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Contents

Study of Interleukin-18 in Chronic hepatitis C virus related liver diseases

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Key words: Interleukin, Hepatitis C, ELISA, Cirrhosis

Background and study aim: ELISA can determine serum Interleukin (IL)-18 level. It is a sensitive, simple and rapid test, thus help to study changes of serum IL-18 levels in chronic HCV related liver diseases during different stages. The objective of this study was to study serum IL-18 levels in chronic HCV related liver diseases.

Patients and methods: Sera from 60 patients with HCV related chronic liver diseases at various stages of HCV infection (chronic hepatitis, cirrhosis and complications) and sera of 10 normal controls were subjected to measurements of serum IL-18 level by ELISA assay.

Results: There were highly significant increase in the mean values of serum IL-18 in chronic HCV related liver cirrhosis, non complicated and complicated patients in comparison to chronic active hepatitis C patients and healthy subjects and highly significant increase in the mean values of serum IL-18 in complicated patients in comparison to non complicated patients**.** There was highly significant increase in the mean values of serum IL-18 in decompensated liver cirrhosis patients when compared to compensated patients.

Conclusion: Serum IL-18 level shows highly positive significant correlation with severity of liver dysfunction in HCV related liver cirrhosis.

INTRODUCTION

Hepatitis C (HCV) virus infection is a major cause of chronic liver disease worldwide, up to 70% of patients develop chronic infection which, in 20% of cases, will progress to cirrhosis and finally to hepatocellular carcinoma **[1]**.

Indeed, HCV is not directly cytopathic, and the mechanisms by which it causes liver injury are not well established. Immune response that essentially conducted by cytokines may play an important role in the pathogenesis of HCV infection **[2]**.

A strong T-helper lymphocyte-cell response, characterized by the production of interleukin-2, tumor necrosis factor-alpha and interferon-gamma, seems to be associated with HCV clearance; however, in the context of a persistent infection, they may be responsible for liver damage **[3]**.

Cytokines genes are polymorphic at specific sites, and some of these mutations have been associated with inter-individual variations of cytokine expression. The Interleukin (IL)-18 gene is located on chromosome 11q22.2-q22.3 and a variety of single nucleotide polymorphisms have been detected within IL-18 gene sequence. Several of these polymorphisms, especially those located in the promoter region, may be associated with differential levels of gene transcription **[4]**.

IL-18 first identified as an IFN-γ inducing factor, is mainly produced by activated macrophages and Kuffer cells, and plays a strategic role in inflammation and liver injury. It exerts a proinflammatory activity by enhancing

the Fas ligand and perforin-mediated T-cell and natural killer-cell cytotoxicity, stimulating Th_1-cell development, inducing chemokines, and decreasing IL-10 expression in T cells. Besides, IL-18 increases the susceptibility of liver endothelial cells to undergo apoptosis **[5]**.

In chronic hepatitis C and cirrhosis, an increase in the expression of proinflammatory cytokines, in particular IL-18, has been shown, which correlates with IFN-γ production **[6]**.

The objective of this study was to study serum IL-18 levels in chronic HCV related liver diseases.

PATIENTS AND METHODS

This study was carried out in Tropical Medicine Department, Faculty of Medicine, Menofia University Hospitals. The study was conducted on four groups: Group I: Included twenty patients with chronic active hepatitis C were selected from patients waiting the interferon based antiviral treatment; all performed liver biopsy and showed hisopathological features of chronic active hepatitis. Group II: Included twenty patients with chronic HCV related non complicated liver cirrhosis (compensated or decompensated). Group III: Included twenty patients with complicated chronic HCV related liver cirrhosis. Group IV: Included ten healthy persons of matched age and sex as control group. After having an informed consent; each patient underwent: Detailed history taking, thorough clinical examination, laboratory investigations including liver profile tests (ALT, AST, bilirubin, serum albumin and INR), viral markers (HCV antibody, HBsAg), quantitative HCV RNA by the PCR, serum alpha-fetoprotein, imaging study (abdominal ultrasonography and triphasic CT scan for HCC patients) and measurement of serum IL-18 using ELISA kit.

Exclusion criteria:
Patients with chronic liver diseases due to any cause other than chronic HCV and patients with any disease known to affect serum level of IL-18 (chronic infection, diabetes mellitus, renal failure, coronary heart disease, other malignancies, etc) were excluded from the present study.

Statistical analysis:
Data were collected, tabulated and statistically analyzed by computer data using SPSS (Statistical Package for Social Sciences) version 15. Qualitative data were presented as number and percent. Comparison between groups was done by Chi-Square test. Quantitative data were tested for normality by Kolmogrov-Smirnov test. Normally distributed data were presented as mean ± SD. Student t-test was used to compare between two groups. F-test (One Way Anova) was used to compare between more than two groups. Pearson's correlation coefficient was used to test correlation between variables. $P<0.05$ was considered to be statistically significant.

RESULTS

No statistically significant differences were reported among the studied groups as regarding age and sex as shown in Table (1).

There were a highly significant increases in the mean values of serum IL-18 in chronic HCV related liver cirrhosis, non complicated and complicated patients in comparison to chronic active hepatitis C patients and healthy subjects. Also, there was a highly significant increase in the mean values of serum IL-18 in complicated patients in comparison to non complicated patients as shown in Table (2).

It is obvious from our results that IL-18 correlates with progression of cirrhosis, where a highly significant increase in the mean values of serum IL-18 in decompensated liver cirrhosis patients was noticed when compared to compensated patients as shown in Table (3).

The serum IL-18 concentrations were related to the Child–Pugh severity of liver disease in cirrhotic patients as shown in Figure (1).

The complications of liver cirrhosis do have an impact on the level of IL-18. There was a highly significant increase in the mean values of serum IL-18 in cirrhotic patients complicated by HCC followed by cirrhotic patients complicated by hepatorenal syndrome (HRS) as shown in Table (4).

Table (1): Age and sex distribution among studied groups

	Age			Sex			
	N	Mean ± SD	Range	Male		Female	
Group I	20	48.55 ± 5.79	41 – 60	14	70%	6	30%
Group II	20	52.45 ± 7.37	39 – 63	13	65%	7	35%
Group III	20	51.00 ± 6.71	38 – 61	12	60%	8	40%
Group IV	10	51.10 ± 6.90	43 – 62	5	50%	5	50%
Test	**F = 1.170**			**$\chi 2$ = 1.254**			
P value	**0.328**			**0.740**			

Table (2): Mean values of serum IL-18 among the studied groups

Groups / **IL-18**	**Group 1 (n = 20)**	**Group II (n = 20)**	**Group III (n = 20)**	**Group IV (n = 10)**
M ± SD	367.10±64.68	743.95±132.62	1133.55±273.47	289.50 ± 40.26
Range	254 – 450	553 – 1129	815 – 1670	224 – 345
F. test	**91.353**			
P. value	**< 0.001**			
LSD test♦	GI & GII** GI & GIII** GII & GIII** GII & GIV** GIII & GIV**			

♦ Least significant difference test.
* Significant ** Highly significant

Table (3): Mean values of serum IL-18 in group II patients in relation to the state of decompensation

Group II	**IL-18**				
	N	**Mean ± SD**	**Range**	**t**	**P**
Compensated	11	652.09 ± 57.69	553 – 739	5.388	<0.001*
Decompensated	9	856.22 ± 108.76	763 – 1129		

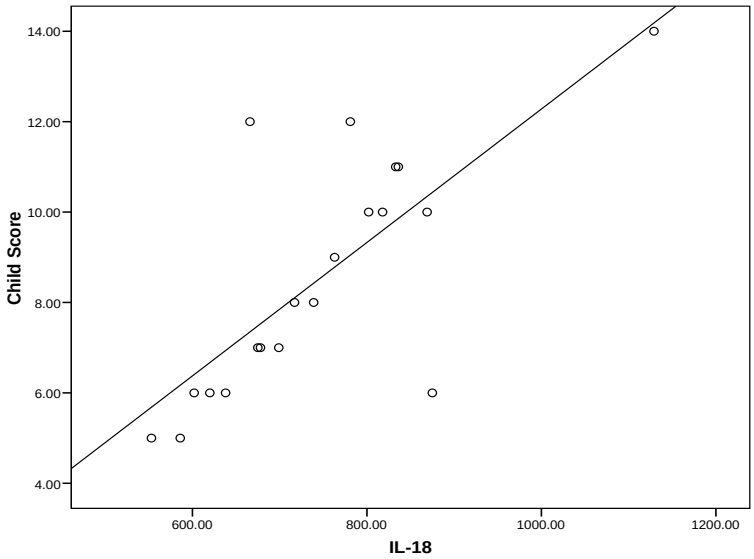

Figure (1): Shows highly significant positive correlation between Child-Pugh score and serum IL-18 level in group II patients.

Table (4): Mean values of serum IL-18 in group III patients in relation to types of complications

Complication / IL-18	HCC (n = 7)	HRS (n = 6)	SBP (n = 7)
M ± SD	1439.14 ± 182.23	1084.43 ± 103.08	870.14 ± 49.34
Range	1262 – 1670	965 – 1238	815 - 950
F. test	**36.737**		
P. value	**< 0.001**		
LSD test	HCC & HRS** - HCC & SBP** - HRS & SBP**		

HCC; Hepatocellular Carcinoma, HRS; Hepatorenal Syndrome, SBP; spontaneous Bacterial Peritonitis

DISCUSSION

HCV infection leads to chronic liver disease in 70% of infected patients, who may develop cirrhosis and HCC during the course of infection. Persistent liver inflammation is strongly associated with HCV-mediated liver disease progression and also increases the risk of an aggravated immune response and fibrosis **[7]**.

IL-18 increases the susceptibility of liver endothelial cells to undergo apoptosis. Significant evidences indicating that IL-18 plays a prominent role in liver injury were reported **[8]**. The present study showed highly significant increase in the mean values of serum IL-18 in chronic HCV related liver cirrhosis, non complicated and complicated patients in comparison to chronic active hepatitis C patients and healthy subjects. These findings were in agreement with Jia et al. **[9]** who reported that patients with chronic hepatitis C had significantly higher serum levels of IL-18 compared with asymptomatic HCV carriers and controls by studying serum IL-18, IL-10 and sIL-2R levels in 27 patients with chronic hepatitis C and 24 asymptomatic HCV carriers in addition 10 healthy individuals. Suggesting that IL-18 and tested cytokines co-participate in the pathogenesis of chronic hepatitis C. Similarly, Sharma et al. **[10]** reported that the mean levels of IL-18 were significantly elevated in patients with chronic hepatitis C and HCV-related cirrhosis when compared with the normal controls.

The difference between the chronic hepatitis and cirrhotic groups was also significant. IL-18 protein production was quantified in serum samples from 50 patients in different stages of HCV infection and compared with 15 normal healthy controls. As necrosis and fibrosis are associated, independent correlations in only six patients, in whom IL-18 levels were strongly related to necroinflammation. Thus, the pathological consequences of infection mediated by IL-18 are clearly detrimental to the host.

Also, Bouzgarrou et al. **[11]** reported that plasma levels of IL-18 were elevated in chronic active hepatitis C patients than healthy controls, cirrhotic patients had higher levels than non cirrhotic patients and elevated in HCC patients than cirrhotic patients. Moreover, Selim et al. **[12]** reported that IL18 levels are elevated in chronic hepatitis C patients than in healthy subjects. IL-18 level is significantly increased with the increase in the histological stage of fibrosis, disease progression is accompanied by an increase in plasma IL-18 and strongly support the involvement of IL-18 in causing liver injury. Thus IL-18 could be used and nominated as an additional non invasive marker for monitoring the degree of liver fibrosis in chronic hepatitis C patients.

Statistical analysis revealed highly significant increase in the mean values of serum IL-18 in decompensated liver cirrhosis patients when compared to compensated patients and highly significant positive correlation between Child-Pugh score and serum IL-18 level in group II patients. These results are in agreement with Urushihara et al. **[13]** who reported that serum level of IL-18 in patients with HCV as disease progressed from non cirrhotic to cirrhotic as well as with deterioration of cirrhosis from Child-Pugh stage A to stage Child-B and then to stage Child-C, further increased IL-18 serum levels were observed. Similarity, Tilg and Diehl **[14]** found that plasma IL-18 levels were increased with disease progression independent of the etiology of chronic liver disease.

IL-18 mRNA expression was significantly up-regulated in the peripheral blood mononuclear cells (PBMC) of cirrhotic patients when compared with other groups, while in the liver, higher levels of IL-18 transcripts were expressed in patients with chronic hepatitis C. The results indicate that IL-18 levels reflect the severity and activity of HCV infection, and may contribute to the pathogenesis and progression of liver disease

associated with HCV, deterioration of cirrhosis from compensated stage to stage of decompensation, further increased IL-18 serum levels were observed **[10]**. Interestingly, IL-18 binding protein (IL-18BP) levels paralleled the increase of IL-18 with disease progression. Only in stage Child C were IL-18BP levels decreased, while IL-18 levels were still increased **[15]**.

Statistical analysis of this study revealed highly significant increase in the mean values of serum IL-18 in patients with HCC as compared to other cirrhotic complications. And highly significant increase in the mean values of serum IL-18 in patients with hepatorenal syndrome (HRS) as compared to patients with spontaneous bacterial peritonitis. Although patients with spontaneous bacterial peritonitis (SBP) show lowest level of serum IL-18 as compared to other cirrhotic complications but higher than patients with liver cirrhosis without SBP. These results are in agreement with Yumoto et al. **[16]** who reported that serum IL-18 and IFN-γ levels were significantly increased in patients with HCC than in patients with chronic viral hepatitis, liver cirrhosis and healthy volunteers. The authors suggested that IL-18 and IFN- γ may be involved in the pathogenesis of hepatic carcinogenesis.

Similarity, Hamouda et al. **[17]** reported that serum IL-18 increased significantly in patients of HCC complicating HCV related cirrhosis more than chronic HCV patients (with or without cirrhosis) and healthy subjects. The study was conducted on fifty subjects by measuring serum IL-18 level in patients and control classified into four groups (chronic HCV infection without liver cirrhosis, chronic HCV infection with liver cirrhosis, HCC on top of chronic HCV liver cirrhosis and healthy subjects). Moreover, Ahmed et al. **[18]** reported that serum levels of IL-18 are elevated in HCC patients than in healthy subjects. IL-18 level is significantly increased with the increase in the tumor size and its concentration may predict the degree of hepatocellular damage. Thus IL- 18 could be used and nominated as an additional non invasive marker for monitoring the degree of liver damage.

The significant increase in the mean value of IL-18 in cirrhotic patients complicated by HRS in the present study were in agreement with Qasem et al. **[19]** who reported that IL-18 in HRS patients were higher than those of patients with liver cirrhosis and chronic hepatitis. The authors reported that serum creatinine is a poor biomarker for early detection of renal impairment in cirrhotic patients while IL-18 was early biomarker of acute kidney injury in cirrhotic patients.

The significant increase in the mean value of IL-18 in cirrhotic patients complicated by SBP in the present study was in agreement with Shin et al. **[20]** who reported that serum IL-18 in patients with SBP was significantly more elevated than in patients with liver cirrhosis and than healthy volunteers. IL-18 concentration was significantly increased in cirrhotic patients who developed SBP, in those with culture-positive peritonitis, and in those who developed organ failure, as compared with the other patients.

CONCLUSION

Serum IL-18 level shows highly positive significant correlation with severity of liver dysfunction in HCV related liver cirrhosis. Serum IL-18 may play a role in pathogenesis of hepatic decompensation and occurrence of hepatic complications in patients with HCV related cirrhosis.

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Vitamin D Profile : Can it Affect the Response to Standard Hepatitis C Treatment in Egyptian Patients ?

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Background and study aim : Vitamin D is a potent immunomodulator. It is reported to be related to the severity of fibrosis and responsiveness to interferon-based therapy in genotype 1 chronic hepatitis C (CHC), so we aimed to evaluate if there is an association between vitamin D metabolism related genes and vitamin D level with the degree of liver damage and the response to treatment of CHC in our locality.

Patients and methods : Two hundred and forty five Egyptian patients (123 patients with sustained virological response and 122 patients with treatment failure) were included. They were subjected to routine investigation needed for treatment, in addition to estimation of 25-OH vitamin D level in serum by ELISA and CYP27B1-1260 gene polymorphism by PCR-RFLP method.

Results: We found that serum levels of vitamin D showed statistically significant increase in responders in comparison with non responders. The distribution of CYP27B1-1260 AC and CC genotypes were significantly presented in the non responders in comparison with the responders. CYP27B1-1260 AC and CC genotypes carriers had a high risk for treatment failure, (OR= 14.7, 95% CI= 2.3-20.1, P<0.001; OR = 20.4, 95% CI= 2.9-21.3, P<0.005 respectively). Serum levels of vitamin D showed statistically significant negative correlations with the activity and fibrosis of the liver in both responders and non responders. Also, there was negative correlation between vitamin D level and viral load in non responder patients (r= -.232, P=0.01). As regard the value of serum vitamin D level in discriminating responders from non responders; area under the ROC curve was 0.708 (95% CI 0.643-0.774). At a cutoff value of 19 ng/dL of serum vitamin D yielded sensitivity 79%, specificity 58%, positive predictive value (PPV) 65%, and negative predictive value (NPV) 73%.

Conclusion: Vitamin D serum level and CYB27B1 -1260 genotype could be used as a predictor to anti HCV treatment response in our locality.

INTRODUCTION

Egypt reports the highest prevalence of hepatitis C virus (HCV) world wide, ranging from 60% to more than 40% among regions and demographic group **[1]**. The recommended therapy for chronic hepatitis C, is pegylated interferon and ribavirin for 24 or 48 weeks **[2]**. Sustained virological response (SVR), defined as undetection of HCV RNA in patient's serum for 6 months after end of treatment, is ranging from 42.9% to 69% in patients with genotype 4 **[3-4]**. Several factors are associated with treatment failure including host and viral predictors such as body weight, ethnicity, liver histology, genotype, viral load and metabolic factors such as elevated fasting glucose **[5,6,7]**.

Vitamin D was initially identified as a calcium homeostatic hormone. Vitamin D is now known to have pleiotropic functions, dealing with both innate and adaptative immunity. Calcitriol mediates its biological effects by binding to the vitamin D receptor (VDR), which is expressed not only by intestine, bone and kidney but also on cell membranes of T lymphocytes, B lymphocytes, dendritic cells and macrophages responsiveness**.** Immunomodulatory actions of vitamin D are elicited through its direct action on T-cell antigen-presenting cell function **[8]**.

Moreover vitamin D improves insulin sensitivity, suppresses proinflammatory cytokines, increases anti-inflammatory cytokines, and improves CD4 T cell hyper-responsiveness **[9,10]**.

Vitamin D deficiency is very common among patients with chronic liver disease (92%), and at least one-third suffer from severe vitamin D deficiency (<12 ng/ml) **[11]**. Serum vitamin D deficiency and the CYP27B1-1260 promoter polymorphism are more prevalent in patients with chronic hepatitis C and related to more fibrosis, and that they are associated with a lower response rate to interferon-alfa based therapy in genotype 1 chronic hepatitis C (CHC) **[12]**.

Our aim is to evaluate the relationship between vitamin D metabolism-related genes and vitamin D level and the response to standard care of treatment for chronic hepatitis C infection in our locality, as genotype 4 is the predominant.

PATIENTS AND METHODS

This cross-sectional study was carried out in Tropical Medicine Department and Medical Biochemistry Department, Zagazig University Hospital, 245 Egyptian patients with compensated liver function out of 270 patients with chronic HCV, their ages ranged from 18 to 65 years, were enrolled in this study during the period from January 2013 to January 2014. All patients were receiving Peg interferon-á-2b (1.5 ug/kg per week) plus ribavirin (1000-1200 mg/d). the studied population included 123 patients with sustained virological response defined as undetectable HCV RNA at 24 weeks post treatment and another 122 patients with treatment failure," a non-responder is someone who does not have disappearance of the HCV RNA, does not ever have a 2-log drop in hepatitis C viral load at 12 weeks, and if HCV RNA was still detectable at week 24 in those patients in whom HCV RNA dropped more than 2 log at 12 week **[13]**. Both groups were matched for age, sex and body mass index**.**

Exclusion criteria :
The patients were excluded from the study if their WBCs less than 4000/mm^3, absolute neutrophil count of <1500 per mm^3, a platelet count of <90 000 per mm^3, hemoglobin level was abnormal, or if they had increased serum bilirubin more than 2 mg/dl **[14]**. Also patients with hepatitis B, auto immune hepatitis, metabolic liver disease, hepato-cellular carcinoma, renal failure and heart failure, decompansated chronic liver disease, or those who had any problem necessities stoppage or interruption of treatment were excluded **[15]**. In addition to those who are previously treated or those were receiving adjuvant medication with immunemodulatory effect.

An informed consent was taken from each patient. All patients were subjected to complete history taking, full clinical examination, ultrasonographic and histopathological evaluation according to Metavair score **[16]**.

Laboratory investigation :
All subjects were subjected to routine laboratory investigation including complete blood picture, liver function test and renal function test. Viral markers including hepatitis C virus antibodies (HCV Abs), hepatitis B surface antigen (HBsAg) and hepatitis B core antibodies (HBcAbs) were tested using ELISA. HCV RNA levels were determined by using the Real time PCR (Step One Real-time PCR System, Applied Biosystem), performed strictly in accordance with the manufacturer's instructions. Serum alpha-feto protein, thyroid hormone (triiodothyronine (T3), thyroxine (T4) and thyroid stimulating hormone (TSH), anti nuclear antibodies (ANA Abs) and 25-OH vitamin D level in serum were measured using ELISA kits (kits provided by Biosource Europe S.A, Belguim).

Isolation of DNA :
Genomic DNA was extracted from EDTA whole blood using a spin column method according to the protocol (QI Aamp Blood Kit; Qiagen GmbH, Hilden, Germany).

Genetic polymorphism detection of the CYP27B1 gene :
The -1260C>A polymorphism (rs10877012) of the CYP27B1 gene was analyzed by restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR) method described by Jennings et al. **[17]**. The 1260 C was amplified in a single product using the primers forward 5-GTGTTCCCTAAGTGTTGTCTC-3 and reverse 5-GCTGACTCGGTCTCCTCTG-3. Fragments were amplified in 50µl reaction mixtures containing 10 µl genomic DNA, 30 µl one step PCR mixture (1 unit Taq polymerase, 10 mM KcL, 10 mM $(NH_4)_2$ SO_4, 20mM Tris Hcl (PH 8.75), 0.1% Triton X-100, 0.1 mg/ml BSA and 200 µm dTNPs) and 2 µl of each primer (BioBasic Inc., Ontario, Canada) and 8 µl DdH_2O. Reaction

conditions used with the thermal cycler (Biometra, Göttingem, Germany) were as follows: an initial incubation at 94ºC for 5 minutes followed by 30 cycles of incubation at 94ºC for 45 seconds, 58ºC for 45 seconds and 72ºC for 45 seconds with a final extension at 72ºC for 7 minutes. Subsequently, it was subjected to digestion with TfiI enzyme, which cleaved the A allele into two fragments of 195 and 103 bp.

Statistical analysis :

Data were analyzed with SPSS for version 15.0 (statistical package for the Social Science, Chicago, IL). Quantitative data were expressed as mean ± standard deviation (SD), data were analyzed by independent sample t and One Way Analysis Of Variance (ANOVA). While qualitative data were expressed as number and percentage and were analyzed by Chi square (X^2) test. Correlation was done using Pearson correlation test. The receiver operating characteristic (ROC) curve and 95% confidence interval (CI) was performed to determine cutoff values for serum level of Vitamin D. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were determined. Odds ratios (ORs) and confidence intervals (CI) were calculated. P-value was considered significant if <0.05 and highly significant if <0.001.

RESULTS

The study included 245 Egyptian patients (123 patients with sustained virological response and 122 patients with treatment failure), their clinical characteristics is shown in table (1). Serum levels of vitamin D showed statistically significant increase in responders in comparison with the non responders.

As regard distribution of of CYP27B1-1260 genotypes, AC and CC genotypes were significantly presented in the non responders in comparison with the responders. CYP27B1-1260 AC and CC genotypes carries had a high risk for treatment failure, (OR=14.7, 95% CI=2.3–20.1, P<0.001; OR= 20.4, 95% CI= 2.9-21.3, P<0.005 respectively).

Serum levels of vitamin D showed statistically significant decrease among those with advanced grading and staging, with a statistically significant negative correlation between vitamin D level and the activity and fibrosis of the liver in both responders and non responders.

Also, there was negative correlation between vitamin D level and viral load in non responder patients (r= -.232, P=0.01, data not shown). As regard the value of serum vitamin D level in discriminating responders from non responders; area under the ROC curve was 0.708 (95% CI 0.643-0.774), (Fig. 1). At a cutoff value of 19 ng/dl of serum vitamin D yielded sensitivity 79%, specificity 58%, positive predictive value (PPV) 65%, and negative predictive value (NPV) 73%.

Table (1) : Baseline patient's characteristics.

Parameter	Responders No=123	Non Responders No=122	T or X^2	P
Age (years)	40.08±9.5	41.37±10.5	0.992	0.322
Sex: Male Female	83 (67.4%) 40 (32.6%)	82 (67.2%) 40 (32.8%)	000	0.96
Viral load (Iu/ml)	598945.5±114	1632321±252	4.332	<0.001
HB (mg/dl)	14.15±1.1	13.93±1.4	2.29	0.13
WBCs/mm^3	6.55 ±1.47	5.13 ±1.32	7.103	0.14
Platelets/mm^3	187.08±49.32	170.54± 46.74	2.667	0.08
AST (Iu/ml)	59.75±34.80	66.29±35.00	1.452	0.15
ALT (Iu/ml)	66.66±35.52	80.58±48.35	2.541	0.12
Alkaline phosphatase (Iu/ml)	85.41±30.89	89.79±40.79	0.938	0.35
Albumin (g/l)	4.41±0.390	4.31±0.461	1.81	0.07
Creatinine (mg/dl)	0.81±0.15	0.87±0.18	1.52	0.13
Activity no (%) Grade 1 Grade 2 Grade 3	 46(37.4%) 62(50.4%) 15(12.2%)	 11(9.0%) 51(41.8%) 60(49.2%)	50.18	<0.001
Fibrosis no(%) Stage 1 Stage 2 Stage 3	 51(41.5%) 52(42.3%) 15(12.2%)	 12(9.8%) 32(26.2%) 78(64.0%)	71.11	<0.001

Table (2) : Vitamin D level in responders and non responders

Parameter	Responders No=123	Non Responders No=122	t	P
Vitamin D level (ng/ml)	30.26±13.89	20.38±10.80	6.151	<0.001

Table (3) : Distribution of CYP27B1-1260 genotypes among responders and non responders

CYP27B1-1260 genotypes	Responders No=123	Non Responders No=122	OR (95%)	P
AA No(%)	30(24.4%)	5(4.1%)		
AC No(%)	28(22.8%)	32(26.2%)	14.7(2.3-20.1)	<0.001
CC No(%)	65(52.8%)	85(69.7%)	20.4(2.9-21.3)	<0.001

Table (4) : Vitamin D level among different histopathological categories.

		Vitamin D Responders	F	P		Vitamin D Non responders	F	P
Activity (Grade)	Grade 1 N (46)	41.6±13.8*	39. 7	<0.001	Grade 1 N (11)	40.5±5.8*	29.1	<0.001
	Grade 2 N (62)	23.9±9.3			Grade 2 N (51)	20.1±10.5		
	Grade 3 N (15)	21.7±4.3			Grade 3 N (60)	17.3±7.8		
Fibrosis (Stage)	Stage 1 N (51)	38.9±14.1*	23.4	<0.001	Stage 1 N (12)	40.5±5.8*	28.4	<0.001
	Stage 2 |N (51)	24.7±10.9			Stage 2 N (32)	16.95±7.19		
	Stage 3 N (15)	21.7±4.3			Stage 3 N (78)	19.29±9.92		

Table (5) : Correlation between vitamin D level and histopathological findings.

Parameter	Responders N=123		Non responders N=122	
	r	p	r	P
Activity	-.581	<0.001	-.467(**)	<0.001
Fibrosis	-.501	<0.001	-.340(**)	<0.001

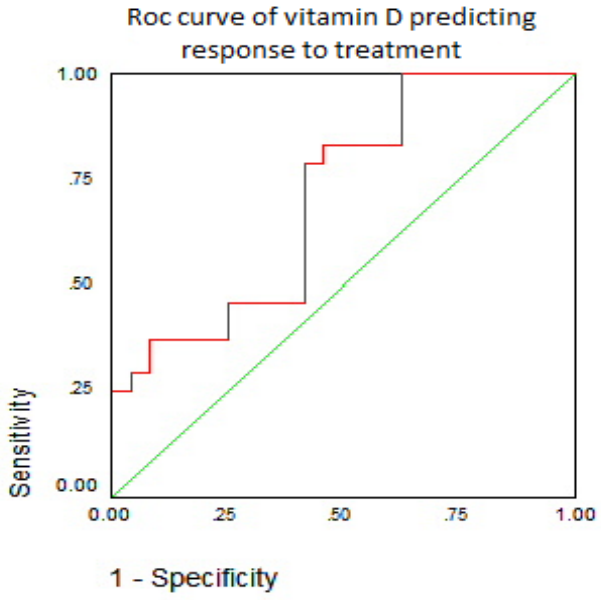

Fig. (1) : ROC curve of vitamin D as a predictor of response to treatment. At cut off level 19 ng/dl of vitamin D it has Sensitivity 79%, specificity 58%, PPV 65% and NPV 73%. Area under curve (AUC)= 0.708(0.643-0.774)

DISCUSSION

Hepatitis C virus genotype 4 (HCV-4) is the most common type of HCV in the Middle East and Africa, in particular Egypt, the response of HCV-4 to the standard regimen of treatment (pegylated interferon/ribavirin) lags behind other genotypes and has become the most resistant type to treat. The development of therapeutic strategies for all patients with HCV-4, have experienced a virological breakthrough **[18]**. Vitamin D is a potent immunomodulator that favour innate immunity and cell differentiation. Increased production of 1,25-dihydroxy vitamin D3 results in the synthesis of cathelicidin, a peptide capable of destroying many viral infectious agents as well as Mycobacterium tuberculosis **[19,20],** so thought that evaluation of vitamin D metabolism related genes and vitamin D level could help in predicting the degree of liver damage and the response to treatment of chronic hepatitis C in our locality.

In this study non responder patients showed statistically significant decrease in vitamin D levels in comparison with the levels of the responders. It was reported that there is an association between vitamin D status and outcome of antiviral therapy in patients with chronic HCV viral infection **[12]**. Also, Bitetto and his colleagues **[21]** found that vitamin D supplementation improved the response to antiviral treatment for recurrent HCV in liver transplant recipients. It had been shown the beneficial effect of vitamin D supplementation on the outcome in patients with chronic HCV genotype 2-3 infection **[22]**.

Current analysis of the genotypes distribution of CYP27B1-1260 among HCV responders and non responders demonstrated significant increase in AC and CC genotypes in non responders. Genotype CC of CYP27B1-1260 impairs the expression of the 1α-hydroxylase, which results in reduced concentrations of bioactive vitamin D **[23,24]**. Thus, one may speculate that the "poor-response" CYP27B1-1260 CC genotype may result in lower local concentrations of calcitriol in the HCV-infected liver, resulting in reduced responsiveness to IFN-α or impaired adaptive immune responses. Consistently, the CC genotype of CYP27B1 is associated with poor response to interferon-α-based treatment of chronic hepatitis C **[25]**.

The present study demonstrated that vitamin D levels significantly decreased among those with advanced grading and staging of the liver with a statistically significant negative correlation in both responders and non responders. Bioactive vitamin D is an important immune modulator, because T cells and macrophages depend on calcitriol in various conditions **[26,27,28]**. Importantly, 1α-hydroxylase is expressed in inflamed tissue and even in immune cells, where it serves as a local, inducible producer of calcitriol **[29]**, this explains why low serum vitamin D level to be related to necroinflammatory activity and progression of liver fibrosis in chronic HCV patients **[12]**. As persistent HCV infection modulates the balance between immune stimulatory and inhibitory cytokines which can prolong inflammation and lead to fibrosis and chronic liver diseases **[30]**.

The negative correlation between vitamin D and IL-23 and -17, at least in part, show how these cytokines might be involved with vitamin D in immune responses in HCV genotype IV-related liver disease and may explain how vitamin D deficiency plays a role in increasing liver fibrosis **[31]**. Another opinion highlighted that Vitamin D is metabolized by the liver and is converted to 1,25 dihydroxy vitamin D3, which is the active form of the vitamin**.** Those with chronic liver disease may have poor conversion from vitamin D3 or any of its other biologically active metabolites**.** Vitamin D deficiency is very common among patients with chronic liver disease [(92%), and at least one-third of them suffer from severe vitamin D deficiency (<12 ng/mL)**[11]**.

This study showed that there is negative correlation between vitamin D levels and viral load in non responders after treatment. Previous study had shown that vitamin D3 increases vitamin D receptors protein expression and inhibits viral replication in cell culture **[32]**. Also, vitamin D acts by improvement of insulin resistance or immune function by up regulation of toll-like receptors involved in the immune response in HCV-infected patients **[33]**.

As regard the value of serum vitamin D level in discriminating responders from non responders; we analyzed the receiving operating curve (ROC) and found that area under the ROC curve was 0.708 (95% CI 0.643-0.774). At a cutoff value of 19 ng/dL of serum vitamin D yielded sensitivity 79%, specificity 58%, positive predictive value (PPV) 65%, and negative predictive value (NPV) 73%. Future studies can combine vitamin D with

other predictors to improve its validity in prediction of treatment response. Vitamin D insufficiency (defined by a 25-hydroxyvitamin D [25(OH)D_3] serum concentration <20 ng/mL) has been proposed as a predictor of failure of treatment of chronic hepatitis C with PEG-IFN-α and ribavirin in others genotypes **[12]**. These findings may have important implications for the management of chronic hepatitis C, as vitamin D status is a potentially modifiable determinant of treatment outcome **[33]**. So, we concluded that vitamin D levels were decreased with the increase in disease severity. Vitamin D serum level concentration and CYB27B1 -1260 genotype could be used as a predictors to HCV treatment response in our locality.

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Screening for Opportunistic Intestinal Parasites in HIV/AIDS Patients, Attending the Services of Medical Care in Three Different Hospitals, Southern Ethiopia

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Key words: *HIV/AIDS, hospital, morbidity, opportunistic, mortality, patients, parasites*

Background and study aim: Diagnostic examination of stools for opportunistic intestinal parasites in HIV/AIDS patients is given less attention than it should be. The suspected opportunistic intestinal parasites such as *Cryptosporidium parvum*, *Cyclospora cayentanensis*, *Toxoplasma gondii*, *Isospora belli* and the symptom of explosive watery diarrhea they cause as well as others including *Strongyloides stercoralis* are the threat against the well-being of HIV/AIDS patients. The objective of this study is to demonstrate the indispensable necessity to free HIV/AIDS patients (who are under medical care in 3 different hospitals, Southern Ethiopia), from opportunistic intestinal parasites using diagnostic examination of stools followed by prompt curative treatment during every safety time interval.

Patients and methods: Fresh stools samples from a total sample size of 710 HIV/AIDS patients were taken and examined in the parasitology laboratory, Dilla University, for the suspected intestinal opportunistic parasites. The methods employed to identify the intestinal parasites included observations in : wet mount, formalin-ether concentration technique, and permanent slide preparation as well as Baermann apparatus method for *Strongyloides stercoralis*.

Result: Out of 710 HIV/AIDS patients examined 196 were found to be positive for 6 different species of the suspected intestinal parasites (infection rate of these parasites in the population of HIV/AIDS patients of the 3 different hospitals being

$$\frac{196}{710} \times 100 = 27.6\%$$

The six species of parasites isolated from fresh stools samples were: *Ascaris lumbricoides*, *Strongyloides stercoralis*, *Entameba histolytica*, *Giardia lamblia*, *Balantidium coli*, and *Trichuris trichiura*.

Conclusion: Reasonably planned successive safety time intervals must be attended continuously by HIV/AIDS patients without interruption to utilize the services of medical care in order to avoid/ neutralize the potential opportunistic infections and reinfections; otherwise, the fulminant death can turn to be true. The safety and well-being of those HIV-infected patients who attend all the medical services & advices provided by clinical experts is not different from that of HIV-noninfected individuals without any trace of exaggeration.

INTRODUCTION

Diagnostic examination of stools for opportunistic intestinal parasites in HIV/AIDS patients is of great validity in providing the service of prompt curative treatment so as to safe guard the patients against chronic morbidity **[1-9]**. The curative treatment of the opportunistic intestinal infections does get control of the present infection (illness) but cannot prevent the possibility of reinfection. Even after the regular administration of free Anti-Retroviral Therapy (ART) in short intervals of time, opportunistic intestinal parasites cannot be ignored. Therefore, the HIV-infected individuals should be screened for opportunistic intestinal parasites within reasonably safe intervals of time by way of diagnostic examination of stools **[10-20]**. After 30 years of HIV epidemic, parasites have become one of the most common opportunistic infections and one of the most important causes of morbidity and mortality against the well-being of HIV-infected individuals **[21-23]**.

Screening for intestinal parasites followed by quick curative treatment or prevention of harmful infections improves the safety and duration of life of the HIV-infected persons **[24,25]**. The prevalence of harmful intestinal helminth infections in asymptomatic HIV- infected individuals has been identified and with this truth in mind screening HIV-infected patients for opportunistic intestinal parasites cannot be ignored by any means **[26-30]**. The HIV/AIDS epidemic so compromises the immune system of its victims that they are left virtually defenseless. Even relatively benign parasites that cause only mild symptoms, in a healthy person, can be quite devastating to a patient suffering from AIDS **[31-35]**. The suspected opportunistic intestinal parasites such as *Cryptosporidium parvum*, *Cyclospora cayentanensis*, *Toxoplasma gondii* and *Isospora belli* with their symptom of explosive watery diarrhea and others including *Strongyloides stercoralis* are the threat against the well-being of HIV/AIDS patients.

The purpose/objective of this study is to demonstrate the indispensable necessity to free HIV/AIDS patients (who are under medical care in 3 different hospitals found in Southern Ethiopia), from opportunistic intestinal parasites using diagnostic examination of fresh stools followed by prompt curative treatments during every successive safety time interval. Here, the objective means that if the HIV-infected patient is found to be positive for one or more species of the suspected intestinal parasites he/she will be given the best choice of treatment quickly and cured. However, it cannot promise/guarantee that other parasitic infections or reinfections with the same species of parasite/s do not occur until the end of the next (subsequent) safety time interval. But if the suspected parasitic infections occur within this length of reasonably predetermined time interval, it is very likely that the opportunistic parasites cannot overpower (i.e., they cannot build up resistance against) promptly successful curative treatment.

PATIENTS AND METHODS

Three different hospitals were the sites of sample taking. The 3 different hospitals where the HIV/AIDS patients had been under medical care were:

- Dilla University, Teaching and Referral Hospital,
- Yirgalem General Hospital, and
- Yirgacheffe Health Center.

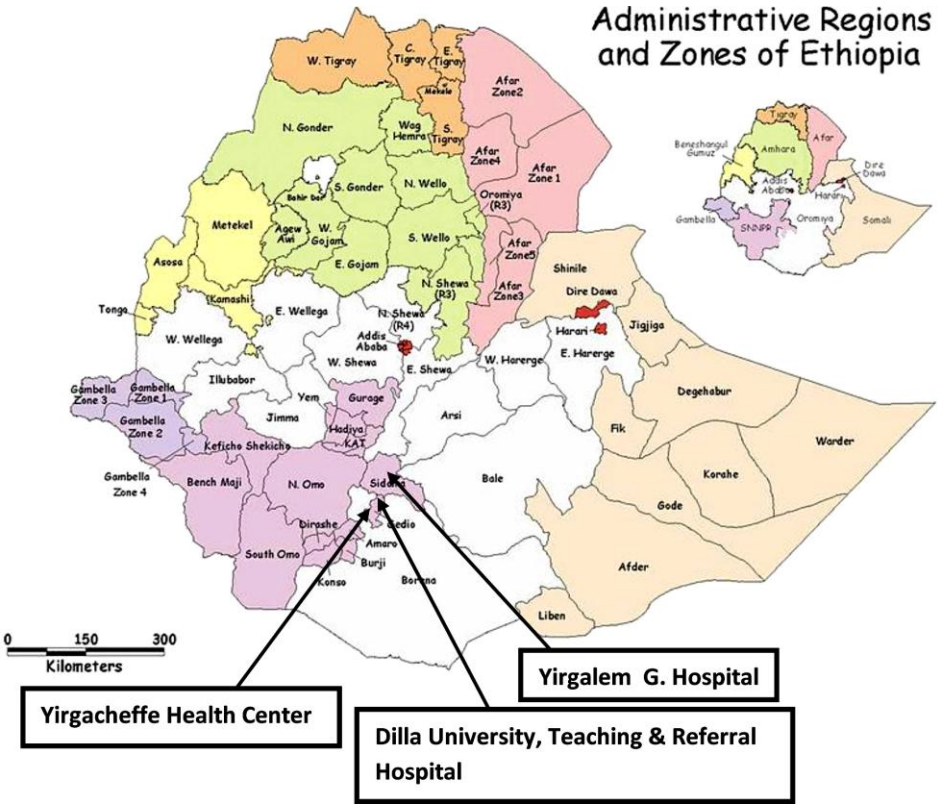

Figure (1): Map of Ethiopia to show the locations of the 3 different hospitals where the HIV/AIDS patients were under medical care.

The total sample size of 710 HIV/AIDS patients was decided to be examined for the suspected harmful intestinal parasites of man. The samples taken from HIV infected patients of the hospitals cited above were fresh stools. Collection of stools samples from the hospitals and documenting the findings had been carried out daily, Monday through Friday (i.e., every week) from October 2013 to June 2014. The statistics selected, being relevant to interpret and analyze the results of this study were **percentage** and **histogram**. The fresh stools sample of each HIV/AIDS patient was examined with a compound light microscope at three stages:

- Direct wet mount,
- Concentration technique, and
- Permanent staining.

Procedure

Direct wet mount:

- About 2.5 ml of fresh stools sample was taken in a small vial from each HIV/AIDS patient of the hospitals selected. Immediately after that, 0.85% NaCl solution in distilled water and warmed to 370ºC was added to each vial of fresh stools sample taken. Then 1 drop of 0.85% warm (370ºC) aqueous NaCl (saline solution) was placed on a clean slide.
- Following that, about 1 drop of the stools specimen (from that of any single HIV/AIDS patient) was added to the slide and mixed with the drop of NaCl solution.
- The saline wet mount was covered with a cover slip and examined under a suitable objective lens. This procedure of using warm saline solution was to allow determining the motility and gross morphology of trophozoites. In the mean time, care was taken not to allow the sample on the slide to dry or cool; otherwise, the motility of trophozoites could have ceased. The stools specimen of any particular HIV/AIDS patient who was positive for the suspected parasites was preserved in 5% formalin (for protozoans) or in 10% formalin (for nonprotozoan parasites) to be used in the stages of Concentration Technique and examination of Permanent Staining Preparation. The stools specimen of each patient was prepared, observed, and preserved exactly in this way.

Concentration technique:

- Involved concentrating the number of the diagnostic stages of the suspected parasites, primarily of cysts, eggs or larvae in the stools specimen that was collected and preserved in 5% or 10% formalin. These concentrated and preserved specimens were part of the complete examination and the detection of small numbers of the parasites that could have been difficult. In order to concentrate the number of the diagnostic stages of the parasites, diethyl-ether had been mixed with the suspension of the stools sample. Then, the speed and concentration time were set at 1000 rpm for 2 minutes.
- Next, the parasites particularly the cysts, eggs, or larvae were expected to sediment at the bottom of the centrifuge tube and the floating stools debris was discarded.

Examination of permanent staining preparation:

- Detection and identification of intestinal protozoan and helminth parasites preserved in 5% or 10% formalin respectively would be enhanced by the examination of permanently stained smears under the oil immersion objective lens.
- These stained slides would provide a permanent record of the suspected intestinal parasites of man.
- The identifications in the stages (steps) of Direct Wet Mount and Concentration Technique would be reconfirmed by the permanently stained slide.
- The staining was with Safranin. Safranin is a stain used in histology to stain **tissues** and to stain gram-negative bacteria as a counterstain. Safranin is not known at all to stain protozoa or any other parasite here before. When it was tried to stain the worms of *S. stercoralis*, for the first time, it gave a very good dyeing effect. It stained the worms red. It also stains well the cysts of *E. histolytica*, *G. lamblia*, and nearly all other diagnostic stages of human intestinal parasites. This is an unexpected advantage as it increases the spectrum of options in techniques of staining.
- About 3 drops of Safranin solution was added to the stools specimen suspension preserved in formalin in a bottle of about 50 ml and waited for about 6 hours to get the diagnostic stages of the parasites stained.
- A drop of Yetwin Mounting Medium melted at 650ºC was placed on a clean slide, then on this drop of mounting medium, a drop of the stools specimen preserved in formalin and stained with Safranin was added and mixed well with the tip of a needle. Next, the specimen was covered with a coverslip and left on a table for

about 24 hours to let the mounting medium solidify and harden.

- Thereafter, the specimen in the hardened mounting medium was examined under the oil immersion objective lens to check the presence of the suspected intestinal parasites of man.

Additional methods:

- Baermann apparatus technique was employed for the diagnostic tests of *S. stercoralis*. Here, the Low or Middle Power objective lens was used in observing under the compound light microscope.
- In all methods of this paper, from all fields of vision (i.e., low power to oil immersion) of the microscope, microphotographs of the parasites diagnosed were taken using a digital camera and transferred to computer for further processing.

Treatment

Curative drugs of choice were used against *E. histolytica*, *G. lamblia*, and *B. coli*. Albendazole was the drug available to neutralize the infections with *S. stercoralis* and other helminths.

RESULTS

A horrifying death of a 26 years old HIV/AIDS patient with exhaustive watery diarrhea has been recorded. Another 13 years old male HIV/AIDS patient has also been observed to be threatened by watery diarrhea. Out of 710 HIV/AIDS patients examined for the suspected harmful intestinal parasites, 196 patients were found to harbor six different species of parasites.

There were 11 HIV/AIDS patients with multiple infections. Each of these 11 patients were identified to harbor two different species of intestinal parasites. For instance, the 26 years old HIV-infected lady who died in a terrifying situation because of her own carelessness to attend the services of medical care & advices provided by medical experts in the hospital, was infected by two intestinal parasites, namely: *Balantidium coli*, and *Trichuris trichiura*. The 13 years old HIV-positive child who had been challenged by watery diarrhea and cured by a quick curative treatment was also infected by two intestinal parasites; i.e., *Balantidium coli* and *Entameba histolytica*.

Figure 2, Table 1, and Figure 3 are forwarded on the next three consecutive pages.

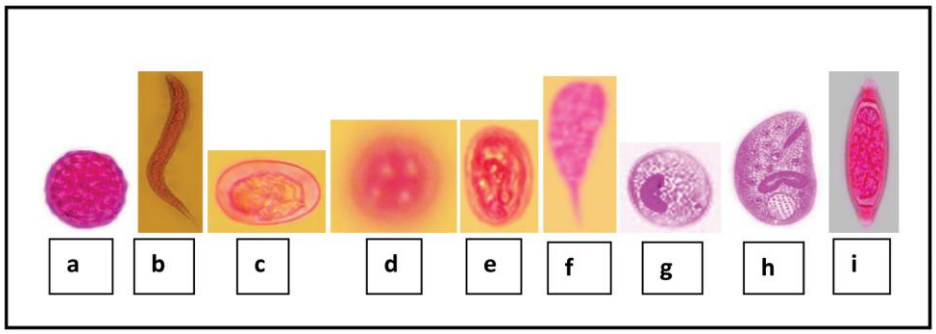

Figure (2) : The list of the most important diagnostic stages (except those of labels: c, f,& h) of intestinal parasites isolated from fresh stools samples of HIV/AIDS patients in 3 different hospitals, Southern Ethiopia.
(a) Ascaris lumbricoides egg, magn†. X640;
(b) Strongyloides stercoralis rhabditiform larva, magn. X640;
(c) Strongyloides stercoralis egg, magn. X640;
(d) Entameba histolytica cyst, magn. X640;
(e) Giardia lamblia cyst, magn. X1600;
(f) Giardia lamblia trophozoite, X640
(g) Balantidium coli cyst, magn. X640
(h) *Balantidium coli* trophozoite, magn. X640;
(i) *Trichuris trichiura* egg, magn. X640.

Each of these six pictures was colored using a computer Adobe Photoshop▪CS and transformed from its original magnified size to the resolution of 1200 pixels/inch with the quality of 12 (maximum) and large file compatible with A4 page format.

Egg of *Strongyloides stercoralis*, and the trophozoites of *Giardia lamblia* and *Balantidium coli* are not the most important diagnostic stages, but in this study they were isolated from fresh stools samples.

Table (1) : The list of human intestinal parasites isolated from fresh stools samples of HIV/AIDS patients under medical care in 3 different hospitals, Southern Ethiopia.

Total sample size of HIV/AIDS patients examined for intestinal parasites	Intestinal parasitic species identified in HIV/AIDS patients	Total number of HIV/AIDS patients positive for the arrowing parasite
710	1. *Ascaris lumbricoides* →	74 (10.42%)≠
	2. *Strongyloides stercoralis* →	45 (6.34%)
	3. *Entameba histolytica* →	43 (6.10%)
	4. *Giardia lamblia* →	25 (3.52%)
	5. *Balantidium coli* →	19 (2.68%)
	6. *Trichuris trichiura* →	1 (0.14%)

≠The percentile quantity in parenthesis adjacent to the value that meant "total number of HIV/AIDS patients positive for the arrowing parasite:" represented the infection rate of the intestinal parasitic species in the preceding column but in the same row.

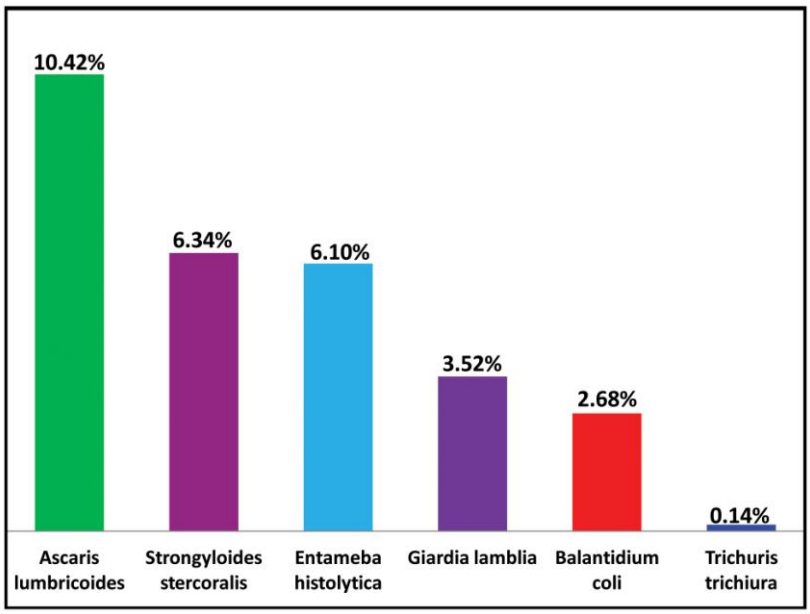

Figure (3) : The Statistic of Histogram, demonstrating the infection rates of intestinal parasites in the population of HIV/AIDS patients in 3 different hospitals, Southern Ethiopia.

DISCUSSION

Out of 710 HIV/AIDS patients examined for the suspected harmful intestinal parasites, 196 patients were found to be positive. This meant that the infection rate of intestinal parasites in the population of HIV/AIDS patients of the 3 different hospitals is: $\frac{196}{710} \times 100 = 27.6\%$

One of the HIV/AIDS patients in one of the 3 hospitals has died in a horrifying situation because of an obvious failure of the patient herself to follow the advice of medical care to maintain her safe and alive. She was advised to come by the end of every one month to the Anti-Retroviral Therapy center of the hospital for medical check in order to avoid any harmfully opportunistic infection or illness. However, she stopped coming to the anti-retroviral therapy center of the hospital, i.e., she stopped being treated with Ethiopian Anti-Retroviral Therapy

Guidelines adopted from WHO Guidelines which involve the administration of CD4 to increase (boost) the immunity of the HIV/AIDS patients. After abandoning attending the services of medical care and advice for six months, she came to the anti-retroviral therapy center of the hospital when she was in the process of dying. At that moment her body was left with skeleton and watery diarrhea (not different from urine). It was at that time (7 March 2014), fresh stools sample of this very patient was taken and examined in which the causative agents of the watery diarrhea were identified to be *Balantidium coli* and *Trichuris trichiura*. Curative treatments of the best choices were given, battling to save/rescue her life. However, in spite of all those attempts she died after a while.

Another 13 years old male HIV/AIDS patient was found to be challenged with watery diarrhea. His fresh stools sample (watery diarrhea) was examined and the causative agents of the challenge were verified to be *Entameba histolytica* and *Balantidium coli*. The child recovered from this double intestinal parasitic infection via the curative treatment as he was not late with the opportunistic infection and because he was punctual in attending the service of the medical care provided by anti-retroviral therapy division of the hospital.

The list of intestinal parasites isolated from fresh stools samples of HIV/AIDS patients in this study consisted of: *A. lumbricoides*, *S. stercoralis*, *E. histolytica*, *G. lamblia*, *B. coli*, and *T. trichiura*. Among these, the most dangerous opportunistic parasite to cause lethal risk to HIV/AIDS patients is *S. stercoralis* if there is failure and interruption in attending the services of medical care according to the recommended safety time intervals. The infection rate of each of the six species of parasites investigated in this study is far less than that in the population of student children of elementary schools (verified and reported herebefore by the researcher of this study project) at Dilla Town and its peripheral villages **[36]**. Some of the suspected opportunistic intestinal parasites such as *Cryptosporidium parvum*, *Cyclospora cayentanensis*, *Toxoplasma gondii*, *Isospora belli* and the symptom of explosive watery diarrhea they cause did not appear in the HIV/AIDS patients examined. Hookworms and *Schistosoma mansoni* are endemic in this region where HIV/AIDS patients tested resided **[36]**; however, these helminths are not found in the HIV/AIDS patients of this project. One of the major reasons for why:

- These suspected protozoan and helminth intestinal parasites (*Cryptosporidium parvum*, *Cyclospora cayentanensis*, *Toxoplasma gondii*, *Isospora belli*, hookworms and *Schistosoma mansoni*) are not found in the HIV/AIDS patients examined,
- The infection rate of each of the six species of parasites investigated in the patients of this study is far lower than that of schools children, and
- The number of different species of parasites harbored in a single person with respect to multiple infection was greater in schools children (5 different species of parasites in a person) than in this study of HIV/AIDS patients (only 2 different species of parasites in a person) must be the protective effect of Anti-Retroviral Therapy that included the administration of CD4 given in the care taker hospitals.

Healthy intaking of mixed diet against weight loss of HIV/AIDS patients was an important component of the medical care and advice offered by care provider health professionals. Based on this, in each of the 3 hospitals the body weight of every HIV/AIDS patient was recorded in every safety time interval in order to take action to increase body weight of the client to normal level by eating more food (either by eating larger portions and/or eating meals more frequently, using a variety of foods) when weight loss was being observed **[37]**.

Getting the stools samples of HIV/AIDS patients examined for opportunist intestinal parasites by experienced parasitologists/medical laboratory experts is very important and useful in giving feedback to care provider physicians for their better patient management so as to avoid fatality.

In conclusion, HIV/AIDS patients must be punctual to the preplanned safety time intervals to attend the services provided by public health institutions. The safety time interval is so named because it is the length of time during which it cannot be late or difficult to treat and get the patient cured from any onset of opportunistic infection if it has happened. It is the recommended segment of duration for medical checkup of the HIV/AIDS patient; example, an interval of one month. This time interval is very important because an opportunistic infection can be cured before it is late and out of control for medication, but reinfection with the same

opportunistic parasite or with others is possible in each of the following successive safety time intervals. Therefore, the subsequently successive safety time intervals that are estimated and approved by health professionals must be attended continuously by HIV/AIDS patients without interruption to utilize the services of medical care in order to avoid/neutralize the potential opportunistic infections and reinfections. The length of safety time interval is variable and is decisively determined by the physician, who is assigned to take care of the HIV-infected patient, based on the degree of severity in health status of the client. Finally, based on what has been spectacularly observed in this study, the following truth can be stated. If an HIV-infected person who is under medical care fails to attend continuously all the services given (in safety time intervals) by health professionals, he/she must be aware of the fact that fulminant death due to HIV infection can turn to be true soon. On the other hand, the safety & well-being of those HIV-infected patients who attend all the medical services and advices provided by clinical experts is not different from that of HIV-noninfected individuals without any trace of exaggeration.

Conflict of interest

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

Financial support

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

Ethics

Ethical permission/clearance to perform the research work to contribute to the well-being of HIV/AIDS patients was obtained from Dilla University, the Office of Gedeo-Zone Administration, and the Directors of the hospitals involved in the study.

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Study of Acid-Base Disturbances in Patients with Liver Cirrhosis

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Key words:
liver cirrhosis, acid base balance disorders, respiratory alkalosis

Background and study aim : Acid base disturbances occur frequently in the setting of liver diseases. As liver's metabolic function worsen, particularly in the setting of renal dysfunction, haemodynamic compromise, and hepatic encephalopathy, acid base disorders ensue. The aim of this study was to assess acid base disturbances in patients with liver cirrhosis.

Patients and Methods: The present study was conducted on 60 cirrhotic patients, as well as 20 healthy persons of matched age and sex as a control group. Diagnosis of cirrhosis was done by clinical examination, ultrasonographic findings and laboratory investigations. Patients and controls were subjected to the following:Full history taking, Thorough clinical examination, Abdominal ultrasound examination, Laboratory investigations including: CBC, Liver function tests, serum creatinine, Arterial blood gases to evaluate blood PH, HCO_3, pressure of carbon dioxide and pressure of oxygen.

Results: There was highly significant difference in PH, PCO_2, PO_2, and HCO_3 and SO_2 between both groups. The common acid base disorder was respiratory alkalosis. However, other disorders can be seen. Change in acid base balance was as following: Child's A group; no change in acid base balance. Child's B group; 75% no changes in acid base balance with 20% respiratory alkalosis and 5% metabolic alkalosis. Child's C group; 70% respiratory alkalosis with 15% respiratory acidosis and 15% metabolic alkalosis.

Conclusion: These result declare the presence of multiple and mixed acid base disorder in cirrhotic patients

INTRODUCTION

The liver is an important organ in acid-base physiology. It is a metabolically active organ which may be either a significant net producer or consumer of hydrogen ions. The acid- base roles of the liver include: carbon dioxide production from complete oxidation of substrates, metabolism of organic acids anions such as lactate, ketone and amino acids. The conversion of ammonium (NH4) to urea in the liver, synthesis of plasma proteins: except immunoglobulins **[1]**.

Albumin is one of plasma proteins synthesized by the liver having many roles in acid–base physiology as it is considered as the major nonvolatile weak acid present in plasma, hypo-albuminemia causes a metabolic alkalosis. It is the major unmeasured anion in the plasma which contributes to the normal value of anion gap. It acts as an extra cellular buffer for CO_2 and fixed acids, thus an abnormal level can cause metabolic acid-base disorder **[2]**. Hepatic disorders are often associated with acid base disorders. The most common disturbances in chronic liver diseases are respiratory alkalosis followed by metabolic alkalosis **[3]**. Acid–base disturbances in patients with chronic severe hepatitis, liver cirrhosis, ascites and patients with hepatic encephalopathy is often alkalosis. Alkalosis indicates that the kidneys have increased their HCO_3 – reabsorption **[4]**. This may result from toxic stimulation of respiratory center by ammonium from administration of alkalies as citrate in transfusions or with potassium supplements or from hypokalemia. Since urea synthesis consumes bicarbonate thus progressive loss of urea cycle capacity is associated with increased plasma bicarbonate level (metabolic alkalosis) and ammonia excretion by the kidney **[5]**.

Acid-base and potassium disorders occur frequently in the setting of liver disease. As the liver's metabolic function worsens, particularly in the setting of renal dysfunction, hemodynamic compromise, and hepatic encephalopathy, acid-base disorders ensue **[6]**. Effective treatment of acid-base disturbance will be valuable in prevention of hepatic encephalopathy **[7]**. The aim of present study was to evaluate the acid base disturbances in arterial blood gases in patients with liver cirrhosis.

PATIENTS AND METHODS

A total of 60 patients with liver cirrhosis were selected after giving a written informed consent, they were selected out from 200 patients admitted at Mansoura University Hospitals in the period between April 2013 and December 2013. They were 33 (55%) males and 27 (45%) females and their ages were ranging from 33 to 70 years, in addition 20 healthy persons of matched age and sex served as a control group. Diagnosis of cirrhosis based on clinical examination, ultrasonographic findings and laboratory investigations.

Patients and controls were classified into the following groups:

Group I : Comprised 60 cirrhotic patients, they were subdivided into 3 subgroups according to modified Child-Pugh classification :

- Group Ia: Comprised 20 cirrhotic patients (Child's grade A)
- Group Ib: Comprised 20 cirrhotic patients (Child's grade B)
- Group Ic: Comprised 20 cirrhotic patients (Child's grade C)

Group II: Comprised 20 healthy controls.

Patients and controls were subjected to the following:

1. Through history taking.
2. Clinical assessment with special emphasis on manifestations of liver cell failure.
3. Abdominal ultrasound examination.
4. Laboratory investigations:
   - CBC **[8]**.
   - Urine analysis .
   - Liver function tests namely alanine trans-aminase (ALT), aspartate transaminase (AST), serum bilirubin, serum albumin, INR level **[9]**.
   - Fasting blood sugar and post prandial blood sugar **[10]**.
   - Serum creatinine **[11]**.
   - Serum electrolytes: sodium, potassium.
   - Arterial blood gases (ABG) to evaluate blood PH, HCO_3, pressure of carbon dioxide (PCO_2) and pressure of oxygen(PO_2). Any venous samples was discard **[12]**.

Statistical analysis:

Data were tabulated, coded then analyzed using the computer program SPSS (Statistical package for social science) version 17.0. Descriptive statistics were calculated in the form of: Mean, Standard deviation (±SD), Minimum and maximum and Frequency (No and %). In the statistical comparison between the different groups, the significance of difference was tested using one of the following tests: ANOVA (analysis of variance): Used to compare between more than two groups of numerical (parametric) data followed by posthoc LSD for multiple comparisons. Inter-group comparison of categorical data was performed by using chi square test (X^2-value). A P value <0.05 was considered statistically significant. And a P value <0.0001 was considered highly significant in all analyses.

RESULTS

Demographics of the studied groups

There was no significant difference between the liver cirrhosis group and healthy controls and among subgroups of liver cirrhosis regarding the age and sex of patients. They were 33 (55%) males and 27 (45%) females and their ages were ranging from 33 to 70 years with a mean value of 41.35±5.81 years in Group Ia, 53.95±7.51 years in Group Ib and 55.35 ± 9.4 years in Group Ic. Manifestations of liver cell failure were present in various proportions of cirrhotic groups. HCV infection was present in the majority of cirrhotic patients, while HBV infection was absent. HCV-Ab and HBsAg were absent in all Group II persons. Ultrasonographic features of cirrhosis were present in all cirrhotic groups. Cirrhosis was mixed with peri-portal fibrosis in some patients of Group Ia, Ib and Ic.

Biochemistry of the studied groups

The mean value of hemoglobin concentration and platelet counts in cirrhotic groups (Ia,Ib,Ic) were significantly lower than that of Group II. On the other hand, there was no significant difference between the studied groups as regard mean total leucocytic and RBCs counts (Table 1). The mean value of hemoglobin concentration in cirrhotic groups (Group Ia, Group Ib and

Group Ic) were significantly lower than hemoglobin concentration in Group II (non cirrhotic). The mean value of platelet count of Group Ib and Group Ic were significantly lower than that of Group Ia.

The mean values of serum Bilirubin in Group Ib and Group Ic were significantly higher than that of Group Ia and Group II. The present study showed that the mean values of serum bilirubin in Group Ib and Group Ic were significantly higher than that of Group II and Group Ia. The mean value in Group Ic was significantly higher than that of Group Ia. While, there was no significant difference between Group Ia and Group II (Table 2).

The mean values of serum albumin in Group Ib and Group Ic were significantly lower than that of Group Ia and Group II. The mean values of INR were significantly higher in Group Ib than that of Group Ia and significantly higher in Group Ic than in Group Ia and Group Ib. Meanwhile the values of INR in Group II were lower than that in Group Ib and Group Ic (Table 2).

The mean values of ALT in Group II and Group Ic were significantly lower than that in Group Ia and Group Ib. The mean values of AST in Group II were significantly lower than that in Group Ia, Group Ib and Group Ic (Table 3).

Acid base and electrolyte disturbances of the studied patients

In the present study initial assessment ABGs (Table 4) were done and both groups showed that significant difference in PH between Group Ic and Group Ia and Group Ib. As well as significant difference between Group II and Group Ic (Fig. 1). There was a highly significant difference in arterial PH, PCO_2, PO_2, $HCO_3$and SO_2 between both groups. Also there was significant difference between Group Ic and Group Ia and Group Ib in PH, PCO_2 and HCO3. But there was no significant difference between both groups regarding serum Na^+ and serum K^+ levels.

The reported acid-base disorders in the studied groups revealed that Group Ia was normal, Group Ib: 20% respiratory alkalosis, 5% metabolic alkalosis and 75% normal, Group Ic: 70% respiratory alkalosis, 15% metabolic alkalosis and 15% respiratory alkalosis. There was highly significance difference between Group I and group II (Table 5).

The common acid base disorder was respiratory alkalosis (Table 5), however, metabolic alkalosis, respiratory acidosis and metabolic acidosis all could also be seen (Fig. 2).

Also PCO_2 there is significant difference (Table 4) between Group Ic and Group Ia and Group Ib. Again, there is significant difference between Group II and Group Ic (Fig. 3).

HCO_3 there is significant difference (Table 4) between Group Ic and Group Ia, Group Ib and significant difference between Group II and Group Ic (Fig. 4).

As regard SO_2 there is significant difference between Group Ib, Group Ic in relation to Group Ia as well as significant difference between Group II and Group Ib and Group Ic (Table 4).

There is low significant increase in serum Na^+ between Group Ib and Group Ic than Group II (Table 4).

As regard K^+ there is low significant decrease (Table 4) in Group Ic in relation to Group Ia and Group II in relation to Group Ic (Fig. 5).

Table (1): Haematological profile in studied groups

		Hemoglobin		RBC		WBC		PLATLETS	
		Mean± SD	**Range**	**Mean± SD**	**Range**	**Mean± SD**	**Range**	**Mean± SD**	**Range**
Group I	GIa	11.85±1.46	8.80-14.30	4.49±.46	3.80-5.30	6.63±1.79	4.20-9.70	169.15±34.76	112.00-234.00
	GIb	10.39±.93[a]	8.70-12.30	4.78±.66	3.70-6.20	7.00±1.88	3.60-9.60	121.10±33.35[a]	81.00-190.00
	GIc	9.00±.90[ab]	7.60-10.70	4.49±.47	3.80-5.30	7.42±1.31	4.90-9.80	93.40±10.14[ab]	76.00-118.00
Group II		13.02±1.26[abc]	11.20-15.40	4.60±.48	3.80-5.30	6.64±1.81	3.90-9.70	260.00±42.77[abc]	193.00-321.00
F		45.265		1.404		0.963		100.502	
P		<0.001		0.248		0.415		<0.001	

G: group, RBCs: Red blood cells, WBCs: White blood cells, SD: standard deviation, P: Probability, a: significance relative to GIa, b: significance relative to GIb, c: significance relative to GIc

Table (2): Results of Bilirubin –Albumin –INR of studied groups

		Bilirubin		Albumin		INR	
		Mean± SD	**Range**	**Mean± SD**	**Range**	**Mean± SD**	**Range**
Group I	GIa	0.98±.22	0.70-1.40	4.23±.40	3.60-4.90	1.04±.11	0.87-1.20
	GIb	1.36±.25[a]	1.10-1.90	3.68±.49 [a]	2.70-4.30	1.45±.24[a]	1.10-1.90
	GIc	2.26±.52[ab]	1.60-2.90	2.34±.48 [a b]	1.60-3.20	1.70±.19[ab]	1.30-1.90
Group II		0.88±.17[bc]	0.60-1.20	4.25±.43 [b c]	3.74-4.90	0.89±.23[bc]	0.00-1.10
F		76.634		78.973		70.714	
P		<0.001		<0.001		<0.001	

G: group, SD: standard deviation, P: Probability, a: significance relative to GIa, b: significance relative to GIb c: significance relative to GIc

Table (3): Results of Alanine amino transferase, Aspartate amino transferase and creatinine

		ALT		AST		Creatinine	
		Mean± SD	**Range**	**Mean± SD**	**Range**	**Mean± SD**	**Range**
Group I	GIa	50.85±19.91	23.00-89.00	58.65±18.62	33.00-89.00	.95±.20	.60-1.30
	GIb	54.90±15.63	32.00-87.00	60.05±14.30	24.00-82.00	1.03±.23	.60-1.40
	GIc	34.85±14.53[ab]	12.00-68.00	39.35±10.84[ab]	19.00-61.00	1.14±.20[a]	.76-1.40
Group II		26.90±7.25[ab]	12.00-38.00	27.85±5.96[abc]	16.00-36.00	.90±.17[c]	.67-1.20
F		15.4		27.6		5.4	
P		<0.001		<0.001		0.002	

G: group, SD: standard deviation, P: Probability, a: significance relative to GIa, b: significance relative to GIb, c: significance relative to GIc

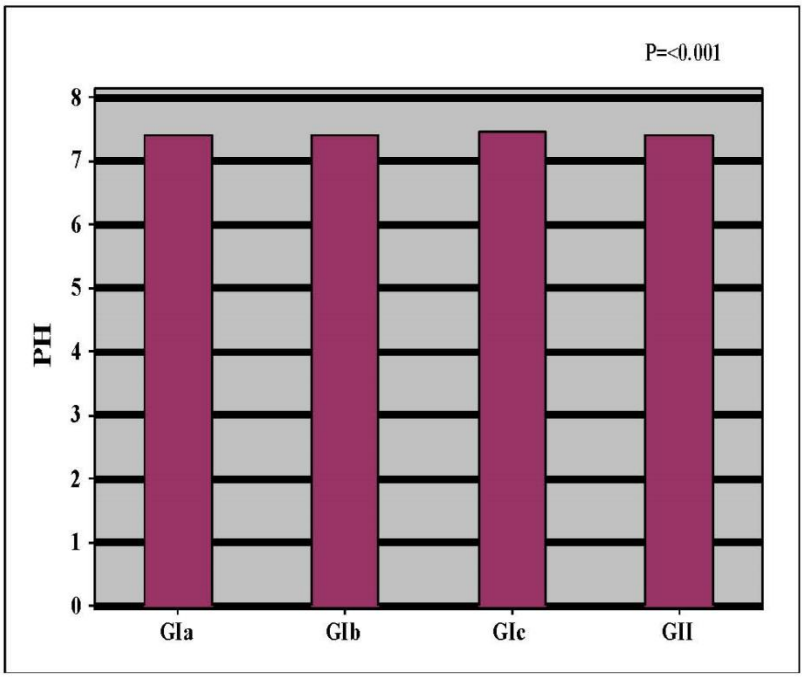

Fig. (1) : Results of PH of studied groups (G: group)

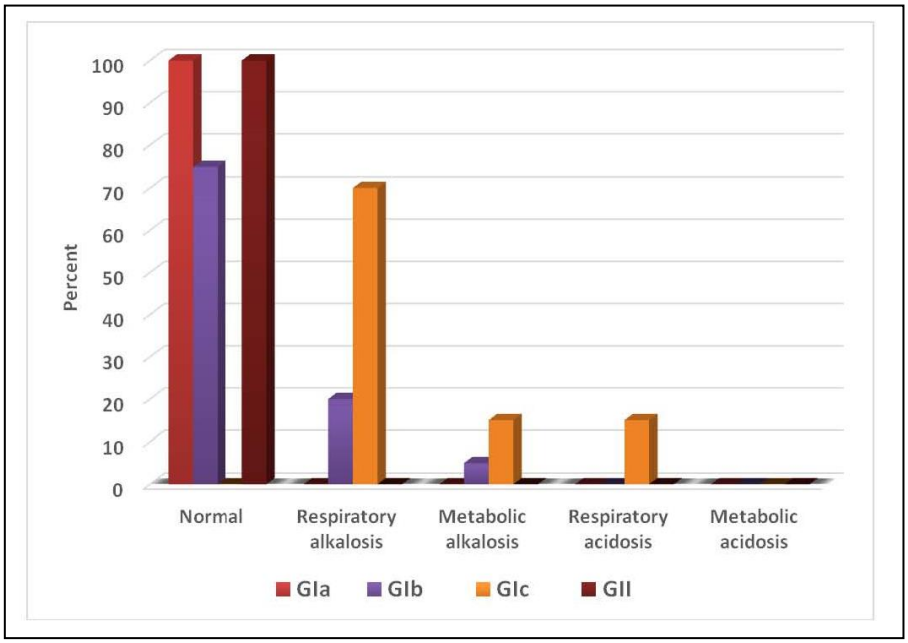

Fig. (2) : Acid-base status in the studied groups

Table (4) : Results of Arterial blood gases of studied groups

	Group I						Group II		F	P
	Group Ia		Group Ib		Group Ic					
	Mean± SD	Range	Mean± SD	Range	Mean± SD	Range	Mean± SD	Range		
PH	7.40±.03	7.36-7.45	7.42±.04	7.35-7.49	7.46±.07^{ab}	7.28-7.52	7.40±.02^{c}	7.36-7.43	8.012	<0.001
PCO2	39.10±2.75	36.00-44.00	36.90±6.82	24.00-45.00	28.60±6.87^{ab}	20.00-51.00	39.05± 1.96^{c}	35.00-42.00	18.886	<0.001
PO2	90.85±2.08	88.00-94.00	88.00±2.85^{a}	83.00-92.00	86.50±3.47^{a}	79.00-91.00	92.80±2.17^{bc}	90.00-96.00	21.88	<0.001
HCO3	24.10±1.43	22.00-26.20	24.65±2.14	21.00-28.50	27.32±3.28^{ab}	21.00-31.00	23.87±10.01^{c}	22.00-25.80	10.989	<0.001
SO2	96.60±1.27	95.00-99.00	93.85±2.18^{a}	91.00-97.00	92.65±2.83^{a}	86.00-96.00	96.65±10.09^{bc}	95.00-99.00	20.701	<0.001
Na+	139.10±2.31	135.00-145.00	140.55±3.90	133.00-146.00	141.00±5.48	130.00-148.00	138.05±1.73^{bc}	136.00-141.00	2.734	0.049
K+	4.31±.33	3.89-4.80	4.23±.47	3.56-5.04	3.98±.53^{a}	3.22-4.84	4.45± 0.27^{c}	4.12-4.94	4.596	0.005

SD: standard deviation, P: Probability, a: significance relative to GIa. b: significance relative to GIb. c: significance relative to GIc.

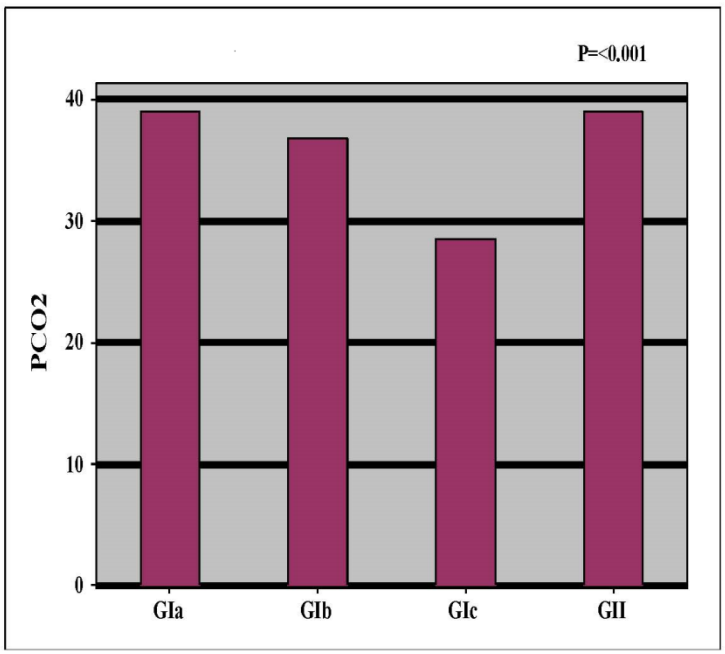

Fig. (3) : Results of PCO_2 of studied groups (G: group)

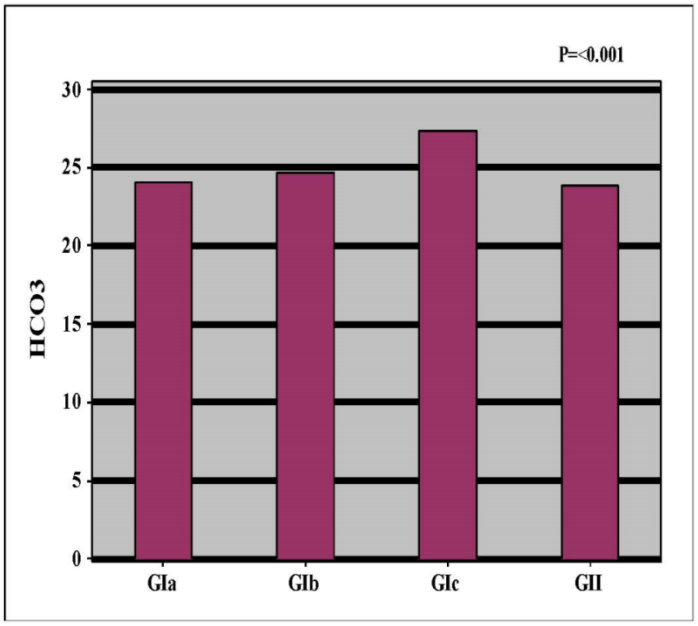

Fig. (4) : Results of Bicarbonate of studied groups (G: group)

Table (5): Acid-base status in the studied groups

Acid-base status	Group I						Group II		X^2	P
	Group Ia		Group Ib		Group Ic					
	No.	%	No.	%	No.	%	No.	%		
Normal	20	100	15	75	0	0	20	100	63.7	<0.001
Respiratory alkalosis	0	0	4	20	14	70	0	0		
Metabolic alkalosis	0	0	1	5	3	15	0	0		
Respiratory acidosis	0	0	0	0	3	15	0	0		
Metabolic acidosis	0	0	0	0	0	0	0	0		

X^2: Qui-square test, P: Probability

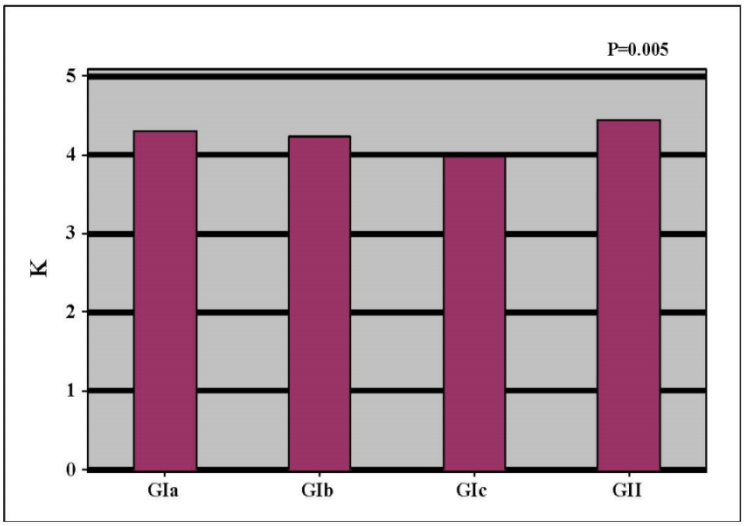

Fig. (5) : Results of Potassium of studied groups (G: group).

DISCUSSION

Acid base disturbances are well recognized in chronic liver disease **[13]**. In the initial stages of portal hypertension development, there is splanchnic arterial hypertension associated with production of nitric oxide **[14]**. In compensated liver disease, there is an increase in cardiac index, heart rate and plasma volume. In setting of decompansated liver disease, arterial pressure decrease which lead to an increase in ADH receptor activity, plasma catecholamines, aldosterone and rennin aldosterone system and ADH levels causing sodium and water retention **[15]**. As liver's metabolic function decreases acid base disturbances often ensue **[16]**. Hyperventilation is almost universal finding with advanced liver disease leading to respiratory alkalosis **[17]**.

In this study, the mean values of hemoglobin concentration and platelets counts in cirrhotic groups were significantly lower than that of healthy subjects. Also, in Group Ib and Group Ic were significantly lower than that of Group Ia and that of Group Ic was significantly lower than that in Group Ib. This could be attributed to splenic sequestration and destruction of platelets and red blood cells **[18]**. Also there is a redistribution of platelet mass located in enlarged spleen **[19]**.

In this study, the mean values of serum albumin in Group Ib and Group Ic were significantly lower than that in group Ia and Group II. This reflects sever liver damage and decreased albumin synthesis **[20]**. These findings are consistent with the findings of Tack, et al. **[21]** who found that serum albumin was lower in cirrhotic patients than that of healthy control patients.

As regard INR it was significantly higher in Group Ib than in Group Ia and significantly higher in Group Ic than in Group Ia and Group Ib. Meanwhile the INR in GII lower than that in Group Ib and Group Ic, while prothrombin time was higher in cirrhotic patients than that of control patients. This could be explained by poor utilization of vitamin K owing to parynchymal liver disease **[22]**.

The mean values of ALT and AST in Group Ic were significantly lower than that in Group Ia and Group Ib. This may reflect the massive destruction and loss of viable hepatocytes **[23]**.

In present study initial assessment ABGs were done and both groups showed that significant difference in PH between Group Ic and Group Ia and Group Ib. As well as significant difference between Group II and Group Ic. This could be attributed to hypoxaemia, anaemia, hepato-pulmonary syndrome, hepatic hydrothorax, hyperventilation which may be due to brain hypoxia and direct stimulation of respiratory centre by elevated progesterone level. Estradiol increase the number of progesterone receptors in animal and hence increase its overall actions **[16]**.

Also metabolic alkalosis may be due to the use of loop diuretics which lead to increase urinary hydrogen loss. High level of aldosterone may increase urinary hydrogen loss **[24]**.

There is low significant increase in serum Na^+ between Group Ib and Group Ic than Group II. This could be explained by high level of aldosterone in case of hepatic fibrosis.

As regard K^+ there is low significant decrease in Group Ic in relation to Group Ia and Group II in relation to Group Ic which may be explained by by use of loop diuretics and high level of aldosterone.

Respiratory alkalosis is thought to be the most common acid base derangement found in patients with liver disease as a result of hyperventilation and an increase in blood ammonia levels. Mulhausen, et al. **[25]** observed the relationship of acid-base status in 91 patients with liver disease; 64% had respiratory alkalosis but all varieties of acid-base abnormalities were observed.

Funk, et al. **[26]** studied 50 patients with stable liver disease and 10 healthy subjects and observed that patients with the mildest form of liver disease (Child-Pugh class A) had a normal acid-base state, whereas those with class B or C had respiratory alkalosis. Proposed causative factors for respiratory alkalosis include hypoxemia in the setting of massive ascites, anemia, hepatopulmonary syndrome, hepatic hydrothorax, or bacterial infection; the exact cause of hyperventilation remains unclear but high progesterone levels owing to liver disease seems the best explanation. Patients with fulminant hepatitis and hepatic coma can have a pH greater than 7.50 **[27]**.

The cause of hyperventilation is not clear; proposed causes include brain hypoxia and direct stimulation of the respiratory center by increased progesterone levels. In addition, estradiol has been proposed to be associated indirectly with respiratory alkalosis because it increases the number of progesterone receptors in animals and hence may increase its overall actions **[16]**.

Progesterone is a respiratory stimulant in human beings and is degraded by the liver. Eiseman and Clark observed that there is a direct correlation with hyperventilation and ammonia; however, subsequent studies with intravenous infusion of ammonia did not produce any increase in ventilation. Chronic respiratory alkalosis, similar to hyperchloremic metabolic acidosis, presents with hypobicarbonatemia and hyperchloremia. In the absence of blood gas determination, this combination often is diagnosed erroneously as a chronic metabolic acidosis. In this respect, the urine anion gap is useful in distinguishing these 2 disorders: if a metabolic acidosis other than distal RTA is present then the urine anion gap should be negative. A positive urine anion gap in this setting suggests the presence of a respiratory alkalosis on distal RTA. A negative urine anion gap helps to rule out chronic respiratory alkalosis. In this disorder a positive urine anion gap is expected as a result of suppressed urinary acidification, which is an adaptive response to chronic alkalemia. Although the definitive diagnosis requires a blood gas, the urine gap provides an index of suspicion alerting to the possible presence of a chronic respiratory alkalosis. There are no studies validating the use of the urine anion gap in patients with respiratory alkalosis in the setting of chronic liver disease, in which ammonia levels may be increased. Respiratory acidosis may occur also, but this is rare exceptions when the patient is exposed to sedatives or in the context of associated lung disease **[28]**.

Metabolic alkalosis is another common base disorder found in patients with liver disease, often as a result of therapy with loop diuretics. This occurs owing to increased urinary hydrogen loss from enhanced distal hydrogen secretion. High aldosterone levels and hypokalemia further increase distal hydrogen secretion. Haussinger, et al. **[24]** suggested that metabolic alkalosis occurs as a result of abnormal hepatic bicarbonate disposal and urea synthesis in cirrhosis.

However, Shangraw and Jahoor **[29]** showed that impaired urea synthesis may not precipitate metabolic alkalosis. Metabolic alkalosis often results from diuretic therapy with loop diuretics or thiazides and often is accompanied by hypokalemia. The administration of potassium or the use of potassium-sparing diuretics such as spironolactone may prevent or reduce metabolic alkalosis. Metabolic alkalosis also may occur in the setting of vomiting. As mentioned earlier, alkalosis, similar to hypokalemia, is thought to exacerbate hepatic coma; an increase in extracellular pH increases the conversion of ammonium to ammonia **[29].**

CONCLUSION

Respiratory alkalosis either alone or associated with metabolic acidosis is most common acid base disorders. So we should put in our considerations the conditions which increase this state as gastric aspiration and vomiting. Other factors implicated in mediating changes in PH should be studied carefully such as septic shock or haemorrhage which may lead to a metabolic acidosis. We should put in our consideration that most minor therapies such as infusion of normal saline, administration of albumin, glucose infusion and initiation of diuretic therapy, vasopressin analogs and lactulose therapy may alter delicate acid-base balance.

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

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

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Risk of Hepatic Encephalopathy in Diabetic Decompensated Liver Diseased Patients with Post- HCV Liver cirrhosis

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Key words:
Diabetes mellitus, hepatic encephalopathy, HCV, HBA1c

Background and study aim: Hepatic encephalopathy (HE) is a complex and variable neuropsychiatric syndrome that is seen in patients with acute and chronic liver diseases. Diabetes mellitus (DM) is more prevalent in patients with post HCV cirrhosis**.** Because diabetes mellitus may be associated with delayed gastrointestinal transit and promoting constipation, increasing intestinal bacterial overgrowth and increasing glutaminase activity, we speculated that its presence in patients with HCV related cirrhosis would predispose to and exacerbate hepatic encephalopathy.

Patients and Methods: This study included 264 patients with severely decompensated post-HCV cirrhosis, 132 diabetic cirrhotic patients and 132 non-diabetic cirrhotic patients as control group. History is taken for all patients regarding the number of attacks of encephalopathy he experienced in the past three months, the duration of diabetes and the anti-diabetic medication he uses. All patients in the study performed liver function tests, abdominal ultrasound, complete blood count and HBA1c level for diabetic patients as well as psychometric tests for hepatic coma.

Results: Diabetic patients had higher frequency of all grades of hepatic encephalopathy mean number of attacks for each patient in the past three months is 1.9±0.3 vs 0.8±0.1 in non-diabetics with unclear precipitating factor in 43% of diabetic patients versus 23% in non-diabetic patients. Patients on oral hypoglycemic drugs represented 14.3% of diabetic patients. Patients with HBA1c >11% were 43% among patients on oral hypoglycemic drugs vs 23% with insulin. Patients on oral hypoglycemic drugs had higher frequency of hepatic coma. The mean number of attacks experienced by each patient rises with increased concentration of HBA1c from 0.8±0.2 at level <7% to 6.4±3for level >11%. The mean number of attacks increased with the duration of diabetes from 1±0.4 for <5 years to 6.4±3.1for >15 years.

Conclusion: The frequency of HE was higher in diabetic patients without other obvious precipitating factor. Patients with uncontrolled diabetes and patients on oral hypoglycemic drugs, and those with longer duration of diabetes seem to have higher risk of developing HE.

INTRODUCTION

Hepatic encephalopathy (HE) is a complex and variable neuropsychiatric syndrome that is seen in patients with acute and chronic liver diseases **[1]**. The presence or severity of HE does not always show a strong and consistent relationship with the severity of liver disease or portal hypertension suggesting that other predisposing or precipitating factors may be involved **[2]**. It has been suggested that DM may contribute to the presence and severity of HE independent of the severity of liver disease in patients with HCV cirrhosis **[3]**.

Diabetes mellitus is very common in the cirrhotic population because of shared etiologies such as obesity, chronic HCV, iron overload, and alcohol as well as insulin resistance associated with cirrhosis **[4]**. Diabetes mellitus is more prevalent in patients with post-hepatitis C cirrhosis than in those with cirrhosis due to other etiologic agents **[5]**.

There are many factors that precipitate HE in patients with cirrhosis including constipation, gastrointestinal bleeding, dietary protein overload, electrolyte abnormalities (hypokalemia, azotemia), medications (opiates, benzodiazepines, and anti-psychotic agents), and infections. Usually HE is reversible in this situation when the precipitating factors are eliminated or corrected. The presence of DM may be another factor in the pathogenesis of HE at least in patients with HCV cirrhosis **[6]**.

An increased ammonia level of gut bacterial origin is an important mediator in the pathogenesis of hepatic encephalopathy (HE), and constipation is a frequent precipitant of hepatic coma. Because diabetes mellitus (DM) may be associated with delayed gastrointestinal transit, we speculated that its presence in patients with HCV-related cirrhosis would predispose to and exacerbate HE **[6]**. Diabetes mellitus and insulin resistance are characterized by releasing and enhancing pro-inflammatory cytokines. Type 2 diabetes mellitus could impair hepatic encephalopathy by different mechanisms that include: a) increasing glutaminase activity; b) impairing gut motility and promoting constipation, intestinal bacterial overgrowth and bacterial translocation **[7].**

This study aimed at evaluating if diabetes mellitus has impact on the incidence of hepatic encephalopathy in patients with severely decompensated liver disease. This study aimed also to evaluate the effect of diabetes control and the drugs used to achieve control on the incidence and severity if hepatic encephalopathy.

PATIENTS AND METHODS

This study was conducted in Tropical Medicine Department in Zagazig University Hospitals**.** The present study included 264 post-HCV liver cirrhosis patients. Patients were categorized into 2 groups:

- **Group I (test):** post-HCV liver cirrhosis with diabetes mellitus (132 patients).
- **Group II (control):** post-HCV liver cirrhosis without diabetes (132 patients)**.**

Inclusion criteria:

Patients who have post-HCV liver cirrhosis with severely decompensated liver disease (Child's grade C) with and without diabetes mellitus were included in this study.

Exclusion criteria:

- Patients with renal impairment or renal medical disease.
- Patients with malignancies.
- Patients with SBP.
- Patients with Child's grade A or B.
- Patients with cirrhosis due to causes other than HCV e.g. HBV, autoimmune, metabolic or alcoholic.
- Patients with type I diabetes.

All patients were subjected to the following:

- Complete history taking with special stress on:
  - Past history of frequency and severity of hepatic encephalopathy,
  - Duration of DM.
  - Drugs used for control of DM
  - Precipitating factors of encephalopathy
  - Thorough clinical examination
  - Psychometric testing for HE

- The following laboratory investigations:
  - Complete blood count, liver function test, kidney function test, prothrombin time, INR, serum uric acid, fasting and postprandial blood glucose.
  - Glycosylated haemoglobin level: The reference range for healthy adults is 4.8–5.9%.The decision limits for non-pregnant adults, according to the American Diabetes Association, are as follows: For patients with DM, the goal of therapy is less than 7.0%.The diagnostic criterion for diabetes is greater than or equal to 6.5% National Glycohemoglobin Standardization Program (NGSP) units **[8]**.
  - Viral markers
  - Autoimmune markers: ANA, SMA, anti-LKM
  - Bilharzial Antibody titre
  - Imaging investigations: Abdominal Ultrasound

Statistical analysis:

Data were expressed as mean ± SD for quantitative data and number and percentage for qualitative data and comparison was done by Standard normal deviate (Z) for the quantitative data and Chi-square test (X^2) for categorical and qualitative data and ANOVA (F) for multivariate analysis.

RESULTS

There were no significant differences between the two studied groups as regards demographic data, hematological parameters, coagulation profile, uric acid as well as liver and kidney function tests. There were also no significant differences as regards the prevalence of ascites among both groups as well as mean splenic size and portal vein diameter as shown in tables (1,2,3). Table 4 shows that patients with diabetes and cirrhosis (group I) had significantly higher incidence of hepatic encephalopathy of all grades than non-diabetic cirrhotic patients (group II) also it shows patients of group I had higher mean number of attacks during the last three months than patients in group II. Comparison of the precipitating factors of the attacks showed that there is higher incidence of unknown precipitating factor among patients of group I as shown in table 5.

Table (6) shows that 42% of patients using oral hypoglycemic had hemoglobin A1c level higher than 11 g/dl the finding that refers to failure of control associated with the use of oral hypoglycemic drugs. Also, there was higher incidence of encephalopathy of all grades among patients receiving oral hypoglycemic drugs than those receiving insulin for control of diabetes in group I as shown in table 7.

Patients of group I with different levels of HBA1c had no significant difference as regards the incidence of different grades of encephalopathy. However, the mean number of attacks experienced by each patient in group I rises significantly with higher levels of HBA1c as shown in table 8. Patients with diabetes for longer than 15 years among patients of group I had significantly higher incidence of severe encephalopathy grade III and IV. Also, the mean number of attacks for each patient rises significantly with longer duration of diabetes as shown in table 9.

Table (1): Patients characteristics of the studied group

		Group I No=132		Group II No=132		Test value	P	Sig.
		No	%	No	%			
Sex	Male	58	43.9	70	53.0	X^2=2.18	0.139	NS
	Female	74	56.1	62	47.0			
Age (years) Mean± SD		57.1 ±7.4		55.5 ±7.1		t=1.789	0.075	NS

Table (2): Comparison between Group I and Group II as regard CBC, Coagulation profile, liver and kidney function tests and uric acid level

	Group I No.=132	Group II No.=132	Z	P	Sig.
HB (g/dl) Mean ± SD	10±1.2	9.9±1.1	0.753	0.452	NS
WBC (cellsx10^3/uL) Mean ±SD	6.6±1.7	6.5±1.7	0.305	0.761	NS
PLT (cellsx10^3/uL) Mean ±SD	83.9±30.4	85.2±31	0.363	0.717	NS
PT (sec) Mean ±SD	23.9±5.4	25±5.1	1.621	0.106	NS
PC (%) Mean ±SD	41.6±11	39.9±12	1.44	0.231	NS
INR Mean ±SD	2.3±1.5	2.4±1.5	0.489	0.619	NS
ALT(IU/L) Mean ±SD	46.7±22.7	48.9±23	0.682	0.496	NS
AST(IU/L) Mean ±SD	82.3±41.7	82.8±40	0.078	0.938	NS
Albumin (g/dl) Mean ±SD	2.2±0.4	2.1±0.4	1.735	0.084	NS
T.bil (mg/dl) Mean ±SD	5.1±1.9	5.3±2.1	1.084	0.279	NS
D.bil (mg/dl) Mean ±SD	3.1±1.1	3.3±1.2	1.99	0.159	NS
Creatinine (mg/dl) Mean ±SD	0.8±0.2	0.8±0.1	0.123	0.902	NS
Uric acid (mg/dl) Mean ±SD	7.1±2.3	6.9±2	0.618	0.537	NS

Table (3): Comparison between Group I and Group II as regard U/S

		Group I No.=132		Group II No.=132		Test value	P	Sig.
		No.	%	No.	%			
Ascites	Absent	6	4.5	7	5.3	X^2=0.08	0.776	NS
	Detected	126	95.5	125	94.7			
PVD (mm) Mean ±SD		14.9±1.3		15±1.1		Z=0.674	0.501	NS
Spleen size(cm)Mean ±SD		16.2±1.5		16.3±1.4		Z=0.33	0.742	NS

Table (4): Comparison between Group I and Group II as regard assessment of Encephalopathy in the last 3 months

		Group I No.=132		Group II No.=132		Test value	P	Sig.
		No.	%	No.	%			
(+ve) Psychometric test		9	6.8	4	3.0	χ^2 2.02	0.154	NS
Grade of encephalopathy	G1	47	35.6	35	26.5	χ^2 17.23	<0.001	HS
	G2	45	34.1	31	23.5			
	G3	29	22.0	19	14.4			
	G4	22	16.7	18	13.6			
No. of attacks/each patient/3 mo Mean ±SD		1.9 ±0.3		0.8 ±0.1		Z=5.063	<0.001	HS

Table (5): Comparison between the two groups as regards precipitating factors of hepatic encephalopathy

Precipitating factors	Group I No=132		Group II No=132		X^2	P	Sig.
	No	%	No	%			
Constipation	25	18.9	17	12.9	1.81	0.178	NS
Protein diet	8	6.1	10	7.6	0.24	0.625	NS
Diuretics	14	10.6	15	11.4	0.04	0.843	NS
Hematemesis and/or melena	6	4.5	12	9.1	2.15	0.142	NS
Tapping	1	0.8	3	2.3	1.02	0.314	NS
Gastroenteritis	3	2.3	7	5.3	1.66	0.197	NS
Chest infection	18	13.6	13	9.8	0.91	0.393	NS
UTI	19	14.4	18	13.6	0.03	0.859	NS
Unknown cause	58	43.9	22	16.7	23.24	<0.001	HS

Table (6): Correlation between anti diabetic drugs and HBA1C in group I

HBA1c level % NGSP units	Oral hypoglycemics No = 19		Insulin No = 113		X^2	P	Sig.
	No	%	No	%			
<7	1	5.3	10	8.8	0.27	0.6	NS
7-9	4	21.1	43	38.1	2.05	0.152	NS
9-11	6	31.6	36	31.9	0.06	0.809	NS
>11	8	42.1	24	21.2	3.86	0.049	S

Table (7): Correlation between encephalopathy and anti-diabetic drugs in group I

	Oral hypoglycemic drugs No = 19		Insulin No = 113		X^2	P	Sig.
	No	%	No	%			
Psycho +ve	6	31.6	3	2.7	21.42	<0.001	HS
Grade I	8	42.1	12	10.6	12.54	<0.001	HS
Grade II	12	63.2	19	16.8	19.44	<0.001	HS
Grade III	15	78.9	25	22.1	24.87	<0.001	HS
Grade IV	19	100.0	33	29.2	34.15	<0.001	HS

Table (8): Correlation between encephalopathy and HBA1C in group I

Grades of encephalopathy	HBA1c level (g/dl)								X^2	P	Sig.
	< 7		7-9		9 -11		>11				
	No =11		No=47		No=42		No=32				
	No	%	No	%	No	%	No	%			
Psycho +ve	0	0.0	2	4.3	5	11.9	2	6.3	3.02	0.389	NS
Grade I	5	45.5	7	14.9	4	9.5	5	15.6	2.72	0.48	NS
Grade II	4	36.3	8	17.0	7	16.7	9	28.1	3.44	0.33	NS
Grade III	5	45.5	11	23.4	13	30.9	11	34.4	2.51	0.473	NS
Grade IV	5	45.5	10	21.3	14	33.3	15	46.9	6.44	0.0992	NS
No of attacks/ patient Mean ±SD	0.8±0.2		3.4±1.1		5±2.2		6.4±3		F=7.2	<0.001	HS

Table (9): Correlation between encephalopathy and duration of diabetes in group I

Grades of hepatic encephalopathy	Duration of diabetes in years								X^2	P	Sig.
	<5years		5-10		10-15		>15				
	No=48		No=33		No=29		No=22				
	No	%	No	%	No	%	No	%			
Psycho +ve	1	2.1	3	9.1	3	10.3	2	9.1	2.71	0.439	NS
Grade I	7	14.6	5	15.2	6	20.7	7	31.8	3.34	0.343	NS
Grade II	5	10.4	8	24.2	9	31.0	10	45.5	11.11	0.011	S
Grade III	6	12.5	9	27.3	11	37.9	13	59.1	16.99	<0.001	HS
Grade IV	4	8.3	11	33.3	15	51.7	17	77.3	35.59	<0.001	HS
No of attacks/ patient Mean ± SD	1 ±0.4		2.1±0.9		5.5±2.1		6.7±3.1		F=8.32	<0.001	HS

DISCUSSION

This study was designed to detect the effect of diabetes itself, its duration, its control and the drugs used to achieve this control on the severity and the frequency of the hepatic encephalopathy encountered by patients with severely decompensated liver cirrhosis due to HCV. HCV seems to have a strong relation with diabetes. Most of the previous literature says that the diabetes and insulin resistance associated steatosis can hasten the progression of HCV related liver damage and HCV can induce insulin resistance in patients with chronic hepatitis C.

In this study the diabetic patients group had no significant difference from non-diabetics as regards age, gender as well as the entire routine laboratory parameters. This finding indicates that the deterioration of the liver function was not the only risk factor that causes the higher frequency of hepatic encephalopathy of all grades noticed in patients of the diabetic group. This is in agreement with Sigal et al. who said that patients with diabetes tend to develop encephalopathy even with milder liver decompensation. The frequency of encephalopathy attacks in the past three months is also significantly higher in diabetic patients **[6]**.

In this study we focused on the history of the possible precipitating factor for encephalopathy attacks like protein diet, constipation, tapping for ascites and diuretics, gastroenteritis, urinary tract and chest infections and upper GIT bleeding. If the history taking revealed unknown mode of precipitation we also mentioned it as an attack with unknown precipitating factor. We found that the patients with diabetes had significantly higher frequency of unknown mode of precipitation (43%) compared to non-diabetic patients (23%). Although diabetes is associated with higher risk of infection, infection doesn't seem to take the upper hand as a precipitating factor. This agrees with Ampuero et al. who said that patients with diabetes showed raised risk of overt hepatic encephalopathy in comparison with non-diabetics because type 2 diabetes mellitus could impair hepatic encephalopathy by different mechanisms that include: a) increasing glutaminase activity; b) impairing gut motility and promoting constipation, intestinal bacterial overgrowth and bacterial translocation **[7]**. This is also agreed with by Thuluvath **[9]** who said that diabetes causes autonomic neuropathy that affects the motility of the gut leading to excess ammonia production.

Asking the patients about the medication they use to control diabetes revealed that a small percent of them use different types of oral hypoglycemic drugs (14%). This small percent of patient had high frequency of all grades of HE. This is in disagreement with Ampeuro et al. who found that metformin decreases the frequency of encephalopathy by decreasing the production of glutamine **[10]**. This disagreement may be due to the use of different types of oral hypoglycemic including metformin by patients in our study rather than focus on metformin only like in Ampuero et al. study.

In this study, we also noticed that 42% of patients on oral hypoglycemic had HBA1c level above 11% NGSP units a mark of bad control of diabetes over the last three months, and about 5 % of them only managed to achieve the target of less than 7%. This finding says that the increased frequency may be related to the bad control of diabetes. This finding also confirms that the patients on insulin had a better chance for managing and controlling diabetes than those on oral hypoglycemic. The glycosylated hemoglobin gives us an idea about the control of diabetes in the last three months. In our study, we investigated about the number and the severity of attacks of hepatic encephalopathy during the past three months to be able to link it to the diabetes control in this period. In this study, we also noticed that 42% of patients on oral hypoglycemic versus 21% of patients on insulin had HBA1c level above 11% NGSP units as a mark of bad control of diabetes over the last three months, and about 5 % of patients on oral hypoglycemic versus about 9% of patients on insulin managed to achieve the target of less than 7% NSGP units. This finding confirms that the patients on insulin had a better chance for managing and controlling diabetes than those on oral hypoglycemic. It is also clear that the increased frequency of hepatic encephalopathy with oral hypoglycemic use is related to the bad control of diabetes rather than oral hypoglycemic drugs themselves. This finding agrees with Gundling et al 2013 who studied different types of oral hypoglycemic drugs with liver cirrhosis versus insulin and concluded that glycemic control was insufficient in 73% of the patients receiving insulin therapy versus 88% of patients receiving oral antidiabetic drugs.[11]

Comparing the frequency of different grades of HE in diabetic patients with variable levels of HBA1c showed that there was no significant difference in the frequency of all grades of HE. However, the number of attacks experienced by each patient during the past three months increases with the increase in the level of glycosylated hemoglobin. This also confirms that the increased frequency of HE associated with oral hypoglycemic drugs use is related to the failure of control rather than the use of those drugs itself. This finding agrees with that in the study by Gundling et al. who said that patients achieving satisfactory control experienced a lower rate of certain cirrhosis-related complications such as hepatic encephalopathy (HE) [HE 36.6% (diabetics) vs. 20.7% (non-diabetics][**11**].

In our study we also tried to find the relation of the duration of diabetes to the severity and frequency of HE. We found that patients with longer duration of diabetes exceeding 15 years have higher frequency of attacks of severe encephalopathy (grade II and IV). Also, we found that the number of attacks experienced by each patient during the past three months increases with the increase in the duration of diabetes. This is in agreement with Nathan et al, 2005 who said that the longer the duration of diabetes the more profound the changes in the nerve tissues and their blood supply. We can also say that patient with long duration of diabetes must have been experiencing cumulative brain and nerve tissue damage through this long period that make these patients more liable to severe and more frequent attacks of hepatic coma **[12]**.

CONCLUSION:

The frequency of HE was higher in diabetic decompensated liver diseased patients without obvious precipitating factor. Patients on oral hypoglycemic drugs, patients with uncontrolled diabetes and patients with longer duration of diabetes seem to have higher risk of developing HE.

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Diagnostic and Prognostic Validity of Serum Golgi Protein 73 in Egyptian Patients with Hepatocelluar Carcinoma

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Key words: HCC, GP73, AFP

Background and study aim : Hepatocellular carcinoma (HCC) is common all over the world. Most HCC are diagnosed at an advanced stage . We aimed to detect the serum Golgi protein (GP 73) in patients with cirrhosis and HCC as non-invasive marker for diagnosis and prognosis of HCC.
Patients and Methods: This study was conducted on 81 subjects: They were divided into 3 groups : 27 patients with HCC, 27 patients with liver cirrhosis and 27 healthy control subjects. Serum alphaphetoprotein (AFP) and GP 73 were estimated by ELISA. In addition, GP 73 was remasured after therapy in patients with HCC who were treated by percutaneous ethanol injection.
Results: GP 73 was elevated in patients with HCC and liver cirrhosis ; serum level was high in HCC patients ($p<0.01$) when compared with the other studied groups. GP 73 had sensitivity of 81.4% and specificity of 100% at a cut-off value 4.12 ng/ml with area under the receiver operator characteristics (AUC) of 0.964 when compared with AFP that showed a sensitivity 77.7% , specificity 85.1% at a cut-off value >200 and (AUC) 0.774. when AFP was combined with GP73 for the diagnosis of HCC, sensitivity and specificity were increased to 87.6% and 100% respectively. At six week after ethanol injection, a significant decrease in GP73 occurred.
Conclusion: Serum GP 73 can be used as a useful biomarker to confirm the diagnosis of HCC especially if combined with AFP and GP73 had promising prognostic value as it decreased after the treatment of HCC and is correlated to tumor size

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer type, and the third leading cause of cancer mortality worldwide **[1],[2]**. Reports show that HCC is becoming more wide-spread and has dramatically increased in North America , Western Europe and Japan **[3,4]**. Additionally there is an increasing incidence of the disease among younger age groups that warrants further investigations **[1].**

Egypt has the rising rates of hepatocellular carcinoma (HCC). Egypt's unique nature of liver disease presents questions regarding the etiology of HCC. The currently increasing incidence of HCC in Egyptians may be due to shift of the relative importance of HCV as primary risk factors **[5]**. HCC is the second most frequent cause of cancer incidence and mortality among men at Egypt **[6]**.

Many observational studies have reported that HCC is diagnosed at an earlier stage in patients who received surveillance **[7]**. Although HCC surveillance programs are controversial, most international societies - the European Association for the Study of the Liver and the American Association for the Study of Liver Diseases – recommend the use of ultrasound and alphafetoprotein (AFP) in patients with a high risk of developing the condition, at 6-12 months frequency **[8]**. The use of AFP alone is strongly discouraged, and its use, in addition to ultrasound is controversial. Patients with abnormal screening tests require additional investigations.

Therefore, there is a strong demand by clinicians for new HCC specific biomarkers. Golgi protein (GP73) is a resident type transmembrane protein expressed primarily in human epithelial cells **[9]**. In the normal human liver, GP73 is expressed in biliary epithelial cells, but detection is negligible in hepatocytes. However, upregulated expression of GP73 has been identified in hepatic cells in liver disease **[10]**. Previous studies have also shown increased serum GP73 levels in patients with chronic liver disease and, in particular, in HCC patients. This phenomenon may be due to migration of the GP73 protein to the plasma membrane and diffusion into the circulation **[11,12]**. Thus, the aim of this study is to assess the value of Golgi Protein 73 (GP73) as a non-invasive marker for diagnosis and prognosis of HCC.

PATIENTS AND METHODS

This study was carried out in Tropical Medical, Radiotherapy and Clinical Pathology Departments, Zagazig University Hospital. This study was conducted on eighty one subjects. The subjects were classified to three groups :

Group I: 27 patients with HCC.

Group II: 27 patients with liver cirrhosis without HCC.

Group III: 27 normal individuals as a control group.

Inclusion criteria:

1. Patients with HCC (diagnosed by ultrasound and triphasic computed tomography (CT) criteria)
2. Cirrhotic patients with no evidence of hepatic focal masses in ultrasound evaluation, included in group B.
3. Diagnosis of cirrhosis was based on clinical, laboratory, and imaging. Patients with cirrhosis and elevated AFP, but no evident focal hepatic lesion on ultrasound, were subjected to triphasic CT performed within 3 months before and 6 months after the enrollment in the study.

Exclusion criteria :

All patients who had a prior locoregional therapy, systemic therapy and/or any surgical intervention (liver resection or transplantation) were excluded from the analysis. Also Patients with any other hepatic or non hepatic malignancy.

All patients were subjected to:

1. Full history taking:
2. Complete general examination.
3. Local examination.
4. Investigations including:

a) Laboratory investigations:

- Complete blood picture (CBC).
- Liver profile: S. bilirubin, SGOT, SGPT, ALP, total protein and S.albumin.
- Kidney profile: S. creatinine, Bl. Urea, and uric acid.
- Coagulation profile: PT, PTT and INR.
- Viral markers:
  - a- Hepatitis B surface antigen (HBsAg).
  - b- Hepatitis C immunoglobulin G (HCV IgG).
- Alpha-feto protein (α-FP): It was determined by ELISA Kit For Alpha-feto protein (α-FP) provided by Ray Biotech, Inc., the catalogue no ELH-AFP. The RayBio® Human AFP (Alpha Fetoprotein) ELISA kit is an in vitro enzyme linked immunosorbent assay for the quantitative measurement of human AFP in serum, plasma, and cell culture supernatants.
- Golgi Protein 73 (GP 73): It was determined by ELISA Kit For Golgi Protein 73 (GP73) provided by Uscn, Life Science (Inc-USA), the catalogue no E91668Hu. The kit is a sandwich enzyme immunoassay for the in vitro quantitative measurement of GP73 in human serum, plasma and other biological fluids.

b) Imaging studies:

- **Abdominal ultra-Sonography (U/S) :** It was done for examination of the liver for criteria of cirrhosis, presence of focal lesions and measurement of their bipendicular dimensions, patency of portal vein, presence of splenomegaly and ascites.
- Tri-phasic CT

Follow up: GP73 was repeated after the end of percutaneous ethanol injection (PEI) by one and half month. The number of PEI sessions depended on the size of each focal lesion according to the equation: 4/3*22/7* (1/2 r+1/2) 3. Tri-phasic CT was repeated 6 months after therapy.

Statistical analysis :

All data were analyzed using SPSS 15.0 for windows (SPSS Inc., Chicago, IL, USA) & Med Calc 13 for windows (MedCalc Software bvba).

Continuous variables were expressed as the mean ±SD and median (range), and the categorical variables were expressed as a number (percentage). Continuous variables were checked for normality by using Kolmogorov-Smirnov test. Independent Student t-test was used to compare normally distributed variables between two groups. Mann Whitney U (MW) test was used to compare non-normally distributed variables between two groups. GP73 pre-treatment and post-treatment were compared using Wilcoxon signed ranks test. Percent of categorical variables were compared using the Chi-square (χ^2) test.

Spearman's rank correlation analysis was done between GP73 levels and all study parameters. Receiver operating characteristic (ROC) curve analysis was used to identify optimal cut-off values of GP73 with maximum sensitivity and specificity for differentiation of patients with HCC from those without HCC.

P<0.05 was considered statistically significant (S), p<0.005 was considered highly statistically significant (HS), and p≥0.05 was considered non statistically significant (NS). p1 denote p value of test of significance between group I & group II, p2 denote p value of test of significance between group I & group III & p3 denote p value of test of significance between group II and group III.

RESULTS

This study was conducted on 81 subjects, divided into 3 groups 27 subjects in each group, group I (HCC patients), group II (liver cirrhosis) and group III (Healthy control group).

As regard the demographic data, there was a highly significant difference between the three studied groups according to the age with mean age 58.44 years in group I, 53.96 years in group II and 50.48 in group III (p1<0.002, p2<0.001 and p3<0.003). Meanwhile, according to sex, there was no significant difference between the three groups. There were 23 males (85.2%) and 4 females (14.8%) in group I, 22 males (81.5%) and 4 females (18.5%) in group II and 22 males (81.5%) and 5 females (18.5%) in group III. In addition to, the laboratory data, there was a highly significant difference between the three studied groups in the level of total bilirubin, serum albumin, ALT, AST, hemoglobin, platelet count and prothrombin concentration when HCC and liver cirrhosis group compared to the control group (p2<0.001 and p3<0.001) (Table 1).

As regard to the clinical data, there was no significant difference between the HCC group and liver cirrhosis group according to jaundice, hepatomegaly, splenomegaly, lower limb edema and ascites (p>0.05).

Comparison between group I and group II as regard Child-Pugh classification, ascites, encephalopathy, viral etiology revealed no significant difference (p>0.05). In group I there were 5 patients with Child A (18.5%) and 22 patients with Child B (81.5%), in group II, there were 6 patients with Child A (22.2%) and 21 patients with Child B (77.8%). In group I, there were 19 HCV positive patients (70.4%), 5 HBV positive patients (18.5%) and 3patinets with positive HBV & HCV (11.1%). In group II, there were 18 HCV positive patients (66.7%), 7 HBV positive patients (25.9%) and 2 patients with positive HBV & HCV (7.4%) (Table 2).

All the HCC patients included in this study were with single focal lesion (100%); with mean dimensions 2.67 cm x 2.67 cm ± 0.52 and mean biperndicular diameter 7.39 cm^2 ± 2.77 and all patients show no PVT.

Comparison between the three studied groups as regard AFP and GP73 shows high significance with mean level of AFP 552.35 ng/ml in group I, 16.43 ng/ml in group II and 1.98 ng/ml in group III (p1, p2 and p3 <0.001). Meanwhile, GP73 showed mean level of 8.86 ng/ml in group I, 4.53 ng/ml in group II and 2.19 g/ml in group III (p1,p2 and p3 <0.001) (Table 3).

Correlation between tumor markers and all study parameters in the HCC group showed high significance correlation between GP73 and AFP and vice versa and between size of tumor with both AFP and GP73 (p<0.001). The size of HCC foci ranged from 1.7 to 3.7 cm with a mean diameter of 2.7±1.0 cm (Figure 2,3)

At a cut-off value 4.12 ng/ml, GP73 showed a sensitivity of 81.4%, 100% specificity and AUC of 0.964 with accuracy of 90.7% when compared to the control group. And at a cut-off value of 6.7 ng/ml, GP73 showed a sensitivity of 81.4% and 92.5% specificity and AUC of 0.812 with accuracy of 87% versus cirrhotic control (Table 4). The sensitivity and specificity of AFP at a cut-off value 200 ng/ml was 77.7% and 85.1% respectively with AUC of 0.774 versus healthy

control (accuracy 81.4%). Meanwhile, versus cirrhotic patient, AFP showed sensitivity and specificity of 74.1% and 66.7% respectively with AUC 0.698 (accuracy 70.4%) (Table 5).

However, when GP73 used in combination with AFP in early diagnosis of HCC, they increased the sensitivity to 87.6% and the specificity to 100% with increasing of AUC to .977 versus the control group and 88.9% and 95.7% for the sensitivity and the specificity versus cirrhotic group respectively with increasing AUC, also to 0.905 (Table 6).

All the chosen HCC patients were treated with percutaneous ethanol injection. The mean level of GP73 before and after treatment showed decrease with mean level 8.86 ng/ml and 5.8 ng/ml pre-treatment and post-treatment respectively (WSR= -3.436, p<0.001) (Table 7).

Table (1) : Comparison between the studied groups as regard the demographic and laboratory data

Demographic and laboratory data	**Group I (HCC patients) (n=27)**		**Group II (Liver cirrhosis) (n=27)**		**Group III (Control group) (n=27)**		**P1**	**p2**	**P3**
Age (years)							**t**	**t**	**t**
Mean ± SD	58.44 ± 5.49		53.96 ± 4.40		52.48 ± 3.91		**>0.05 (NS)**	**>0.05 (NS)**	**>0.05 (NS)**
Sex NO.	**NO.**	**%**	**NO.**	**%**	**NO.**	**%**	**X^2**	**χ^2**	**X^2**
Male	23	85.2%	22	81.5%	22	81.5%	1.000 (NS)	1.000 (NS)	0.726 (NS)
Female	4	14.8%	5	18.5%	5	18.5%			
Total bilirubin (mg/dl)							**t**	**T**	**t**
Mean ± SD	1.68 ± 0.93		1.71 ± 0.77		0.62 ± 0.23		0.440 (NS)	**<0.001 (HS)**	**<0.001 (HS)**
AST (U/L)							**t**	**T**	**T**
Mean ± SD	103.63 ± 39.29		76.04 ± 19.52		16.67 ± 2.94		**0.002 (HS)**	**<0.001 (HS)**	**<0.001 (HS)**
ALT (U/L)							**t**	**T**	**t**
Mean ± SD	118.15 ± 36.92		62.63 ± 17.005		15.96 ± 3.04		**<0.001 (HS)**	**<0.001 (HS)**	**<0.001 (HS)**
Median	122		58		16				
Albumin (g/dl)							**t**	**T**	**t**
Mean ± SD	2.81 ± 0.33		2.83 ± 0.29		4.69 ± 0.28		0.876 (NS)	**<0.001 (HS)**	**<0.001 (HS)**
Prothrombin concentration							**t**	**T**	**t**
Mean ± SD	61.81 ± 9.70		62.81 ± 4.48		98.74 ± 1.45		0.630 (NS)	**<0.001 (HS)**	**<0.001 (HS)**
Hemoglobin (g/dl)							**t**	**T**	**t**
Mean ± SD	10.28 ± 0.91		11.49 ± 0.61		13.48 ± 0.30		**<0.001 (HS)**	**<0.001 (HS)**	**<0.001 (HS)**
Platelet count ($\times 10^3/mm^3$)							**t**	**T**	**t**
Mean ± SD	151 ± 32.30		103.11 ± 26.20		269.26 ± 16.33		**<0.001 (HS)**	**<0.001 (HS)**	**<0.001 (HS)**
Creatinine (mg/dl)							**t**	**T**	**T**
Mean ± SD	0.92 ± 0.20		0.93 ± 0.19		0.84 ± 0.14		0.839 (NS)	0.216 (NS)	0.114 (NS)
Urea (U/L)							**t**	**T**	**T**
Mean ± SD	19.37 ± 4.69		19.63 ± 3.09		18.96 ± 1.84		0.351 (NS)	0.600 (NS)	0.341 (NS)

P value by man-whitney test)

χ^2: Chi-square test **t:** independent Student t-test p< 0.05 is significant

p1 denote p value of test of significance between group I & group II

p2 denote p value of test of significance between group I & group III

p3 denote p value of test of significance between group II & group III

Table (2) : Comparison between group I and group II as regard Child-Pugh classification and viral etiololgy

	Group I HCC (n=27)	**Group II Cirrhosis (n=27)**	**P**
Child-pugh class.			
A	5(18.5%)	6(22.2%)	NS
B	22(81.5%)	21(77.8%)	NS
C	0	0	NS
Viral infection			
HBV	5(18.5%)	7(25.9%)	NS
HCV	19(70.4%)	18(66.7%)	NS
HBV & HCV	3(11.1%)	2(7.4%)	NS

Table (3) : Comparison between the studied groups as regard AFP and GP73

Tumor markers	**Group I (HCC patients) (n=27)**	**Group II (Liver cirrhosis) (n=27)**	**Group III (Control group) (n=27)**	**p1**	**p2**	**p3**
AFP (ng/ml)				**t**	**T**	**T**
Mean ± SD	552.35 ± 169.05	16.43 ± 3.49	1.95 ± 0.11	<0.001	<0.001	<0.001
Range	208 – 865	9.45 – 23	1.72 – 2.13	(HS)	(HS)	(HS)
GP73 (ng/ml)				**MW**	**MW**	**MW**
Mean ± SD	8.86 ± 3.24	4.53 ± 1.86	2.19 ± 0.74	<0.001	<0.001	<0.001
Range	2.2 – 12.5	1.99 – 11	1.0 – 4.12	(HS)	(HS)	(HS)

t: independent Student t-test
MW: Mann Whitney U test
p< 0.05 is significant

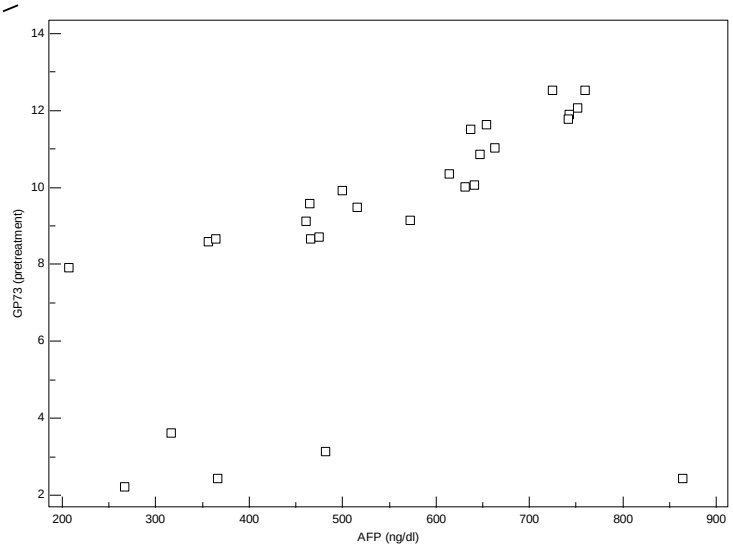

Figure (1): Scatter plot with regression line shows correlation between AFP (ng/ml) & pretreatment GP73 (r=+0.968, p<0.001).

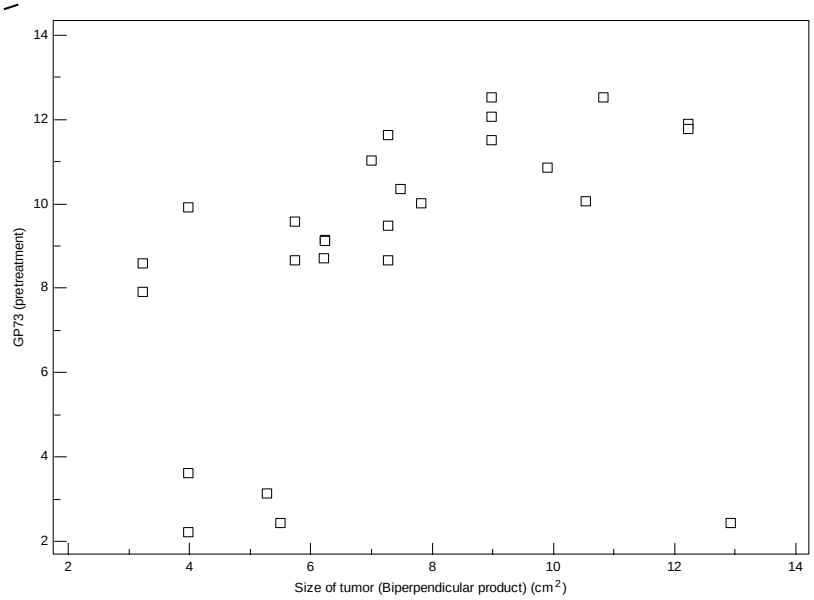

Figure (2): Scatter plot with regression line shows correlation between size of tumor (Biperpendicular product) & pretreatment GP73 (r=+0.861, p<0.001).

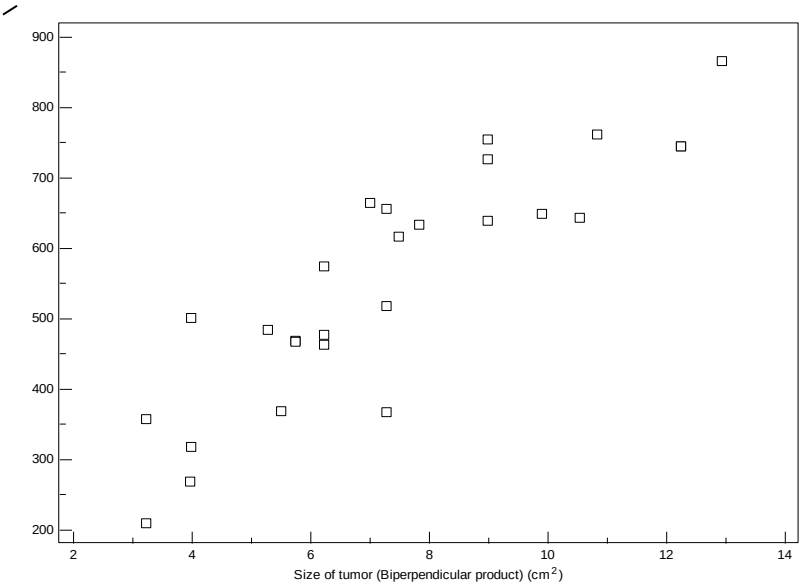

Figure (3): Scatter plot with regression line shows correlation between Size of tumor (Biperpendicular product) & AFP (r=+0.876, p<0.001).

Table (4) : GP73 as a diagnostic marker for HCC (versus cirrhosis)

Cut-off value	Sens. % (95% CI)	Spec. % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	AUC* (95% CI)
> 6.7	81.4 % (61.9-93.7)	92.5 % (75.7-99.1)	91.7 % (73-99)	83.3 % (65.3-94.4)	0.812‡ (0.683-0.905)

* Accuracy of GP73 as a diagnostic marker for HCC (versus cirrhosis) = 87% (68.8 – 96.4) (Excellent).
‡ **p<0.0001 (HS)**

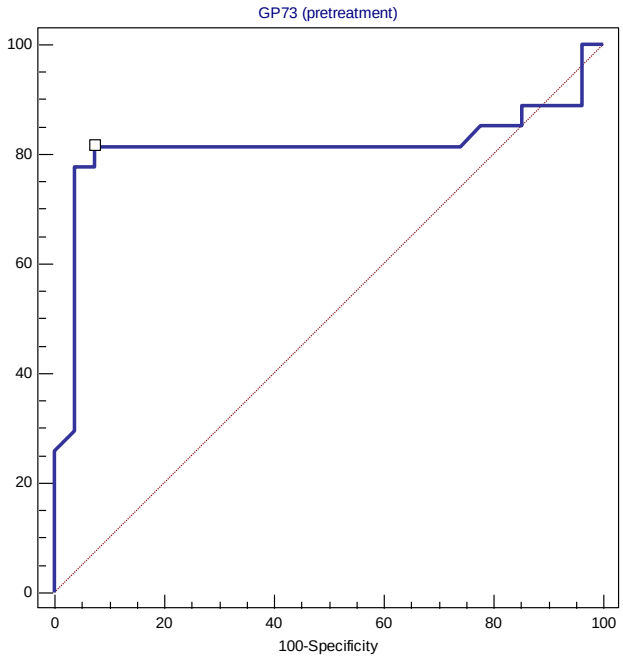

Figure (4): Receiver operating characteristic (ROC) curve of GP73 as a diagnostic marker for HCC (versus cirrhosis).

Table (5) : Validity of GP73 versus AFP in prediction of HCC among HCC (versus healthy)

Cut-off values	Sens. % (95% CI)	Spec. % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	AUC* (95% CI)
GP73 > 4.12	81.4 % (61.9-93.7)	100 % (87.2-100)	100 % (84.6-100)	84.4 % (67.2-94.7)	0.964‡ (0.875-0.996)
AFP > 200	77.7 % (57.7 – 91.4)	85.1 % (66.3 – 95.8)	84 % (63.9 – 95.5)	79.3 % (60.3 – 92)	0.774§ (0.640 – 0.877)
Both	87.6%	100%	100%	90.1%	0.977

* Accuracy of GP73 as a diagnostic marker for HCC (versus healthy control) = 90.7% (74.6 – 96.9) (Excellent); Accuracy of AFP as a diagnostic marker for HCC (versus healthy) = 81.4% (62 – 93.6); Accuracy of both GP73 and AFP 93.4%
‡ **p<0.001 (HS)**
§ **p<0.001 (HS)**

Table (6): Validity of GP73 versus AFP in prediction of HCC among HCC (versus cirrhosis)

Cut-off values	Sens. % (95% CI)	Spec. % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	AUC* (95% CI)
GP73 > 6.7	81.4 % (61.9-93.7)	92.5 % (75.7-99.1)	91.7 % (73-99)	83.3 % (65.3-94.4)	0.812‡ (0.683-0.905)
AFP > 400	74.1 % (53.7 – 88.9)	66.7 % (46 –83.5)	69 % (49.2 – 84.7)	72 % (50.6 – 87.9)	0.698§ (0.558 – 0.816)
Both	88.9%	95.7%	94.3%	88.9%	0.905

* Accuracy of GP73 as a diagnostic marker for HCC (versus cirrhotic) = 87% (68.8 – 96.4) (Excellent). Accuracy of AFP as a diagnostic marker for HCC (versus cirrhotic) = 70.4% (49.9 – 86.2).
‡ p<0.001 (HS)
§ p<0.001 (HS)

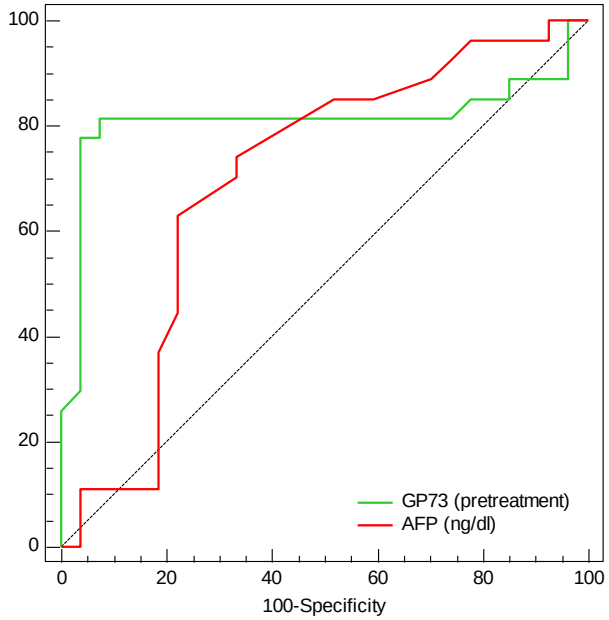

Figure (5): Receiver operating characteristic (ROC) curve of GP73 and AFP as diagnostic markers for HCC (versus cirrhosis).

Table (7): Comparison between GP73 before and after therapy

	Pre-treatment GP73 (n=27)	Post-treatment (n=27)	WSR	p
Mean ± SD	8.86 ± 3.42	5.80 ± 2.71	-3.436	**<0.001 (HS)**
Median (Range)	9.5 (2.2 – 12.5)	5 (1.99 – 7.5)		

WSR: Wilcoxon signed ranks test.
p< 0.05 is significant

DISCUSSION

Hepatocellular carcinoma is usually asymptomatic in early stages and tends to be invasive. Therefore, most patients are presented with an incurable disease at the time of detection which makes its early diagnosis critical for a good prognosis. Surgical resection remains the treatment of choice for these tumors, but unfortunately only 10-20% of primary HCCs are resectable at time of diagnosis. Continuous researches are ongoing worldwide to find and evaluate an early sensitive and specific marker for HCC **[13]**. In Egypt, chronic HCV is the main cause of liver cirrhosis and liver cancer,

which is one of the top five leading causes of death **[14]**.

Serum AFP is the most widely used biomarker for diagnosis of HCC. The normal range for serum AFP levels is 10-20 ng/ml and a level >400 ng/ml is usually regarded as of diagnostic value. However, two thirds of HCC patients with the nodule less than 4 cm have serum AFP levels less than 200 ng/ml and up to 20% of HCC patients do not produce AFP. Therefore, the lack of AFP sensitivity and specificity **[15]** has elucidated the need for a new tumor marker for differentiating HCC from benign hepatic disorders.

In the present study there was a statistically significant difference between the mean value of GP3 in patients with HCC compared to patients with liver cirrhosis and control with mean values of 8.86 ± 3.24, 4.53± 1.86 and 2.19 ±0.74 ng/mL respectively with a p value <0.001. These findings were in agreement with Riener et al. **[16]**, Gu et al. **[17]** who demonstrated that elevation of serum levels of GP73 was detected in patients who had developed an HCC on the background of HCV infection in comparison with cirrhotic control. Mao et al. **[18]** who studied GP73 in viral hepatitis have found that the elevation of serum GP73 is mildest in virus carriers, moderate in patients with cirrhosis and dramatic in patients with HCC. This indicates the performance of GP73 might depend on the etiology of underlying disease. Therefore, serum GP73 can be used to monitor disease progression from HBV infection to cirrhosis to HCC.

These results didn't come in agreement with Ozkan et al. **[19]** who found that levels of GP73 weren't significantly higher in HCC and cirrhotic patients compared to controls where the median of GP73 was 0.27ng/ml in controls, 0.32ng/ml in cirrhotic patients and 0.21ng/ml in those with HCC with a p value >0.05 which could support the presence of GP73 specific auto antibodies interfering with ELISA analysis.

Regarding the diagnostic value of GP73, the sensitivity and specificity varied with different cut-off points. In our study, GP73 had a sensitivity of 81.4 % and a specificity of 100% at the optimal cut-off value of 4.12 ng/ml with AUROC of 0.964 if compared versus healthy control and had a sensitivity of 81.4% and a specificity of 92.5% at the optimal cut-off value of 6.7 ng/ml if compared versus cirrhotic patient with AUC of 0.812 which was similar to the results of Marrero and Lok **[20]**, Gomaa et al. **[21]** postulated that, GP73 is up-regulated in HCC and measurement of serum GP73 revealed a sensitivity and specificity of 69% and 75% respectively.

In the present study, AFP had a sensitivity of 81.4% and a specificity of 92.5% at a cut-off >400. Sarwar et al. **[22]** found that AFP sensitivity was 42.7% and its specificity was 100% at a cut-off value of >400 ng/ml.

In this study the combination of AFP and GP73 led to enhancing the sensitivity of detection of HCC to 87.6%, the specificity to100% and AUROC curve was 0.9777 with an accuracy of 93.4% which is better than either of them alone. This was in agreement with Wang et al., (2009) [23] and Mao et al. **[18].**

In our study, the size of focal lesions ranged from 1.8-3.7 cm as we selected the cases only fit for percutaneous ethanol injection. Daniele et al. **[24]** said that PEI would be more effective in small focal lesions less than 3 cm.

In our study, there was a decrease in the level of sGP73 after PEI in the HCC group with mean level of 8.86 ng/ml before PEI and of 5.80 ng/ml after PEI (p<0.001) and this was in consistent with Mao et al. **[18]** who demonstrated that surgical resection of the tumor results in diminished serum GP73 levels and that tumor recurrence correlates with the recurrence of elevated GP73 in the blood. Reappearance of serum GP73 indicates the existence of tumor lesions and thus may serve as an indicator for the recurrence of HCC.

CONCLUSION

In conclusion, in combination, measurement of both GP73 and AFP has the promise to further improve the detection of HCC. GP73 has a promising prognostic value as it decreased after the treatment of HCC and is correlated to tumor size.

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Ethical Approval: This study was approved by the ethical committee of Faculty of Medicine, Zagazig University and all patients provided

written informed consent before participation in any protocol specific procedure.

Conflicts of interest : There are no conflict of interests

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Video case: Multiple sessile gastric polyposis in ulcerative colitis patient

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43 years old man presented with epigastric pain and bleeding per rectum. Upper gastrointestinal endoscopy (Video1) revealed multiple sessile gastric polyposis. Histopathological examination of one polyp revealed H. pylori related inflammation . Colonoscopy (Video2) detected ulcerations and hyperemic inflammations of the rectal and sigmoid mucosa. Histopathological examination of colonic and rectal biopsies revealed ulcerative colitis with mild activity .Finally the question is: Is there any relation between gastric and colonic lesions?

Image Case:Hepatic Hydatid Cyst ; an Incidental Finding in Patient with Blunt Abdominal Trauma

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We reported a 13 years old male with blunt abdominal trauma presented with right upper quadrant pain at Emergency Department, Zagazig University Hospitals. It was diagnosed as hepatic hematoma and treated conservatively. Two weeks later, on abdominal ultrasound examination the liver showed large left lobe cyst with separated tissues and daughter cyst inside the sand consistent with Hydatid cyst (Figure 1), the case was further studied by abdominal CT that showed thick walled cyst (Figure 2) and confirmatory MRI was done (Figure 3).

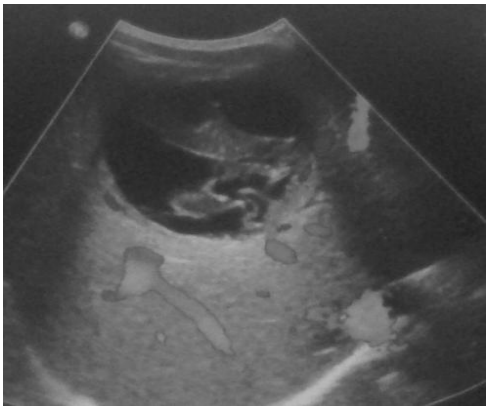

Figure 1: Ultrasonic appearance of the Hydatid cyst: A large cystic lesion with debris within it. There are a floating detached endomembrane.

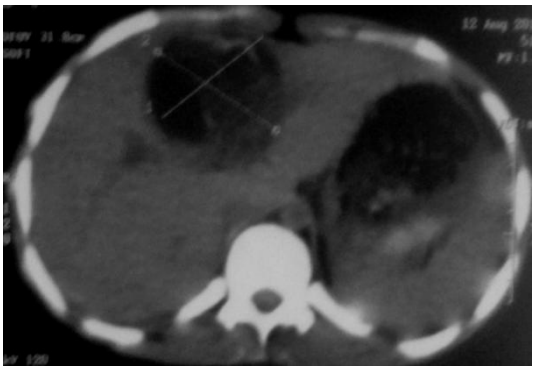

Figure 2: CT scan appearance showing large cystic mass at segment IV of the LT liver lobe with detachment of the laminated membrane from the pericyst. No calcifications of the cyst wall

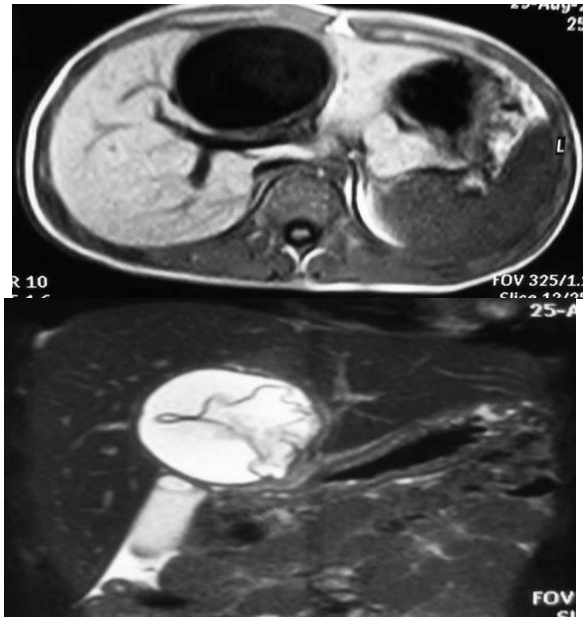

Figure 3: MRI scan with axial T1WI and coronal T2WIs showing the LT liver lobe cyst displaying low signal intensity on T1WI and high SI on T2WI. At the coronal T2WI clear demonstration of the detached laminated membrane from the pericyst.