twenty seven patients were lost during the whole follow up period, 19 patients died and 8 patients were lost. Deceased and lost patients were replaced by other patients. Finally at the 8^{th} week 4 patients were lost at each group and were not replaced.

The follow up of the patients was done at the endoscopy unit, ICU and the in-patient department. The telephone was another way of contact with the patients.

Statistical analysis

Data were checked, entered and analyzed using SPSS version 19 EPI-INFO 6 for data processing and statistics. The quantitative data were presented as mean ($\overline{X}$) and standard deviation and were compared using student t test. The categorical data were presented as number and percentage and were compared using Chi-square test (X^2). For all above-mentioned statistical tests done, the threshold of significance was fixed at 5% level (P-value).

RESULTS

There were no significant differences between the test group (group I) and the control group (group II) as regards mean age and gender distribution as shown in table (1). Table (2) shows that, there are no statistically significant differences between cases and controls regarding the baseline clinical presentations including ascites, jaundice, hepatic encephalopathy, lower limb edema and fever. There were no statistically significant differences between group I and group II regarding all sonographic data e.g. liver size, portal vein diameter, presence and amount of ascites and spleen size as shown in table (3).

Table (4) shows that, there are no statistically significant baseline differences between cases and controls regarding laboratory parameters including (hemoglobin concentration, total leukocytic count, platelet count, albumin level, total and direct bilirubin levels), ALT, AST, PT, INR, and serum creatinine level. There was also no significant difference between the two groups as regards Child's grade as shown in table (5).

There were no significant differences found between the two groups as regards their preliminary endoscopic findings such as number of OV cords, grade of OV and risky signs, amount of sclerosant material used in sclerotherapy, grade of portal hypertensive gastropathy and duodenopathy as shown in table (6). While Table (7) shows that, there are no statistically significant differences between cases and controls regarding post sclerotherapy symptoms including (dysphagia, odynophagia, retrosternal pain, epigastric pain, heart burn, and dyspepsia) after first endoscopic sclerotherapy setting.

Table (8) shows that, there are no statistically significant differences between cases and controls regarding all three hematological parameters all through the follow up period.

There were no significant differences between the two studied groups as regards all the endoscopic findings such as risky signs, grade and number of OV cords, amount of sclerosant agents used to secure OV as well as portal hypertensive gastropathy and duodenopathy and frequency of sclerosant ulcer all through the period of follow up as shown in tables 9 and 10.

The incidences of post sclerotherapy symptoms such as dysphagia, odynophagia, heart burn, retrosternal and epigastric pain and dyspepsia were significantly lower in the test group than in the controls all through the period of follow up as shown in table (11). There was also significant increase in the rate of moderate severity recurrent bleeding at the second week and at the end of the follow up period as shown in table (11). There were no significant differences between the studied groups as regards incidence of ascites, lower limb edema, jaundice, abdominal tenderness, encephalopathy, fever, chest or urinary tract infections as shown in table (12)

Table (1): Demographic data

	Group I No.=50		**Group II No.=50**		**Test value**	**P**	**Sig.**
	No.	**%**	**No.**	**%**			
Gender							
Male	28	56.0	27	54.0	X^2= 0.04	0.841	NS
Female	22	44.0	23	46.0			
Age in years (Mean ± SD)	51±8.3		51.7±7.7		t= 0.212	0.832	NS

NS: non-significant

Table (2): Baseline clinical presentations of studied groups

			Group I No.=50		Group II No.=50		X2	P	Sig.
			No.	%	No.	%			
Ascites			18	36.0	23	46.0	1.22	0.269	NS
Jaundice			6	12.0	4	8.0	0.8	0.371	NS
H E			9	18.0	8	16.0	0.12	0.732	NS
LL. edema	Mild		14	28.0	17	34.0	0.58	0.446	NS
	Moderate		8	16.0	10	20.0	0.44	0.505	NS
Fever (No. of days of fever over 38.3)		1	4	8.0	2	4.0	1.33	0.248	NS
		2	4	8.0	4	8.0	0.25	0.617	NS
		3	1	2.0	3	6.0	2.0	0.157	NS
		4	1	2.0	1	2.0	1.0	0.317	NS
		5	0	0.0	1	2.0	2.0	0.157	NS

Table (3): Comparison of U/S presentation of studied groups

		Group I No.=50		Group II No.=50		Test value	P	Sig.
		No	%	No	%			
Liver	Shrunken	15	30.0	10	20.0	X^2= 2.0	0.157	NS
	Average	35	70.0	40	80.0	X^2=0.67	0.414	NS
Portal vein diameter (mm)	≤13mm	2	4.0	4	8.0	X^2=0.71	0.399	NS
	> 13mm	48	96.0	46	92.0			
	Mean ±SD	14.3±1.3		14.6±1.5		T=0.775	0.44	NS
Ascites	Present	22	44.0	25	50.0	X^2=0.36	0.548	NS
	Absent	28	56.0	25	50.0			NS
Spleen (cm)	≤13mm	4	8.0	3	6.0	X^2=0.15	0.695	NS
	> 13mm	46	92.0	47	94.0			
	Mean ±SD	15±1.8		14.9±1.8		T=0.173	0.863	NS

Table (4): Comparison of laboratory parameters of studied groups

	Group I No.=50	Group II No.=50	t	P	Sig.
	Mean ± SD	Mean ± SD			
WBC (cellX10^3/ml)	8±3.8	8.2± 3.8	0.254	0.8	NS
Hb(g/dl)	7.2± 0.9	7.1± 0.9	0.552	0.582	NS
Platelet (X10^3/ml)	85.1± 31.3	88.4± 36.5	0.486	0.628	NS
ALT(IU/ml)	64.5± 19.8	65.2± 29.1	0.145	0.885	NS
AST(IU/ml)	83.9± 27.9	87.8± 45.1	0.512	0.61	NS
Total bilirubin (mg/dl)	2.1 ± 1.2	1.9± 1.1	0.75	0.387	NS
Direct bilirubin (mg/dl)	0.7± 0.3	0.65± 0.3	0.243	0.808	NS
Albumin (g/dl)	3.1± 0.3	3± 0.3	0.314	0.754	NS
INR	1.4± 0.3	1.4± 0.2	0.0	1.0	NS
PT(second)	17.2±2.3	17.4±2	0.518	0.606	NS
Creatinine (mg/dl)	1±0.5	1±0.4	0.195	0.846	NS

Table (5): Comparison of Child Pugh grade of studied groups

	Group I No.=50		Group II No.=50		X2	P	Sig.
	No.	%	No.	%			
A	22	44.0	20	40.0	0.19	0.663	NS
B	28	56.0	30	60.0	0.14	0.71	NS

Table (6): Upper GIT endoscopy presentation of group I and group II

		Group I No.=50		Group II No.=50		X2	P	Sig.
		No.	%	No.	%			
Risky signs	**Present**	20	40.0	24	48.0	0.73	0.394	NS
No. of Oesophageal varices cords	**2**	18	36.0	17	34.0	0.06	0.811	NS
	3	27	54.0	27	54.0	0.04	0.847	NS
	4	5	10.0	6	12.0	0.18	0.669	NS
Oesophageal varices (OV) grade	I	2	4.0	0	0.0	2.17	0.538	NS
	II	16	32.0	15	30.0			
	III	24	48.0	26	52.0			
	IV	8	16.0	9	18.0			
Amount of EO (Mean ± SD)		10.2±4.3		10.9±5		t=0.752	0.454	NS
PHG grade	I	4	8.0	3	6.0	0.29	0.593	NS
	II	23	46.0	22	44.0	0.04	0.833	NS
	III	23	46.0	25	50.0	0.16	0.689	NS
Duodenopathy		16	32.0	18	36.0	0.24	0.628	NS

Table (7): Post sclerotherapy symptoms of studied groups after first injection sclerotherapy

	Group I No.=50		Group II No.=50		X2	P	Sig.
	No.	%	No.	%			
Dysphagia	30	60	32	64	0.17	0.68	NS
Epigastric pain	43	86.0	47	94.0	1.78	0.182	NS
Heart burn	40	80.0	38	76.0	0.23	0.629	NS
Odynophagia	29	58.0	33	66.0	0.68	0.409	NS
Retrosternal pain	47	94.0	43	86.0	1.78	0.182	NS
Dyspepsia	40	80.0	45	90.0	1.96	0.161	NS

Table (8): CBC of group I and group II after 2 weeks follow up

		Group I No.=50	Group II No.=50	T	P	Sig
After two weeks	WBC ($X10^3$/ml) Mean ± SD	5.5± 1.9	5.6± 2.2	0.137	0.891	NS
	Hb (g/dl) Mean ± SD	9.3± 1	8.8± 2.2	1.331	0.186	NS
	Platelet ($X10^3$/ml) Mean ± SD	83.3± 21.5	80.4± 27.2	0.583	0.562	NS
Afterfour weeks	WBC ($X10^3$/ml) Mean ± SD	7.1±2.3	6.9±2.6	0.17	0.685	NS
	Hb (g/dl) Mean ± SD	9.5±1	9.1±1.5	2.46	0.119	NS
	Platelet ($X10^3$/ml) Mean ± SD	81.6±22.9	82.5±25.1	0.172	0.864	NS
After six weeks	WBC ($X10^3$/ml) Mean ± SD	7.4±2	6.9±2.5	1.22	0.272	NS
	Hb (g/dl) Mean ± SD	10.2±4	9.1±1.2	3.47	0.065	NS
	Platelet ($X10^3$/ml) Mean ± SD	79.2±20.7	80.9±22.6	0.379	0.706	NS
After eight weeks	WBC ($X10^3$/ml) Mean ± SD	8.4±4.5	7.1±2.2	3.37	0.069	NS
	Hb (g/dl) Mean ± SD	10±5	8.4±5.9	2.14	0.147	NS
	Platelet ($X10^3$/ml) Mean ± SD	79.8±23.9	80.4±25.2	0.131	0.896	NS

Table (9): Endoscopic findings in studied groups at two and four weeks of follow up

			Group I No.=50		Group II No.=50		X2	P	Sig.
			No.	%	No.	%			
After two weeks	**Risky signs**		16	32.0	24	48.0	3.2	0.074	NS
	No. of Oesophageal varices cords	1	2	4.0	0	0.0	3.33	0.504	NS
		2	12	24.0	15	30.0			
		3	28	56.0	28	56.0			
		4	5	10.0	5	10.0			
		5	1	2.0	0	0.0			
	Oesophageal varices(OV) grade	I	2	4.0	0	0.0	3.63	0.304	NS
		II	18	36.0	15	30.0			
		III	26	52.0	28	56.0			
		IV	2	4.0	5	10.0			
	Amount of EO (Mean ± SD)		8.6±4.3		9±4.8		0.45	0.654	NS
	PHG grade	I	2	4.0	3	6.0	0.21	0.646	NS
		II	22	44.0	20	40.0	0.16	0.685	NS
		III	26	52.0	25	50.0	0.04	0.841	NS
	Duodenopathy		13	26.0	19	38.0	2.25	0.134	NS
	Sclerosant ulcer		2	4.0	6	12.0	2.17	0.14	NS
After four week	**Risky signs**		12	24.0	13	26.0	0.05	0.817	NS
	No of esophageal varices cords	1	0	0	0	0	1	0.3	NS
		2	15	30.0	12	24.0	0.46	0.499	NS
		3	25	50.0	29	58.0	064	0.422	NS
		4	6	12.0	6	12.0	0.09	0.758	NS
		5	1	2.0	0	0.0	1.01	0.315	NS
		I	2	4.0	0	0.0	2.04	0.153	NS
		II	28	56.0	31	62.0	0.37	0.542	NS
		III	17	34.0	16	32.0	0.05	0.832	NS
		IV	0	0	0	0	1	0.3	NS
			6.9±3.7		7.3±3.6		0.62	0.56	NS
		I	4	8.0	7	14.0	0.92	0.338	NS
		II	18	36.0	16	32.0	0.18	0.673	NS
		III	28	56.0	27	54.0	0.04	0.841	NS
	Duodenopathy		12	24.0	13	26.0	0.05	0.817	NS
	Sclerosant ulcer		0	0.0	3	6.0	3.09	0.079	NS

Table (10): Endoscopic findings in studied groups at six weeks and end of follow up

			Group I No.=50		Group II No.=50		X2	P	Sig.
			No	%	No	%			
After six weeks	**Risky signs**		8	16.0	10	20.0	0.27	0.603	NS
	No. of Oesophageal varices cords	1	3	6.0	1	2.0	1.04	0.307	NS
		2	18	36.0	13	26.0	1.17	0.279	NS
		3	19	38.0	26	52.0	1.98	0.159	NS
		4	6	12.0	6	12.0	0.09	0.758	NS
		5	1	2.0	0	0.0	1.01	0.315	NS
	Oesophageal varices(OV) grade	I	7	14.0	6	12.0	0.09	0.766	NS
		II	33	66.0	30	60.0	0.39	0.534	NS
		III	5	10.0	9	18.0	1.33	0.249	NS
		IV	1	2.0	1	2.0	1.0	0.317	NS
	Amount of EO (Mean ± SD)		5.9±4.1		6.5±4.1		0.674	0.502	NS
	PHG grade	I	5	10.0	6	12.0	0.1	0.749	NS
		II	18	36.0	18	36.0	0.04	0.835	NS
		III	27	54.0	26	52.0	0.04	0.841	NS
	Duodenopathy		10	20.0	13	26.0	0.51	0.476	NS
	Sclerosant ulcer		1	2.0	5	10.0	2.84	0.092	NS
After eight week	**Risky signs**		6	12.0	8	16.0	0.33	0.564	NS
	No of esophageal varices cords	1	4	8.0	4	8.0	0.14	0.712	NS
		2	25	50.0	22	44.0	0.36	0.548	NS
		3	13	26.0	14	28.0	0.05	0.821	NS
		4	5	10.0	6	12.0	0.1	0.749	NS
		5	3	6	5	10	0.54	0.461	NS
	Oesophageal varices(OV) grade	I	5	10.0	3	6.0	0.54	0.461	NS
		II	32	64.0	33	66.0	0.1	0.749	NS
		III	6	12.0	5	10.0	0.1	0.749	NS
		IV	0	0.0	0	0.0			
	Amount of EO (Mean ± SD)		4.6±2.7		5±3		0.49	0.485	NS
	PHG grade	I	6	12.0	5	10.0	0.1	0.749	NS
		II	14	28.0	19	38.0	1.13	0.288	NS
		III	30	60.0	26	52.0	0.65	0.42	NS
	Duodenopathy		8	16.0	10	20.0	0.27	0.603	NS
	Sclerosant ulcer		0	0.0	2	4.0	2.04	0.153	NS

Table (11): Post sclerotherapy symptoms studied groups all through follow up period

			Group I (No= 50)		Group II (No= 50)		X^2	P	Sig.
			No	%	No	%			
After two weeks	Dysphagia		20	40	40	80	16.67	<0.001	HS
	Epigastric pain		21	42	43	86	21.01	<0.001	HS
	Heart burn		18	36	40	80	19.87	<0.001	HS
	Odynophagia		13	26	27	54	8.17	0.004	S
	Retrosternal pain		12	24	40	80	31.41	<0.001	HS
	Dyspepsia		18	36	39	78	17.99	<0.001	HS
	Recurrent bleeding	Mild	2	4	0	0	2.04	0.153	NS
		Moderate	1	2	7	14	4.89	0.027	S
		Severe	0	0	1	2	1.01	0.315	NS
		Total	3	6	8	16	2.55	0.11	NS
After four weeks	Dysphagia		13	26	30	60	11.79	<0.001	HS
	Epigastric pain		11	22	33	66	1.64	<0.001	HS
	Heart burn		10	20	24	48	8.73	0.003	S
	Odynophagia		3	6	15	30	9.76	0.002	S
	Retrosternal pain		10	20	30	60	16.67	<0.001	HS
	Dyspepsia		14	28	39	78	25.09	<0.001	HS
	Recurrent bleeding	Mild	1	2	0	0	1.01	0.315	NS
		Moderate	2	4	3	6	0.21	0.646	NS
		Severe	0	0	2	4	2.04	0.153	NS
		Total	3	6	5	10	0.54	0.461	NS
After six weeks	Dysphagia		10	20	24	48	8.73	0.003	S
	Epigastric pain		11	22	27	54	10.87	<0.001	HS
	Heart burn		6	12	14	28	4	0.046	S
	Odynophagia		6	12	14	28	4	0.046	S
	Retrosternal pain		13	26	30	60	11.79	<0.001	HS
	Dyspepsia		13	26	34	68	17.7	<0.001	HS
	Recurrent bleeding	Mild	1	2	1	2	0.51	0.475	NS
		Moderate	0	0	5	10	5.26	0.022	S
		Severe	0	0	0	0	1	0.31	NS
		Total	1	2	6	12	3.84	0.049	S
After eight weeks	Dysphagia		4	8	12	36	4.76	0.029	S
	Epigastric pain		10	20	27	54	12.4	<0.001	HS
	Heart burn		6	12	15	30	25.0	<0.001	HS
	Odynophagia		6	12	14	28	4.0	0.046	S
	Retrosternal pain		10	20	30	60	16.67	<0.001	HS
	Dyspepsia		9	18	30	60	18.54	<0.001	HS
	Recurrent bleeding	Mild	0	0	4	8	4.17	0.041	S
		Moderate	0	0	0	0	1	0.3	NS
		Severe	0	0	0	0	1	0.3	NS
		Total	0	0	4	8	4.17	0.041	S

Table (12): clinical presentation of both studied groups at the end of follow up period

		Group I (No= 50)		Group II (No= 50)		X^2	P	Sig.
		No	%	No	%			
Fever (No. of days of fever over 38.3)	1	11	22.0	12	24.0	0.06	0.812	NS
	2	8	16.0	6	12.0	0.33	0.564	NS
	3	4	8.0	7	14.0	0.92	0.338	NS
	4	5	10.0	8	16.0	0.8	0.372	NS
	5	1	2.0	3	6.0	1.04	0.307	NS
Heptic encephalopathy		16	32.0	13	26.0	0.44	0.509	NS
Ascites	Present	27	54.0	30	60.0	0.37	0.545	NS
	Absent	23	46.0	20	40.0			
Jaundice	Tinge	7	14.0	3	6.0	1.78	0.182	NS
	Clinically apparent	3	6.0	6	12.0	1.1	0.295	NS
LL oedema	Mild	13	26.0	15	30.0	0.2	0.656	NS
	Moderate	5	10.0	9	18.0	1.33	0.249	NS
Abdominal tenderness		8	16.0	6	12.0	0.33	0.564	NS
Diarrhea (No. of days of diarrhea)	1	9	18.0	8	16.0	0.07	0.79	NS
	2	7	14.0	3	6.0	1.78	0.182	NS
	3	2	4.0	1	2.0	0.34	0.558	NS
Chest infection (No. of times of chest infection)	1	7	14.0	4	8.0	0.92	0.338	NS
	2	1	2.0	0	0.0	1.01	0.315	NS

DISCUSSION

Esophageal variceal bleeding is a major cause of mortality in patients with portal hypertension. Endoscopic interventions, either by endoscopic variceal sclerotherapy or better by endoscopic variceal ligation are effective means of control of variceal bleeding [21]. Endoscopic intervention may be followed by ulcer formation, post injection sclerotherapy ulcer or post banding ulcer, that may be exacerbated by gastric acid. These ulcers may lead to further bleeding. Also endoscopic intervention is associated with annoying symptoms like chest pain, dysphagia and heart burn [5,12]. Most of these symptoms are also induced by acid. This study was designed to evaluate the role of rabeprazole as a member of the PPI group in prevention of post sclerotherapy ulcer formation, recurrent bleeding and reduction of the post injection symptoms.

Rabeprazole was selectively used in this study as it is little different in its metabolism, being converted more rapidly to the activated sulphenamide and also dissociated more rapidly from the H^+K^+-ATPase, resulting in both a faster rate of inhibition and a shorter duration of action [22,24]. Rabeprazole is also the PPI less affected by the hepatic CYP2C19 metabolism [23,24]. Rabeprazole was used only for 2 months follow up period to avoid the development of long term use complications of PPIs like increased risk of bone fractures, anemia, and hypo-magnesemia and to limit the cost of the drug as much as possible.

All baseline demographic, clinical, laboratory, sonographic, endoscopic findings, symptoms after first EIS and the Child Pugh class had no statistically significant differences between the 2 groups. Moreover, in the present study there were no statistically significant differences regarding the CBC parameters of both groups during the 2 months follow up period. These results were in agreement with those reported by Dotan et al. [26] who evaluated 468 patients received pantoprazole and 468 controls for the development of thrombocytopenia and found no difference in the incidence of thrombocytopenia between both groups [25].

In this study there were no statistically significant differences between cases and controls regarding endoscopic findings during the follow up period, no statistically significant differences between the two groups in the incidence of sclerosant ulcer development. These results were in concordance with that reported by Shaheen et al. [27] as they evaluated 42 patients after EVL and randomization

into placebo and pantoprazole treated groups (pantoprazole 40 mg for 10 days). They found that subjects receiving pantoprazole after EVL had significantly smaller post-banding ulcers on follow-up endoscopy than subjects receiving placebo. However, the total ulcer number was not different between the groups [26]. Akahoshi et al. [28] also agree with the present study as they found that rabeprazole was associated with faster healing of post sclerotherapy ulcers [27].

In this study there was statistically highly significant decrease in all post injection adverse symptoms in the rabeprazole treated group throughout the follow up period. These results were in agreement with that reported by Akahoshi et al. [28] who compared the results of using rabeprazole 20 mg once daily versus famotidine 20 mg twice daily and found that the H2-blocker group experienced a significantly higher number of days of heartburn and dysphagia than did the PPI group. Finally, they stated that rabeprazole treatment prevents sclerotherapy-associated gastroesophageal reflux and improves the subjective symptoms following EIS [27]. On the other hand, Shaheen et al. [27] used pantoprazole for 10 days on 42 patients and found no significant symptoms improvement and Boo et al. [29] who used pantoprazole for 7 and 14 days and found no significant difference in symptoms [26,28]. This difference could be attributed to the use pantoprazole and the shorter duration of the two studies. Also, the two previously mentioned studies worked using band ligation which by nature has fewer symptoms than sclerotherapy.

In the present study, at the 2^{nd} and 6^{th} weeks of follow up, the incidence of moderate recurrent bleeding was significantly lower in the rabeprazole group, while the incidence of mild and severe recurrent bleeding and total cases with recurrent bleeding were not significantly affected. The total number of patients who developed recurrent bleeding at the end of the follow up period in the treated group was 7 versus 23 in the control group which was highly significantly lowered.

These results were in concordance with the studies conducted by Shaheen et al. [27] and Boo et al. [29], in their studies 3 patients and 2 patients bled from post banding ulcer respectively all were in placebo group. The larger number of recurrent bleeding in the present work may be due to larger sample size and longer follow up period and the fact that sclerotherapy is done on patients with active variceal bleeding unlike banding which is done usually for primary prophylaxis [26,28].

At the end of our follow up period, there were no significant differences between both groups regarding the infectious or non infectious complications that can be induced by the use of rabeprazole. There was no significant difference regarding the development of fever, hepatic encephalopathy, SBP, diarrhea and chest infection. It was found that 32% of cases suffered from hepatic encephalopathy versus 26% of the controls. 14% of cases complained of one attack of chest infection versus 8% of controls and 2% of cases complained of 2 attacks of chest infection versus 0% of controls. 18% of PPI treated cases had one day of diarrhea versus 16% of controls, 14% of cases had 2 days of diarrhea versus 6% of controls, and 4% of cases had 3 days of diarrhea versus 2 % of controls. 57.1% of the admitted cases who had diagnostic follow up ascitic fluid aspiration had developed SBP with TLC of the ascetic fluid equals to or greater than 400 versus 50% of the controls. Conversely, Gipiuliano et al. [30] and Johnstone et al. [31] found increased risk of community acquired pneumonia with the PPI use; in this study no significant difference between both groups in risk of development of pneumonia [29,30]. This difference may be due to short duration and single small dose of rabeprazole use in our study.

Deshpande et al. [32] and Howell et al. [33] found increased risk of colistridium difficile induced diarrhea associated with PPI use. However in this study no significant difference between both groups regarding diarrhea may be because not all cases of diarrhea with PPI use must be due to colistridium difficile infection, besides, in the present study the colistridium difficile toxin wasn't evaluated. Also Deshpande et al. [32] had used prophylactic antibiotics with PPI, which is also associated with increased risk of colistridium difficile diarrhea [31]. Howell et al. [33] used PPI daily or greater than daily dose and for longer duration than ours [32].

In this study there was no significant difference between both groups regarding the incidence of development of SBP, unlike the study conducted by Bajaj et al. [34] in which the incidence of development of SBP in PPI group was 30% [33]. This difference may be due to larger population size, longer follow up period and concomitant

use of antibiotics and the presence of a decompensated cirrhosis group in their study.

In this study no significant difference was found between the 2 groups regarding the development of hepatic encephalopathy, unlike the results recorded by Lin et al. [35] who found that patients with HE had a significantly higher rate of PPI use (89.1%) compared with non-HE patients (63.6%). The difference could be attributed to the larger sample size [34]. Also there were concomitant drug use as lactulose in the present work.

CONCLUSION

The use of rebeprazole at a dose of 20 mg daily after sclerotherapy can help controlling the post sclerotherapy adverse symptoms and reduce the risk of recurrent bleeding. These effects are not reflected on the endoscopical findings. This beneficial effect is mediated without significant increase of the risk of chest, gastrointestinal or ascetic fluid infections and without increasing the rate of hepatic coma.

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Reducing Ventilator-Associated Pneumonia in Neonatal Intensive Care Unit Using "VAP Prevention Bundle"

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Key words: Ventilator-associated pneumonia (VAP), mechanical ventilation, neonatal, bundle approach

Background and study aim : Ventilator-associated pneumonia (VAP) is a serious health care- associated infection, resulting in high morbidity and mortality. It also prolongs hospital stay and drives up hospital costs. Measures employed for preventing ventilator-associated pneumonia in developing countries are scarcely reported. The aim of the current work is to assess the efficacy of our designed "VAP prevention bundle" in reducing VAP rates in the neonatal intensive care unit (NICU).

Patients and Methods: This prospective before-and-after study was conducted at Zagazig university hospital NICU; all neonates who had mechanical ventilation during the period from January 2013 to March 2014 for ≥48 hours were eligible after parental consent. VAP rates were evaluated before (phase-I) and after (phase-II) full implementation of the comprehensive preventive measures specifically designed by our infection control team.

Results: Out of 143 mechanically ventilated neonates, 73 patients developed VAP (51%) throughout the study period (2500 mechanical ventilation days). The rate of VAP was significantly reduced from 67.8% (42/62) corresponding to 36.4 VAP episodes/1000 mechanical ventilation days (MV days) in phase-I to 38.2% (31/81) corresponding to 23 VAP/1000 MV days (RR 0.565, 95% confidence interval 0.408- 0.782, p= 0.0006) after VAP prevention bundle implementation (phase-II). Parallel significant reduction in MV days/case were documented in the post-intervention period (21.50±7.6 days in phase-I versus 10.36 ± 5.2 days in phase-II, p= 0.000). There were trends toward reduction in NICU length of stay (23.9±10.3 versus 22.8±9.6 days, p=0.56) and overall mortality (25% versus 17.3%, p=0.215) between the two phases which didn't reach statistical significance. The commonest micro-organisms isolated throughout the study were gram-negative bacteria (63/66, 95.5%) particularly Klebsilla pneumonia (55/66, 83.4%).

Conclusion: The implementation of our multifaceted infection control bundle has resulted in a significant reduction of VAP rates, length of stay and hospital cost in our NICU. These rates are still far behind the internationally acknowledged ones.

INTRODUCTION

Ventilator-associated pneumonia (VAP) is defined by the Center for Disease Control and Prevention (CDC) as an episode of pneumonia in a patient who requires a device to assist or control respiration continuously through a tracheostomy or endotracheal tube within 48 hours period before the onset of infection [1]. VAP is a serious complication in neonates on mechanical ventilation and account for 6.8%- 32.2% of health-care associated infections among neonates [2,3]. It has a large impact on neonatal morbidity, survival, hospital costs and duration of neonatal intensive care unit (NICU) stay[3,4]. The effect of VAP on health care costs is especially significant in developing countries, whereas most studies on VAP have been conducted in the developed countries [5,6].

Prevention of VAP has been primarily achieved by the "bundle approach". This involves the simultaneous application of several preventive measures for all patients, often aided by tools such as checklist. In some cases there is only theoretical evidence or biologic plausibility for one or more of the elements of the bundle being effective, but application of these bundles is widely used and has been highly successful in the recent years [7].

As neonates have different anatomy, physiology, underlying diseases and they undergo different invasive procedures compared with adults and older children [8], specific studies for evaluating different "VAP bundles" efficacy in preventing VAP in NICU are needed. In Egypt and other developing countries, reports on the success of VAP intervention strategies, particularly among neonates, are scarce. The aim of the present study is to assess the effectiveness of our proposed "VAP prevention bundle" in decreasing different rates of neonatal VAP.

PATIENTS AND METHODS

Setting:
The present study was conducted in the NICU at Children Hospital of Zagazig University, Egypt from January 2013 to March 2014. Our 23 bed NICU is staffed with certified physician 24 hours/day, 7 days/week with a nurse-to-patient ratio 1:3-1:4 depending on the patient acuity. Eight mechanical ventilators and 5 nasal continuous positive airway pressure (CPAP) are available at our unit. A neonatology consultant leads the daily rounds on all NICU patients to review patient's information and updates and develop the care plan.

Design:
This before-and-after intervention prospective study passed through the following periods; **phase-I** at which VAP rate, expressed as the number of VAP episodes per 1000 mechanical ventilator days (VAP/1000 MV days) were calculated for 6 months started at January 2013. Throughout this period we reviewed and summarized recommend-dations by different authors and health institutes regarding strategies for prevention of health care associated infections particularly VAP. Observations were documented by our team members regarding the most prevalent practical errors that may have contributed to increased risk of VAP among our mechanically ventilated neonates. Accordingly, our "VAP preventive bundle" was tailored to stress on our common errors and included common and affordable evidence-based practices recommended by previous studies and agencies [9-15].

Moreover, three months were needed (**inter-mediate phase**), which started at July 2013, until education and full implementation of the bundle by our health care providers were satisfactorily accomplished. During this period we performed several education sessions to discuss evidences about the pathogenesis, risk factors, danger of VAP and its sequel. Training and re-training campaigns were performed for each VAP bundle's item particularly hand hygiene, sterile handling of respiratory equipment, and proper timed mouth care. Finally, signed statement from each staff member acknowledging their understanding of the policy and the mandate to comply with it was taken, to ensure the connection between policy and practice.

VAP prevention bundle :
In addition to routine infection control protocol, our designed bundle was composed of :

- Head-of-bed elevation 30^0-45^0 whenever possible.
- Re-enforcement on hand hygiene practice.
- Sterile suctioning and handling of respiratory equipment.
- Intubation, re-intubation and endotracheal tube (ETT) suctioning as strictly indicated by the unit protocol (document).
- Changing ventilator circuit if visibly soiled or mechanically malfunctioning (document)
- Proper timed mouth care with normal saline and suctioning of oro-pharyngeal secretion.
- Daily evaluation for readiness for extubation to nasal continuous airway pressure (NCPAP) at morning round, and sedation vacation for sedated patients.

Written protocols were performed for strict indications of intubation, re-intubation, suctioning of ETT and change of the ventilator circuits. Documentation was needed in the patient flow sheet for any action delivered. Figure-1 explains the relation between the pathogenesis of VAP and our bundle strategies.

Phase-II was started on October 1st 2013 for six months at which re-evaluation of VAP rate/1000 MV days were performed to assess the efficacy of our infection control bundle.

VAP diagnosis :
VAP was diagnosed by the pediatrician and confirmed by attending neonatology consultant using criteria for less than one year established by Foglia and colleagues [7]. The criteria were as follow, neonatal patients who are mechanically ventilated ≥48 hours must have new onset and persistent abnormal chest radiograph and worsening of gas exchange (desaturations, increased oxygen requirement or increased ventilator demand), and at least three of the following: temperature instability with no other recognized cause; new onset of purulent sputum, change in the character

of sputum, increased respiratory secretions, or increased suctioning requirement; apnea, tachypnea, nasal flaring with retraction of the chest wall or grunting; wheezing, rales or rhonchi; cough; and bradycardia (<100 beat/min) or Tachycardia (>170 beat/min).

Patients:
All neonates admitted to NICU during phases-I and II periods and utilized mechanical ventilation for ≥48 hours were eligible. The patient demographic data, date of admission, underlying disease, duration of MV, length of NICU stay, antibiotics used, and other culture positive infections while on MV were recorded for each case.

Methods:
Complete blood count (CBC), C-reactive protein, blood culture and non- bronchoscopic broncho-alveolar lavage (NB-BAL) by passing 6f-8f sterile catheter through the endotracheal tube and wedging the airway[16] were performed for all clinically suspected VAP. The microbiology lab analyzed the samples using Bact/Alert 3D-Biomerieux-France and provided micro-organism identification followed by antibiotic sensitivity according to the isolate using Vitek MS–Biomerieux-France. Multidrug resistant organisms need special ABX sensitivity order.

Statistical analysis:
Categorical variables were summarized as number and percent while continuous variables were expressed as mean ± standard variation. Chi-square test and student t-test were used for analysis of difference for categorical and continuous variables respectively. Relative risk ratio, 95% confidence interval, and p value were determined for VAP rates outcome. The level of significance was set at $p<0.05$. SPSS statistical software version 16 was used for data presentation and analysis.

RESULTS

A total of 143 neonates were enrolled in the current study as 62 cases in phase-I and 81 cases in phase-II. The two groups were comparable in terms of gender, birth weight, gestational age and mode of delivery as shown in table (1). The leading cause for primary use of mechanical ventilation was prematurity and related complications (46/62 cases, 74.2% versus 63/81, 77.8% in phase-I and phase II respectively). Other causes such as perinatal asphyxia (6/62, 9.7% versus 11/81, 13.6%), respiratory causes other than RDS (4/62, 6.5% versus 3/81, 3.8%), congenital heart diseases (5/62, 8% versus 4/88, 4.9%), others (1/62, 1.6% versus 0%) were diagnosed as primary causes for mechanical ventilation.

Two Thousand Five Hundred days of mechanical ventilation were accrued during the study periods as 1154 MV days in phase-I and 1346 MV days in phase-II. 42/62 (67.74%) episodes of VAP were diagnosed during the pre-intervention period with a rate of 36.4 VAP/1000 MV days. Significant reduction in VAP incidence rate was observed after implementation of our VAP bundle, as31/81 (38.2%) VAP events corresponding to 23 VAP/1000 MV days (RR 0.565, 95%CI 0.408-0.782, Z score 3.437 p=0.0006) were diagnosed in phase II as displayed in table (2). Concomitant significant reduction in MV days/ case was obvious in the post-intervention period when compared to pre-intervention one (21.50± 5.2 days in phase-I versus 10.36±5.2 days in phase-II, p= 0.000). There was a trend toward reduction in NICU length of stay (23.9±10.3 versus 22.8±9.6 days in phase I and phase II respectively, p=0.56) but didn't reach statistical significance. 16/62 (25.8%) mechanically ventilated neonates died in phase-I, 2 cases of them were related to VAP caused by multi-drug resistant klebsilla pneumonia, compared to 14/81 (17.3%) in phase-II, one of them caused by polymicrobial VAP (K. pneumonia and Candida). The difference in overall mortality rates between the two phases didn't reach statistical significance (X^2=1.54, p=0.215).

Seventy-three VAP events were documented throughout the study, 90.4% (66/73) of them revealed positive isolates on culturing their NB-BAL (37/42, 88% in phase-I and 29/31, 93.5% in phase-II). Gram negative bacteria were the most commonly isolated micro-organisms (97.2% versus 93.1% in phase-I and II respectively), klebsilla pneumonia was the leading causative pathogen throughout the study period. No single case of Gram-positive isolates was diagnosed in phase-I cases, compared to 6.9% (2 cases) among those in phase-II. Fungus, namely Candida spp. was the single isolate from one case in phase-I, but were isolated mixed with gram negative bacteria in three cases of phase-II (10.4%) as described in table (3).

Table (1) : Demographic and clinical characteristics of study populations

Character	Phases		Test	p
	I	II		
Number of ventilated neonates	62	81		
Gender (male)				
Number	43	52		
%	69.3%	64.1	χ^2 0.42	0.52
Gestation age (week)				
< 37 Number (%)	45 (72.5)	66 (81.5)	χ^2 1.60	0.21
- 30-37	20 (32.2)	34 (41.9)		
- <30	25 (40.3)	32 (39.6)		
> 37 Number (%)	17 (27.5)	15 (18.5)		
Mean ±SD	32.18±4.5	31.73±4.3	t 0.59	0.57
Birth weight (g)				
≤ 2500 Number (%)	48 (77.4)	63 (77.7)	χ^2 0.00	0.99
- <1500	28 (45)	43 (53)		
> 2500 Number (%)	14 (22.6)	18 (22.3)		
Mean ±SD	1898±954	1803±1074	t 0.54	0.59
Mode of delivery (% C/S)	32.2	43.2	χ^2 1.79	0.18
Days of mech. ventilation				
Min	5	5		
Max	51	35		
Mean ±SD	21.50±7.6	10.36±5.2	t 4.73	0.000
Length of NICU (day)				
Min	7	10		
Max	63	45		
Mean ±SD	23.87±10.3	22.8±9.7	t 0.58	0.56
Mortality Number (%)	16 (25.8)	14 (17.3)	χ^2 1.54	0.22

t student t test, χ^2 chi-square, SD : Standard Deviation, C/S : Caesarian Section, NICU : Neonatal Intensive Care Unit.

Table (2): Ventilator-associated pneumonia rates

Item	Phases	
	Phase I	Phase II
Number MV Neonate	62	81
VAP episodes	42	31
MV days	1154	1346
VAP %	67.8	38.2
VAP/1000 MV days	36.4	23
Relative Risk (RR)	0.565	
95% C.I.	0.408-0.782	
Z score	3.437	
P	0.0006	

MV : Mechanical ventilation, VAP : Ventilator-Associated Pneumonia, CI : Confidence Interval

Table (3): Microbiologic features of VAP pathogens

Pathogen	Phases		Total
	Phase I	Phase II	
Positive Culture number (%)	37 (88)	29 (93.5)	66 (90.4)
Gram-negative number (%)	36 (97.2)	27 (93.1)	63 (95.5)
- K-pneumonia	32 (86.5)	23 (79.3)	55 (83.4)
- P-aeruginosa	2 (5.4)	4 (13.8)	6 (9)
- E-coli	2 (5.4)	- (-)	2 (3)
Gram-positive number (%)	- (-)	2 (6.9)	2 (3)
Fungi number (%)	1 (2.7)	3* (10.4)	4 (6)
MDR number (%)	9 (24.3)	8 (27.6)	17 (25.8)

K : klebsiella, P pseudomonas, E : Escherichia, MDR : Multiple-Drug Resistant,
*** Three cases in phase-II showed combined K pneumonia and Candida spp isolates.**

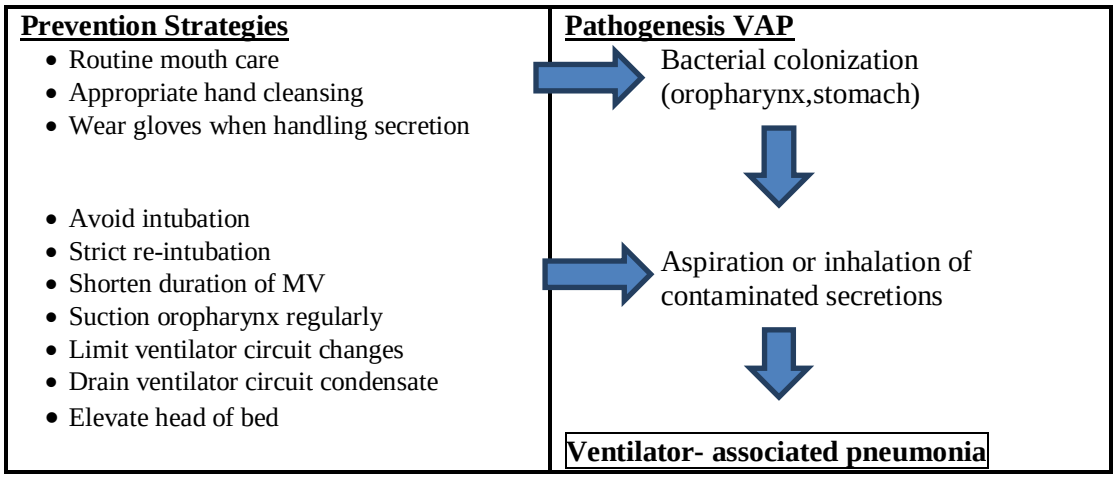

Fig. (1): Relationship between VAP pathogenesis and its preventive strategies

DISCUSSION

Advances in neonatal intensive care have improved survival among very low birth weight infants. As many of them require mechanical ventilation, ventilator-associated pneumonia (VAP) has become a major challenge. It represents an important cause of morbidity and mortality in this high-risk population [17]. Data obtained from the current study runs in parallel with this fact as 50% (71/134) of mechanically ventilated neonates enrolled in the study were very low birth weight (VLBW) and 77% (111/134) were premature. Developmental immaturity in the neonatal immune system including greater permeability of the skin and mucus membrane, lower level of immunoglobulin, and decreased complement activity all of which increase their susceptibility to nosocomial infection. Mechanical ventilation and other invasive treatment measures are very likely to increase risk of oro-pharyngeal or trachea-bronchial colonization with pathogenic bacteria, VAP occurs when bacterial, viral or fungal pathogens enter the sterile lower respiratory tract and lung parenchyma[18].

Several studies have shown a reduction of VAP rate after guidelines implementation into a bundle [9-15]. The power of the bundle is that it brings together several evidence-based practices that individually improve care, but when applied together, may result in an even greater improvement in the desired outcome [18]. "VAP preventive bundle" implemented in the present work was associated with statistically significant reduction in VAP rates in our NICU (36.4/1000 MV days in phase-1 versus 23/1000 MV days in phase-II, p=0.0006). All items involved in our proposed bundle were derived from controlled trials or health institutes recommendations for adults, children or neonatal VAP prevention [9-15].

Most adult VAP prevention bundles recommend elevation of the head of a ventilated patient's bed from 30-45 degrees to reduce the risk of aspiration

of contaminated oro-pharyngeal and gastrointestinal content. Drakulovic and colleagues demonstrated that a semi-recumbent position reduced the rate of clinically suspected and microbiological confirmed VAP [19]. Only one underpowered pediatric trial presented in an abstract form has evaluated this intervention and showed no effect [20]. The logic of head-of-the bed elevation is sound, it is found in almost every VAP reduction bundle and its implementation was easy and accepted by health care providers in our work.

There is unequivocal evidence that hand hygiene is the most important infection control intervention in all health care setting, but also one of the most difficult strategies to maintain. Gram negative organisms which colonize the ETT are frequently carried on the hands of the care-givers [21, 22]. Several hand hygiene training campaigns were conducted throughout the study period, 6-steps hand washing posters were displayed on all sinks, alcohol-based hand rub solution were placed at each bedside, and in the corridor between rooms to improve compliance with hand hygiene.

Breathing circuit condensate contamination can also serve as a mechanism for the pathogenesis of VAP, the condensate that collect in the tubing should be drained away to prevent aspiration [23]. CDC recommended; ensuring proper sterilization of reusable respiratory care equipment, using sterile water in humidification system, periodic drainage of condensate from the breathing circuit and hand hygiene before and after contact with respiratory equipment. CDC guidelines do not recommend changing the breathing circuit unless it is visibly soiled or mechanically malfunctioning [9]. We followed the CDC strategies regarding ventilator care in our bundle. Similarly, recent study concluded that decreasing the ventilator circuit changes from every 7 days to every 14 days has no adverse effect on the rate of VAP in NICU [24]. Yuan and his team reported that the risk factors for the development of neonatal VAP were re-intubation, frequent ETT suctioning, and the duration of mechanical ventilation [25], Tan and his fellows proved the same findings [26]. The use of non-invasive measures such as nasal CPAP and nasal prong ventilation may reduce VAP rate. In time–sequenced cohort studies, reducing days of mechanical ventilation by non-invasive respiratory support decreased VAP incidence [27, 28]. Pneumonia is less common in neonates treated with nasal CPAP when compared with those intubated on MV (1.9/1000 CPAP days versus 12.5/1000 MV days, p=0.04) [27]. Results from the German Surveillance System for VLBW infants supported the protected value of NCPAP against pneumonia, as its incidence was 1/1000 CPAP days compared to 2.5/1000 MV days [29]. In our bundle, attending physician should assess, on daily bases, the readiness of every mechanically ventilated neonate for weaning to NCPAP and every effort was done to wean them as soon as possible.

CDC recommended a comprehensive oral hygiene program for mechanically ventilated patient [9]. A meta- analysis by Pineda and colleagues showed reduction in VAP among adult patients treated by decontamination with oral chlorhexidine [30]. Similar protective results were concluded by meta-analysis by Chlebichi and Safdar in which chlorhexidine rinse was used [31]. Neonates are likely at greater risk for aspiration of contaminated oral secretion, because endo-tracheal tubes used to ventilate them are un-cuffed [18]. As chlorhexidine gluconate is not approved for infants less than 2 months, timed mouth care with normal saline and oro-pharyngeal suctioning were included in our bundle.

The criteria defined by Foglia and his colleagues were used throughout the present study periods to ensure uniformity of the results. The CDC/ NHSN (National Health safety Network) proposed protocol clarification in July 2013, at which leukocytosis (>15.000 WBC's) or leucopenia (<4000 WBC's) and shift to left (>10% band forms) were added [32].VAP rates has been reported from both developed and developing countries, the National Healthcare Safety Network reported that VAP rate in level III NICUs of US hospital in 2010 were in the range 0.4-1.4/1000 MV days [33]. In the International Nosocomial Infection Control Consortium, the mean rate from 36 NICUs around the world between January 2004-December 2009 was 9.0/1000 MV days [34]. In the German Nosocomial Infection Surveillance System, the mean VAP rate was 5.5/1000 MV days [29]. On the other hand, in 55 intensive care units of 8 developing countries between 2002-2005, the overall VAP rate was 24.1/1000 MV days ranging from 10.0-52.7/1000 MV days between units [35]. Data from Asian countries suggested incidence rates varying from 3.5-46/1000 MV days in the newborn period [36].VAP rate in our study during the post-intervention period, 23/1000 MV days, was comparable to that in developing countries, but still significantly higher than the benchmark

(1.5/1000 MV days) in developed countries. The lack of respiratory therapists, overcrowding in hospital, and relatively high nurse-to-patient ratio in our country's NICUs may explain such disparity. In addition, conduct of rigorous nosocomial infection surveillance on a multicenter collaborative network level by NICUs in most developed countries is a major factor in the gap. Significant reduction in mean mecew2hanical ventilation days/ case were achieved in our neonates in the post-intervention period, an important goal especially in premature to reduce the hazards of MV in such population. However, reduction in MV days was not associated with similar reduction in length of NICU stay, a finding which is expected when dealing with neonates particularly premature, as NICU stay is dependent on several factors mainly; the baby's gestational age, severity of underlying illness and hospital course of which, health-care associated infection is an important factor.

The overall mortality rate among our cases showed a trend toward reduction during the post-intervention period, but didn't reach statistical significance (17.3% in phase-II versus 25.8% in phase-I, p=0.215). As we did not match patient to detect adjusted attributable mortality, it is not possible to conclude that the reduction in mortality is attributable to the decrease in VAP rate.

The predominant pathogen of VAP in our study was bacteria, gram negative bacteria outnumbered the gram positive strains. Similar finding was shown in Yuan and colleague work [25] and Xie and team trial [37], while staphylococcus aureus and P. aeruginosa were the most frequently identified pathogen in VAP in western pediatric populations [38,39]. The difference in bacterial spectrum between ours and that reported from western countries may be due to varied practices, especially antibiotic selection. Exposure of NICU patients to different antibiotics favors selection and subsequent colonization with different pathogens that may leads to VAP. Awareness of local microbiological surveillance data on noso-comial infection can improve the selection of appropriate therapy. Even-though, the incidence of VAP was reduced with bundle implementation in our NICU, there was no significant difference in the incidence of multi-drug resistant organisms, probably due to resistant of health care providers to follow strict antibiotics use as advised by many infection control specialist [40].

In summary, this study provided characterization of VAP in an Egyptian NICU. It demonstrated that a bundle of infection control practices can effectively reduce the occurrence of VAP during neonatal ventilation. This "VAP prevention bundle" can be extended to other NICUs in Egypt and other low-income countries.

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Conflict of interest: None.

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Video case: Dense Infiltration by Rhabitiform Larvae of *Strongyloides stercoralis* during Childhood

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The densely concentrated number of rhabditiform larvae of *Strongyloides stercoralis* was isolated from a fresh stools sample of a 13 years old male child . The stools sample was centrifuged at the speed of 1000 rpm (revolutions of the rotor per minute) for 2 minutes.
The supernatant was discarded and then about 3 drops of the supernatant was added to the sediment at the bottom of each centrifuge tube that were shaked well.
Now, the shaked suspensions of the sediment were collected in a vial.
Next, a drop of the shaked suspension was placed on a clean slide with the help of a dropper and covered with a cover slip.
After that, the preparation was focused on under the low power objective lens of the compound light microscope and the motile rhabditiform larvae you have seen were recorded with a digital camera from the field of vision of the microscope.
There were no serious clinical manifestations with the child from whom this heavy load of rhabditiform larvae was obtained.
The child was treated and cured successfully with a single dose of 6 mg ivermectin tablet on an empty stomach.

Image Case: Diffuse Bowel Polyposis during Childhood

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This eight years old female child that presented by long history of anemia and passage of fleshy tissues per anus. She was examined locally by surgeon and there was no local cause for tissue passage. On examination she was pale and showed brown pigmentations of the mucous membrane in the lower lip and cheeks and also the tongue (Figure 1 and 2). We decided to examine her by colonoscopy and there were large sized polyps (Figure 3 and 4) involving the caecum, ascending and sigmoid colon that was successfully snared and hisopathological examination described villous adenoma. Later we examined her by upper endoscopy and surprisingly showed variable sized polpyps involving the gastric corpus, antrum and also the duodenum and upper jeujenum, histopathological examination of the gastric polyps showed also villous adenoma. The polyps and pigmentation raised the probability of **Peutz-Jeghers syndrome**.

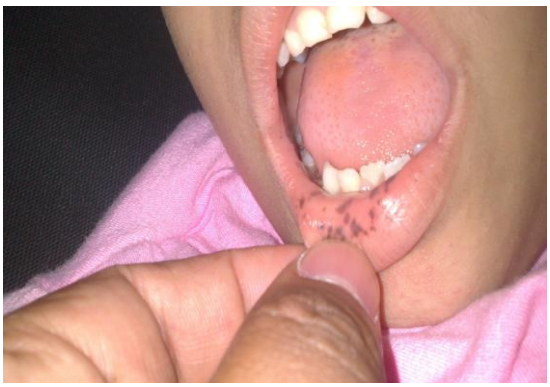

Figure (1): Pigmentations along the inner aspect of lower lip

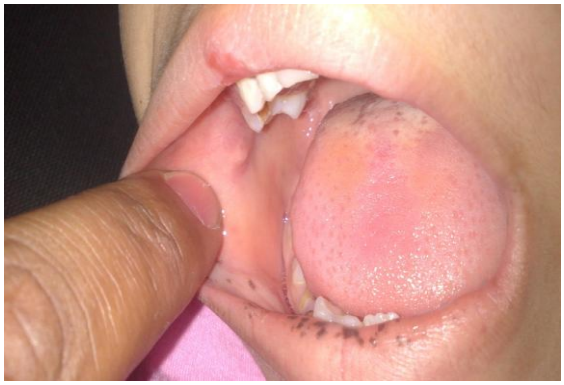

Figure (2): Pigmentations along the inner aspect of the cheek and tongue

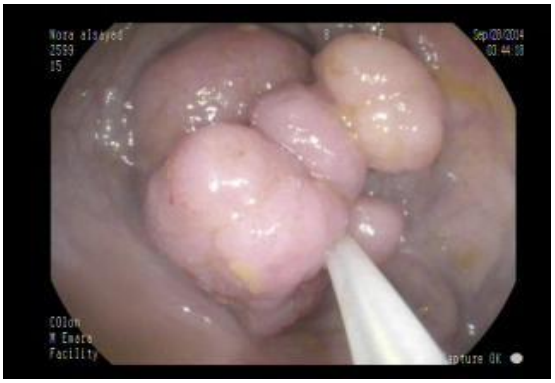

Figure (3): Large polyp (snared) in the ascending colon

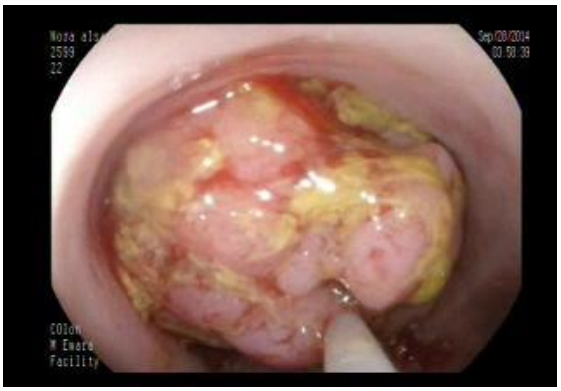

Figure (4): Extraction of snared large polyp from the sigmoid colon