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Contents

Hepatitis C Virus: From Liver to Bone Disease, There are Multiple Stations

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See the full article pages

Background

Osteoporosis is defined as a "progressive systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture" Common fractures are vertebral compression fractures, fractures of the distal radius, and proximal femur [1].

World Health Organization defines osteoporosis as a bone mineral density (BMD) measurement of 2.5 standard deviations or more below the population mean BMD of sex-matched young adults, i.e., a t-score of ≤−2.5. The term "established osteoporosis" includes the presence of a fragility fracture [2].

Osteoporosis can result in spontaneous or low trauma fractures in the patients, adversely affecting morbidity, quality of life. The prevalence of osteoporosis associated fracture ranges from 5% to 20% [3].

Hepatic Osteodystrophy

Osteoporosis and osteopenia are well known complications in patients with chronic liver disease. Its prevalence varies considerably. It ranges from 12 to 55% according to many factors including patient selection, diagnostic criteria, underlying liver disease [4] and it also increases with the increased severity of the liver disease defined as advanced Child-Pugh score [5].

Osteoporosis is a risk factor for development of fractures, which may be a source of morbidity in patients already debilitated by chronic liver disease. Prevention of morbidity of hepatic osteodystrophy is to identify those patients who are predisposed to development of osteopenia and osteoporosis [5].

Hepatic bone disease is caused by a variety of causes ranging from chronic hepatitis as HCV and HBV [6] to cirrhosis. Compensated as well as decompensated cirrhosis whatever its cause affect BMD leading to osteopenia and osteoporosis [7]. Patients awaiting for liver transplantation have different degree of osteopenia as well as osteoporosis [8]. Osteoporosis and osteopenia are alterations in BMD that frequently occur in chronic liver disease; predominantly in chronic cholestatic disease and liver cirrhosis [9].

Hepatitis C Virus and Bone affection

Chronic hepatitis C (HCV) is systemic disease rather than hepatotropic virus. HCV affects more than 170 million people worldwide [10] and is the single important cause of liver disease in Egypt [11]. It is associated with multisystemic manifestations [12]. Liver disease is the most common affectation, and leads to liver cirrhosis and hepatocellular carcinoma [13].

Osteopenia and osteoporosis are common in chronic HCV patients. In most studies suggesting that HCV by itself provokes osteopenia [14]. Some of this research involved non cirrhotic patients [14], others, individuals affected by liver cirrhosis [15], or both cirrhotics and non-cirrhotics [16], and some were restricted to patients awaiting organ transplantation [17] and also in renal transplant patients infected with HCV [18].

The pathogenesis of osteoporosis in chronic liver disease including HCV is still unknown and it is most likely that multiple factors are operating simultaneously [19].

The development of osteoporosis may be related to both increase bone resorption and/ or decrease bone formation [20]. Inhibition of osteoblast (bone forming cell) by retained substances of cholestasis as unconjugated bilirubin, retained bile acids, toxic effect of alcohol, and excessive tissue iron deposition [21].

Various potential inciting factors that either directly or indirectly alter bone mass are insulin-

like growth factor 1 (IGF-1) deficiency, hyperbilirubinemia, hypogonadism, subnormal 25-hydroxyvitamin D levels, vitamin D receptor genotypes, vitamin K, osteoprotegerin (OPG) and receptor activator of nuclear factor interactions and concurrent use of drugs like cholestyramine, diuretics, glucocorticoids and immunosuppressive agents [6-22]. Lifestyle factors (smoking, alcoholism, and immobility), malnutrition and low body mass index [23].

Increase osteoclast activity is cytokine mediated mechanism of bone loss. The proinflammatory cytokines interleukin-1(IL-1) and tumor necrosis factor (TNF) increase osteoclast activity and are increased in hepatic inflammation and fibrosis. TNF increased in viral hepatitis and alcoholic liver disease as well as in patients with cirrhosis [24].

Application of therapy must consider general measures (correction of reversible risk factors, calcium intake and supplementation) and specific treatment for osteoporosis. Bisphosphonates are antiresorptive drugs that can improve BMD in other chronic liver disease, but only limited data are available for osteoporosis in HCV infection [6].

Summary and Comment on the paper

In this issue of the Afro-Egyptian Journal of Infectious and Endemic Diseases Abdelkader et al. [25], discussed the impact of HCV on BMD both in cases with chronic hepatitis and in cases with HCV cirrhosis in comparison to apparently healthy controls. The authors included 80 participants. Of them 30 patients were chronic HCV infection without cirrhosis, 30 patients were chronic HCV infection with compensated cirrhosis and 20 age and gender matched apparently healthy controls. All subjects of the study performed liver function tests, viral markers, liver biopsy, hormonal assay and BMD measurement by Dual energy X-ray absorptiometry (DEXA). They found that in patients with chronic hepatitis C the frequency of osteopenia was 36.7%, osteoporosis was 6.7%, total patients with low BMD were 43.3%. In cirrhotic patients, the frequency increased as follow: osteopenia was 43.3%, osteoporosis was 10.0%, and total patients with low BMD were 53.3% vs 5.0% in the healthy controls. There was also no significant difference between patients with low BMD and patients with normal BMD as regards age, gender, common risk factors, liver function tests or hormonal levels. They concluded that reduced BMD is common in chronic HCV-infected patients and consequently, HCV infection is a risk factor of osteoporosis, and this risk is increased with advancement of the liver disease.

The paper looks interesting because it discuss an important issue that we commonly face in the everyday clinical practice. Liver cirrhosis is a debilitating condition and with increase in the severity it seems that BMD affection increases and more morbidity awaits the patients. We suppose that this study do have some limitations, the first of all is the small sample size. A large sample may be more presentable to the level which present of this big problem. Second, the cross sectional design of this study, a prospective study is more valuable and may address the HCV induced BMD morbidities more clearly. Third, exclusion criteria needs to be more dependable and should include for example smokers, alcohol abusers, patients under hormonal therapy affecting calcium metabolism, patients with thyroid and parathyroid disturbances, severe renal insult and associated HBV infection. Lastly, a selection bias seems to be present. It not obvious how patients were selected and how they were randomized.

Recommendations:

According to the previous discussion, it may be valuable to conduct a prospective study over a long period of time to evaluate the impact of HCV on the bone health. Implication of HCV genotyping during this study may add to our knowledge is there any impact of HCV genotype on BMD?**.** Likewise, little is known about the effect of antiviral agents against HCV on BMD and their potential benefit on bone impairment. Hence, impact of HCV therapy on the HCV induced BMD affection needs further investigation.

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Ascitic Fluid Calprotectin and Serum C-Reactive Protein as Diagnostic Markers for Spontaneous Bacterial Peritonitis

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Key words: Ascitic fluid calprotectin, SBP, CRP

Background and study aim: Spontaneous bacterial peritonitis (SBP) is an important cause of morbidity and mortality in cirrhotic patients with ascites. The diagnosis of SBP is based on PMN leukocyte cell count exceeding 250/µL in ascitic fluid. However, this procedure is time consuming as well as subjective. C-reactive protein (CRP) has been reported to be a reliable predictor of SBP and an index of therapeutic effectiveness in adults. Ascitic fluid calprotectin reliably predicts PMN count >250/µL, which may prove useful in the diagnosis of SBP. This work was planned aiming to evaluate both ascitic fluid calprotectin and serum CRP as accurate diagnostic laboratory markers for detecting SBP

Patients and Methods: From 140 patients; only 124 patients with ascites were included in this study. They were divided into SBP group including 70 patients (49 males and 21 females) and non-SBP group of 54 patients (25 males and 29 females). Serum CRP was determined by latex agglutination and ascitic fluid calprotectin was measured using an enzyme-linked immunosorbent assay.

Results: Ascitic fluid calprotectin and serum CRP were significantly higher in SBP patients in comparison with the non-SBP group (754.67 ±256.06 vs. 280.77 ±230.97 and 62.4 ±28.39 vs. 9.81 ±8.98) respectively. In addition, both were positively correlated with ascitic fluid proteins and PMN count as well as with each other. At a cutoff value of 270 mg/dl, ascitic fluid calprotectin had 86% specificity and 97.5% sensitivity for detecting SBP [Area under the receiver operating characteristics curve (AUC) = 0.924 with negative and positive predictive values (NPV, PPV) for ascitic calprotectin 96% and 69% respectively.

Conclusion: Ascitic fluid calprotectin and serum CRP may be used as accurate and reliable markers for the diagnosis of SBP.

INTRODUCTION

Spontaneous bacterial peritonitis (SBP) is an important cause of morbidity and mortality in cirrhotic patients with ascites. SBP is estimated to affect 10-30% of cirrhotic patients hospitalized with ascites, and mortality in this group approaches 30% [1]. Many of these patients are asymptomatic, and it is therefore recommended that all patients with ascites undergo paracentesis at the time of admission to confirm the SBP status [2]. Although SBP is less prevalent in an outpatient setting, it is reasonable to also evaluate the ascitic fluid of outpatients because of the high mortality associated with SBP.

The diagnosis of SBP is based upon the polymorphonuclear (PMN) leukocyte cell count exceeding 250/µL in ascitic fluid [3]. However, this procedure is time consuming and subjective. Alternative methods using automated PMN counting [4], reagent strips (urine dipsticks) [5], or ascitic lactoferrin [6] have been developed; unfortunately, their diagnostic accuracies are limited and their use is dependent upon availability of laboratory personnel and reagents/components from the commercial source. Therefore, an accurate and convenient method of rapid diagnosis of SBP remains an unmet clinical need.

C-reactive protein (CRP) is an acute phase reactant which binds to different substrates. It activates the complements, takes part in cytokine secretion, and increases the phagocytosis of leucocytes. CRP has been reported to be a reliable predictor of SBP and an index of therapeutic effectiveness in adults [7].

Calprotectin, a calcium and zinc-binding protein, is detected almost exclusively in neutrophils [8], and its presence in body fluids is proportional to the influx of neutrophils [9].

Calprotectin is primarily expressed in neutrophils and macrophages, while it is not usually present in lymphocytes. It has been estimated to account for more than 40% and 5% of cytosolic and total proteins of neutrophils, respectively [10]. Ascitic fluid calprotectin reliably predicts PMN count >250/μL, which may prove useful in the diagnosis of SBP, especially with a readily available bedside testing device [11].

This work was planned aiming to evaluate ascitic fluid calprotectin as an accurate diagnostic laboratory marker for detecting spontaneous bacterial peritonitis (SBP).

PATIENTS AND METHODS

In this prospective observational study, we recruited 140 patients with ascites referred for paracentesis to Tropical Medicine Department-Mansoura University, from October 2012 to March 2013. All patients were subjected to the following evaluations: complete history taking and physical examination, abdominal ultrasound, laboratory assessment including full blood count, liver profile, creatinine, CRP, ESR, and ascitic fluid analysis (WBCs, protein, bacteriologic culture with sensitivity, pathological assessment and calprotectin level). Paracentesis of ascitic fluid was performed for every patient with cirrhosis and ascites that was admitted to our department, independently of the clinical suspicion of AFI, as a routine procedure. AFI diagnosis was based on the presence of ≥250 cells/mL PMN in the ascitic fluid, with or without positive ascitic fluid culture in the absence of a hemorrhagic ascites and secondary peritonitis (by WBCs count, bacteriological culture, LDH and clinical examination)

Exclusion criteria included patients who were immunocompromised and patients who had received antibiotic prior to hospital admission. Moreover, patients with heart failure, diabetes mellitus, hematological disorders and neoplastic disorders and patients with clinically overt hypo- or hyperthyroidism or with clinically and laboratory evident autoimmune diseases were also excluded from this study. None of the study participants had received anticoagulant medications, non-steroidal anti-inflammatory drugs (NSAID) or oral contraceptive drugs before hospital admission.

Sampling

1. Five ml blood was withdrawn by venipuncture, one ml in EDTA tube for CBC and four ml delivered into plastic tube and allowed to clot. Non-hemolyzed sera was separated by centrifugation and used for determination of creatinine, uric acid, CRP, and liver functions (ALT, AST, total bilirubin and albumin).
2. Ascitic fluid sample was taken by paracentesis performed under aseptic conditions from a puncture site in the left or right lower quadrant with the patient in the supine position. All samples for diagnostic testing were immediately collected at the bedside and processed by laboratory personnel without further delay.

Methodology

1- Blood glucose, liver profile, and creatinine concentrations were measured on a Dimension Xpand plus chemistry analyzer (Roche Diagnostics, Basel, Switzerland) using commercially available reagents and an enzyme-based kit.
2- Complete blood picture was measured using CELL-DYN Emerald cell counter (ABBOTT, Germany).
3- Serum CRP was determined using latex agglutination test kit (Omega diagnostics LTD AVITEX CRP Ref OD073/OD023/ OD023/E. Scotland, UK) **[12]**.
4- Ascitic fluid calprotectin was measured by an enzyme-linked immunosorbent assay using immune diagnostic AG ELISA kit ([MRP 8/14] Stubenwald-Allee 8a–D-64625, Bensheim) **[13]**.

This study was approved by the Ethical Committee of Mansoura University and all patients provided written informed consent prior to participation in any protocol-specific procedures. The study was conducted in accordance with the guidelines of the Helsinki Declaration.

Statistical analysis

All statistical analyses were performed using the SPSS version 17.0 software, Chicago, USA. Data were first tested by Kolmogorov–Smirnov test for

distribution of data. Parametric data was expressed in mean and standard deviation (SD). The mean and SD of the differences and the limits of agreement, defined as the mean ± 2 SD of the difference (95%CI), were calculated. Unpaired *t* test was used for intergroup comparisons. A P-value of less than 0.05 indicated statistical significance. Correlations between numerical data were determined with the Pearson's rank correlation coefficient. All hypothesis testing were two-tailed. Analysis of the receiver operator characteristics (ROC) and calculation of the area under the curve (AUC) were used to evaluate the capability of calprotectin to identify a PMN count >250/μL.

RESULTS

***Patient characteristics*:**

From 140 patients, only 124 patients with ascites were included in this study. After three weeks of admission of each case, the final diagnosis (SBP) and the aetiology of ascites were assessed. According to ascitic fluid analysis and clinical data, they were divided into a SBP group including 70 patients (49 males and 21 females) and non-SBP group of 54 patients (25 males and 29 females). A total of 16 patients had malignant ascites (which included 6 leaking hepatocellular carcinoma, four ovarian, two lymphomas, one breast, one stomach, one colorectal and one pancreatic cancer) were excluded from this study.

Patients suffered from liver cirrhosis with different aetiologies (Table 1). 97 chronic hepatitis C related cirrhosis (78.2%), 21chronic hepatitis B related cirrhosis (16.9%), 3 autoimmune-related cirrhosis (2.4%), 2 nonalcoholic steatohepatitis-related cirrhosis (1.6%) and one cryptogenic cirrhosis (0.8%).

Table (1) : Baseline characteristics of patients with liver cirrhosis

Parameters	No. of patients
Aetiology of liver cirrhosis:	
Chronic hepatitis C (CHC)	97 (78.2%)
Chronic hepatitis B (CHB)	21 (16.9)
Autoimmune hepatitis (AIH)	3 (2. 4%)
Nonalcoholic steatohepatitis (NASH)	2 (1.6%)
Cryptogenic	1 (0. 8%)
Child-Turcotte-Pugh class:	
Child A	0
Child B	88 (71%)
Child C	36 (29%)
MELD score	11.3 (10.5-18)

MELD: Model for end-stage liver disease

Fever was the most common presentation found in 49 cases (70%), followed by abdominal pain in 39 patients (55.7%), abdominal tenderness in 34 cases (48.6%), altered mental status in 23 cases (32.9%) and upper GIT bleeding in 19 cases (27.1%), while 21 cases (30%) were asymptomatic (Table 2).

Table (2): Clinical presentation in patients with SBP

Parameters	No. of patients (n=70)
Fever	49 (70%)
Abdominal pain	39 (55.7%)
Abdominal tenderness	34 (48.6%)
Altered mental status	23 (32.9%)
Upper GIT bleeding	19 (27.1%)
Asymptomatic	21 (30%)

GIT: gastrointestinal tract

According to the Child-Turcotte-Pugh Score, 88 (71%) of the patients were classified as stage B and 36 (29%) of the patients were classified as stage C (Table 1).

Laboratory and ascitic fluid cell count:
There was significant increase in WBCs, platelets, CRP, AST and creatinine in the SBP group versus the non-SBP group **(**10.16±2.88 vs. 6.99 ±1.78; 107.52±19.13 vs. 130.9±29.73; 62.4 ±28.39 vs. 9.81 ±8.98; 43.15±13.75 vs. 51.95 ±10.34 and 1.77±0.44 vs. 1.19±0.42 respectively). There was no correlation between hemoglobin (Hb), ALT, serum bilirubin, serum albumin and random blood sugar (RBS) in both groups 10.09 ±0.69 vs. 9.74 ±0.81; 50.5±21.42 vs. 40.31 ±15.33; 1.82 ±0.81 vs. 1.87 ±0.48; 2.68 ±0.33 vs. 2.57 ±0.43 and 141.2 ±38.18 vs. 148.4 ±21.66 respectively) (Table 3).

Table (3): Biochemical parameters in the studied groups

	SBP group (n=70)	**Non-SBP group (n=54)**	**P-value**
Hb (gm/dl)			
Range	8.10 – 11.0	8.9 – 11.4	0.09
Mean ±SD	10.09 ±0.69	9.74 ±0.81	
WBCs (10^3/cmm)			
Range	3.5 – 16.0	4.3 – 11.0	0.001
Mean ±SD	10.16 ±2.88	6.99 ±1.78	
Platelets (10^3/cmm)			
Range	60 – 142	68 – 201	0.001
Mean ±SD	107.52 ±19.13	130.9 ±29.73	
CRP (mg/dl)			
Range	12 – 96	6 – 48	0.001
Mean ±SD	62.4 ±28.39	9.81 ±8.98	
ALT (U/L)			
Range	20 – 96	22 – 65	0.054
Mean ±SD	50.5 ±21.42	40.31 ±15.33	
AST (U/L)			
Range	22 – 75	29– 72	0.01
Mean ±SD	43.15 ±13.75	51.95 ±10.34	
Bilirubin (mg/dl)			
Range	0.8 – 3.3	1.3 – 2.8	0.79
Mean ±SD	1.82 ±0.81	1.87 ±0.48	
Albumin (gm/dl)			
Range	2.0 – 3.0	1.7 – 3.0	0.27
Mean ±SD	2.68 ±0.33	2.57 ±0.43	
Creatinine (mg/dl)			
Range	0.8 – 2.7	0.9 – 2.5	0.017
Mean ±SD	1.77 ±0.44	1.19 ±0.42	
RBS (mg/dl)			
Range	110 – 181	112 – 189	0.24
Mean ±SD	141.2 ±38.18	148.4 ±21.66	
Ascitic fluid analysis			
WBCs (cell/cmm)			
Range	250 – 400	5 – 200	
Mean ±SD	296 ±48.55	66.13 ±38.6	0.001
Protein (mg/dl)			
Range	112 - 288	71 – 122	
Mean ±SD	213±28.23	93.5±11.1	0.001
Calprotectin (ng/ml)			
Range	230 – 1080	120 – 920	
Mean ±SD	754.67 ±256.06	280.77 ±230.97	0.001

Hb: hemoglobin, WBC: white blood cell, CRP: C reactive protein. ALT: alanine aminotransaminase, AST: aspartate aminotransferase, RBS: Random blood sugar

In addition, there was a significant increase in ascitic fluid WBCs, protein, and calprotectin in the SBP group vs. the non-SBP group (296 ±48.55 vs. 66.13 ±38.6; 213±28.23 vs. 93.5±11.1 and 754.67±256.06 vs. 280.77±230.97 respectively) **(**Table 3).

There was positive correlation between serum CRP and ascitic fluid proteins and WBCs (r= 0.686, p= 0.001 and r= 0.793, p= 0.001 respectively) (Figure 1).

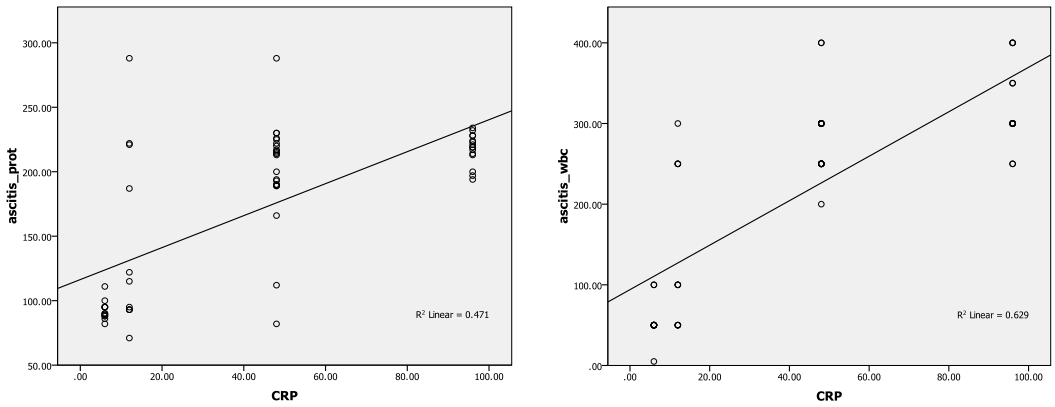

Figure (1): Correlation between serum CRP and ascitic fluid protein and WBCs

Diagnostic value of CRP:

Receiver operating characteristics (ROC) curve for sensitivity and specificity of CRP. At a cutoff value of 30 mg/dl, CRP was shown to have 96% specificity and 90% sensitivity for detecting SBP [Area under the receiver operating characteristics curve (AUC)= 0.91 with negative and positive predictive values (NPV, PPV) for CRP 95% and 70% respectively].

Diagnostic value of ascitic calprotectin:

Receiver operating characteristics (ROC) curve for sensitivity and specificity of calprotectin. At a cutoff value of 270 mg/dl, ascitic fluid calprotectin had 86% specificity and 97.5% sensitivity for detecting SBP [Area under the receiver operating characteristics curve (AUC)= 0.924 with negative and positive predictive values (NPV, PPV) for ascitic calprotectin 96% and 69% respectively].

There was positive correlation between ascitic fluid calprotectin and ascitic fluid proteins and WBCs (r= 0.524, p= 0.001 and r= 0.692, p= 0.001 respectively) (Figure 2).

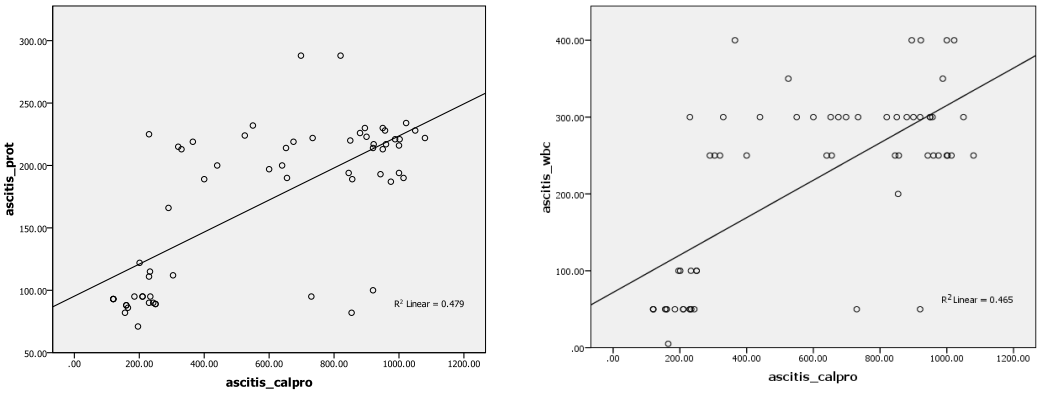

Figure (2): Correlation between ascitic fluid calprotectin and ascitic fluid proteins and WBCs

There was positive correlation between ascitic fluid calprotectin and serum CRP (r =0.793, p= 0.001) (Figure 3).

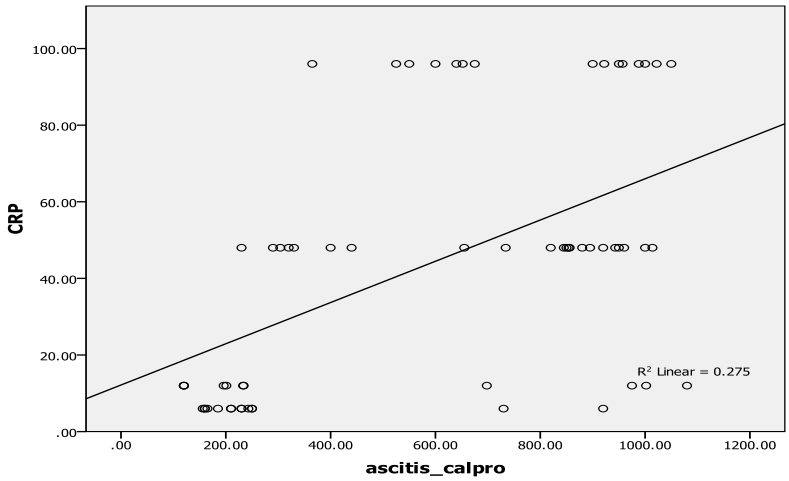

Figure (3): Correlation between ascitic fluid calprotectin and serum CRP

DISCUSSION

Ascites is commonly found in patients with liver cirrhosis and may promote bacterial translocation, enhancing the risk of SBP [1]. SBP in outpatients is rare, but when it occurs it often requires hospitalization to manage to disease course [2].

Spontaneous bacterial peritonitis (SBP) is an important cause of morbidity and mortality in cirrhotic patients with ascites. The diagnosis of SBP is based upon the PMN leukocyte cell count exceeding 250/µL in ascitic fluid, however this procedure is time consuming and subjective. Ascitic calprotectin reliably predicts PMN count >250/µL, which may prove useful in the diagnosis of SBP.

In this prospective observational study which conducted on 124 patients with ascites, most of the patients were in their fourth to sixth decades of life which was consistent with the mean age (49.09±11.3 years) reported by Ajitpal et al. [14]. SBP was found to be more common in males than in females (70% vs. 30%, respectively), a result which is in agreement with Reiberger et al. [15] who similarly reported a 68% male incidence of SBP. The male predominance in our study may be due to higher incidence of bilhariziasis and HCV in our locality.

The most common clinical presentation was fever (70%), followed by abdominal pain (55.7%), abdominal tenderness (48.6%), altered mental status (32.9%), upper GIT bleeding (27.1%) while 30% of patients were asymptomatic (Table 2). These results were consistent with the study conducted by Runyon et al. [16] in which fever was the most common feature (67%), followed by abdominal pain (60%), abdominal tenderness (42%) and encephalopathy (57%). Bandy and Tuttle [17] reported that as many as 30% of patients with paracentesis-proven SBP may be completely asymptomatic.

A significant decrease in hemoglobin levels were found in patients with SBP in comparison with non-SBP patients (9.74±0.81 vs. 10.09±0.69) (Table 3). Syed et al. [18] reported a mean hemoglobin level of 9.6 gm/dl in SBP patients. In the current study, an increase in WBC count was seen in SBP versus non-SBP (10.1±2.8 vs. 6.99±1.7), which is also in agreement with the results reported by Syed et al. [18]. A significant decrease in platelets in the SBP group versus the non-SBP group (107.5±19.1 vs. 130.±29.7) (Table 3) is consistent with findings of the study by Ajitpal et al. [14], who found that platelet count decreased in SBP patients than those without SBP.

The significant increase in creatinine concentration in the SBP group versus the non-SBP group (1.77±0.44 vs. 1.19±0.42, p 0.017). This is in agreement with results of the study conducted by Ajitpal et al. [14] in which the levels of serum creatinine were significantly higher in patients with SBP compared to those without (2.44 ± 0.84 vs. 1.8 ± 1.35, p <0.05).

A significant increase in WBCs and protein in ascitic fluid was reported between the two patient groups (296 ±48.55 vs. 66.13 ±38.6 for WBCs and 213±28.23 vs. 93.5±11.1 for protein) (Table 3). Subhas et al. [19] reported that the highest concentration of protein in ascitic fluid was 1.9gm/dl and the lowest was 0.40gm/dl. Mean ascitic fluid concentration was 0.93 ± 0.44gm/dl. Ascitic fluid analysis at admission by Syed et al. [18] showed mean TLC, polymorphonuclear (PMN) and protein as 903.34±3342/mm3, 411.62 ±1109/mm3 and 1.18±0.746gm/dl respectively. However, our results were much lower than the results reported by the previous studies. This may possibly be due to the difference in immune status as well as etiology of cirrhosis in patients in our study (due to HCV infection), compared to other studies (alcoholic cirrhosis). Runyon [20] demonstrated that cirrhotic patients with ascitic protein concentrations below 1 g/dl were 10 times more likely to develop SBP than individuals with higher concentration.

There was a significant increase in CRP in the SBP group versus the non-SBP group (62.4 ±28.39 vs. 9.81±8.98, p 0.001), with a significant positive correlation being observed between serum CRP and ascitic fluid protein and WBCs count (Figure 1). It was reported that at a cutoff value of 30 mg/dl, CRP had 96% specificity and 90% sensitivity for detecting SBP (Figure 2). Preto-Zamperlini et al [7] reported that the SBP group demonstrated significantly elevated CRP levels, leading to the conclusion that CRP was an independent variable in the prediction of SBP. Being an acute phase reactant, CRP binds to different substrates and stimulates the complements system, has a crucial role in cytokine secretion and increases the phagocytosis of leukocytes. In study by Yildirim, et al. [21] it was founded that CRP was increased in the serum and ascitic fluid of SBP patients.

In this study, ascitic fluid calprotectin was found to be significantly elevated in patients with SBP compared to non-SBP patients (754.67 ±256.06 vs. 280.77 ±230.97, P= 0.001) (Table 3), a result which is consistent with those demonstrated in the studies of Elbanna et al. [22] and Ali et al. [23].

A significant positive correlation was observed between ascitic fluid calprotectin and ascitic fluid protein and WBC count (Figure 3). A study conducted by Ali et al. [23] similarly reported a significant positive correlation between ascitic fluid calprotectin and PMN cell count. Burri et al. [11] reported that ascitic calprotectin levels correlated well and reliably with PMN count. Samples with PMN >250/μL also had higher ascitic calprotectin levels than the samples with PMN ≤250/μL.

The present study demonstrated that at a cutoff value of 270 mg/dl, ascitic fluid calprotectin had 86% specificity and 97.5% sensitivity for detecting SBP (Figure 4). Burri et al. [11] reported that at a cut-off value of 0.63 μg/mL, ascitic calprotectin yielded a sensitivity of 95% and a specificity of 89.2%.

This prospective study evaluated the diagnostic utility of measuring calprotectin in ascites to identify ascitic PMN counts >250/μL in patients referred for paracentesis, and provides the following new information: Patients with an elevated PMN count (>250/μL) had higher ascitic calprotectin levels than those with normal cell counts; this finding indicates that ascitic calprotectin levels correlate well and reliably with PMN count. It is clinically significant that calprotectin levels in ascitic patients can identify elevated PMN counts using ELISA methods. Indeed, ascitic calprotectin may serve as a surrogate marker for PMN count and would be amenable to routine SBP screening, especially when measured by a bedside test.

There are several limitations to the current study that merit consideration. First, we included all patients with ascites, irrespective of the aetiology, and it may be that our results cannot be generalized to all patients with liver cirrhosis. Second, our sample size was small and larger studies are needed to evaluate this test in different clinical settings and to establish a reliable cut-off for ascitic calprotectin for optimal identification of PMN counts >250/μL.

In conclusion, both ascitic fluid calprotectin and serum CRP are significantly elevated in SBP patients in comparison with non-SBP patients. In addition, they also correlate well with the PMN count and protein levels in ascitic fluid and reliably diagnose SBP.

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Osteoporosis in Chronic Hepatitis C

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Background and study aim: Hepatitis C virus infection is a multisystemic disease with many extrahepatic manifestations. Affection of bone matrix density is a common complication of chronic hepatitis and cirrhosis. The pathogenesis of osteoporosis in chronic liver disease is still unknown and is expected to be multifactorial. The aim of this work is to assess the frequency of osteoporosis/osteopenia in patients with chronic hepatitis C virus infection with or without cirrhosis.

Patients and Methods: This study was carried out on 30 patients with chronic HCV infection without cirrhosis (Group II), 30 patients with chronic HCV infection with compensated cirrhosis (Group III) and 20 age and gender matched healthy controls (Group I). All subjects of the study performed liver function tests, viral markers, liver biopsy, hormonal assay and Bone Mineral density measurement (BMD) by Dual energy X-ray absorptiometry (DEXA).

Results: In patients with chronic hepatitis C (group II) the frequency of osteopenia was 11 (36.7%), osteoporosis 2 (6.7 %), total patients with low BMD was 13 (43.3%). In cirrhotic patients (group III), the frequency of osteopenia was 13 (43.3%), osteoporosis was 3 (10.0%), and total patients with low BMD was 16(53.3%) vs 1(5.0%) in the control group (group I). there was also no significant difference between patients with low BMD and patients with normal BMD as regards age, gender, common risk factors, liver function tests or hormonal levels.

Conclusion: Reduced BMD is common chronic HCV-infected patients with and without cirrhosis. HCV infection is a risk factor of osteoporosis.

INTRODUCTION

Chronic hepatitis C (HCV) is systemic disease rather than hepatotropic and affects more than 170 million people worldwide [1]. It is associated with multisystemic manifestations. Liver disease is the most common affectation, and leads to liver cirrhosis and hepato-cellular carcinoma [2]. Osteoporosis is a condition that is characterized by low bone mass and microarchitectural deterioration of bone tissue, leading to enhanced bone fragility and a consequent increase in fracture risk [3].

Osteoporosis and osteopenia are well known complications of chronic hepatitis as well as cirrhosis. Its prevalence varies considerably. It ranges from 12 to 55% according to patient selection, diagnostic criteria, underlying liver disease and its severity [4,5]. Osteoporosis and osteopenia are common in chronic viral hepatitis as HCV and HBV [6]. Compensated as well as decompensated cirrhosis, whatever its cause, affects bone mineral density leading to osteopenia and osteoporosis [7]. Prevalence of osteoporosis in cirrhotic patients is related to the severity of liver disease expressed by the Child-Pugh score [8].

Osteopenia and osteoporosis are common in chronic HCV patients. In most studies suggesting that HCV by itself provokes osteopenia [9-12]. Some of this research involved non-cirrhotic patients [12-14], others, individuals affected by liver cirrhosis [9,11], or both cirrhotics and non-cirrhotics [15,16], and some were restricted to patients awaiting organ transplantation [17,18].

The pathogenesis of osteoporosis in chronic liver disease is still unknown and it is likely that multiple factors are operating simultaneously [19]. The development of osteoporosis may be related to both increase bone resorption and/ or decrease bone formation [20]. Inhibition of osteoblast (bone forming cell) may be mediated by retained substances of cholestasis as unconjugated bilirubin, retained bile acids, toxic effect of alcohol, and excessive tissue iron deposition [21].

Various potential inciting factors that either directly or indirectly alter bone mass are insulin-like growth factor 1 (IGF-1) deficiency, hyper-bilirubinemia, hypogonadism, subnormal 25-hydroxyvitamin D levels, vitamin D receptor genotypes, vitamin K, osteoprotegerin (OPG) and receptor activator of nuclear factor interactions and concurrent use of drugs like cholestyramine, diuretics, glucocorticoids and immunosuppressive agents [22], Lifestyle factors (smoking, alcoholism, immobility), malnutrition and low body mass index [23,24]. Increase osteoclast activity is cytokine mediated mechanism of bone loss. The pro-inflammatory cytokines interleukin-1 (IL-1) and tumor necrosis factor (TNF) increase osteoclast activity and are increased in hepatic inflammation and fibrosis. TNF increased in viral hepatitis and alcoholic liver disease as well as in patients with cirrhosis [25].

Osteoporosis is a risk factor for development of fracture, which may be a source of morbidity in patients already debilitated by chronic liver disease. Prevention of morbidity of hepatic osteodystrophy is to identify those patients who are predisposed to development of osteopenia and osteoporosis [26].

The aims of this study were to assess the frequency of osteoporosis in chronic hepatitis and post HCV cirrhosis in comparison with a group of age- and sex-matched healthy controls and to identify the main risk factors for its development.

PATIENTSAND METHODS

This study was conducted in the Department of Tropical Medicine, Zagazig University Hospitals, Egypt during the period from January 2012 to December 2013 and included 80 subjects. The subjects were divided into three groups: Group I: 20 apparently healthy individuals, Group II: 30 patients with chronic viral hepatitis C without cirrhosis, Group III: 30 patients with post hepatitis C compensated cirrhosis. The evidence of HCV infection was detected by anti-HCV antibody and HCV RNA.

Exclusion criteria:

1- Patients with liver disease due to multiple etiologies or with other liver conditions (HBV, primary biliary cirrhosis, autoimmune or metabolic cause like Diabetes Miletus, hemo-chromatosis, Willson`s disease).
2- Cholestatic liver disease.
3- Decompensated cirrhosis.
4- Postmenopausal women.

All patients and control groups were subjected to:

1- Complete history taking with history of previous bone fractures.
2- History of drug therapy as steroids.
3- History of smoking and alcohol.
4- Thorough physical examination with special emphasizes on the manifestations of chronic liver disease.
5- Laboratory investigations including:
   - Complete blood picture.
   - Liver functions tests: liver enzymes, serum total proteins, albumin, serum bilirubin, alkaline phosphates,
   - Child- Pugh score
   - Abdominal ultrasonography.
   - HCV antibodies by ELISA.
   - HCV RNA by quantatitive PCR.
   - Serum calcium and phosphorus.
   - Hormonal assay: Hormonal immunoassasy: These included Parathyroid hormone level, Gonadal hormones levels (Testosterone and estrogen levels), Thyroid hormone (T3,T4, TSH).
   - Liver histopathology for diagnosis of chronic hepatitis C and cirrhosis: Pathological examination performed at liver histopathology laboratory at Pathology Department, Faculty of Medicine, Zagazig University. Hepatitis grading and staging were evaluated according to the METAVIR scoring system which consists of two separate score, one for necro-inflammation grade (activity score A0-A3) is assessed by an algorithm of both piecemeal necrosis and lobular necrosis and another for the stage of fibrosis (F0-F4) [27].
   - Bone Mineral density measurement (BMD) by Dual energy X-ray absorptiometry (DEXA) (Norland Excell X-ray bone densitometer From Norland Medical system, Inc. USA). Done on femoral neck (FN) and the lumbar spine (L2-L4) region, BMD was expressed as grams per square centimeter. T-score is the number of standard deviation of BMD

value above or below a young reference value for individual of same ethnic background and gender.

WHO classification of osteoporosis:

- Normal BMD is represented by a T score of more than -1 (low risk of fracture).
- Osteopenia (low bone mass) is represented by a T score between -1and -2.5 (medium risk of fracture).
- Osteoporosis is presented by a T score less than -2.5 and a previous history of a fragility fracture (high spontaneous fracture probability) **[28]**.

Statistical analysis:

Data were expressed as mean ± SD for quantitative data and number and percentage for qualitative data and comparison was done by paired t test and ANOVA for the former and corrected X2 for the latter.

RESULTS

The patients of the three studied groups had no significant differences as regards age and gender distribution as shown in Table 1. Males and females are almost equally represented in each group. The frequency of smoking and mean body mass index of each group were represented in Table 2 as common risk factors for osteoporosis with no significant differences found among the three studied groups.

The frequency of osteopenia/osteoporosis in the three studied groups is represented in table (3). The comparison showed that the frequency of osteopenia/osteoporosis is highly significantly higher in group II and significantly higher in group III when compared to the control group I. The results of the hormonal assay for parathyroid hormone, sex hormones and thyroid hormones as well as thyroid stimulating hormone were compared among the three studied groups in table (4). It shows that the parathyroid hormone level was significantly higher in the compensated cirrhosis patients group (group II) than in the two other groups, also serum testosterone level was significantly lower in the compensated cirrhosis patients group (group II) than the other two groups.

In tables 5 and 6 the two patients groups were divided according to the presence or absence of bone disease into two groups. As presented in table 5, the patients without bone disease were compared to those with bone disease according to demographic data as well as common risk factors for osteoporosis/osteopenia and showed no significant difference between the two groups as regards any of these items. In table 6, there's comparison between the two groups as regards platelet count, total and direct bilirubin levels, total protein and albumen levels, prothrombin time, liver enzymes, serum calcium and phosphorus as well as parathormone and testosterone levels, showing no significant difference as regards any of these laboratory parameters.

Table (1): Demographic data of the studied groups

	Group I N=20 (No. &%)	Group II N=30 (No. &%)	Group III N=30 (No. &%)	F	P-value
Age (years)					
X±SD	38.6±9.6	39.6±8.4	39±9.6	0.09	0.914*
Range	22-56	22-55	25-53		
	No (%)	**No (%)**	**No (%)**	**χ^2**	
Female	3(15.0)	4(13.3)	4(13.3)	0.04	0.983*
Male	17(85.0)	26(86.7)	26(86.7)		

* Non-significant

Table (2): Frequency of the common risk factors of osteoporosis in different studied groups

	Group I N=20 (No. &%)	Group II N=30 (No. &%)	Group III N=30 (No. &%)	χ^2	P. value
Smoking	1(5.0)	4(13.3)	0(0.0)	4.62	0.099*
Body mass index (BMI)				**F**	**P.value**
	26±6.4	26.04±3.2	27.2±3	0.788	0.458*

Table (3): Frequency of osteopenia/osteoporosis among studied groups

	Group I N=20		Group II N=30		Group III N=30	
	N	%	N	%	N	%
Normal	19	95.0	17	56.7	14	46.7
Osteopenia	1	5.0	11	36.7	13	43.3
Osteoprosis	0	0.0	2	6.7	3	10.0
Total bone disease	1	5.0	13	43.3	16	53.3
X^2			8.75		12.49	
P			<0.01**		<0.001***	

Table (4): Hormonal assay of different studied groups using analysis of variance by (ANOVA test).

Variables	Group I N=20 $X \pm SD$	Group II N=30 $X \pm SD$	Group III N=30 $X \pm SD$	F	P value
Parathyroid hormones levels					
PTH (7 - 53) pg/ml	41.6± 6.9	40±10.2	47.6±13.1	4.078	0.021**
Gonadal hormones levels					
Total testosterone (ng/ml) (men 2.8_ 8 & women 0.06 - 0.82)	6.4±2.8	5.6± 2.4	4.4±2.1	4.755	0.011**
Estradiol (pg/ml)	49.8± 51.4	53.3±28.3	52±40.4	0.047	0.954*
Thyroid hormones					
Total T3 (1.3- 3.1) nmol/l	2.2±0.6	2.2±0.4	2.3±0.4	0.175	0.84*
Total T4 (66-181) nmol/l	108.8± 40	125.2±30	127.1±20	2.58	0.081*
TSH (0.3 - 4.2) uIU/ml	2.4± 0.97	2.1±1	1.9±1	1.426	0.247*

Table (5): Demographic and clinical factors associated with bone disease in patients of HCV cirrhosis (group III) and chronic viral hepatitis C (group II)

	Without bone disease N=31	With bone disease N=29	Test of significance	P
Age (years)				
$\bar{X}$±SD	38.5±7.8	42.0±701	1.81	0.07*
Range	22-52	28-55		
Gender	No %	No %	X2	
Male	26 83.9	26 89.7	0.08	0.78*
Female	5 16.1	3 10.3		
Smoking	No %	No %		
Negative	29 93.5	27 93.1	0.2	0.65*
Positive	2 6.5	2 6.9		
BMI				0.7*
X±SD	26.6±3.2	26.3±301	T	
Range	22-33	21-33	0.32	

Table (6): Some laboratory parameter and its relation to bone disease

	Without bone disease N=31	With bone disease N=29	t	P
Platelets x10^3/mm $\overline{X}$ ±SD Range	154±56 77-260	135±.55.1 75-247	103	0.19*
Total bilirubin /mg% $\overline{X}$ ±SD Range	1.69±0.5 0.9-2.9	1.73±0.6 0.7-3.1	0.28	0.77*
Direct /mg% $\overline{X}$ ±SD Range	0.36±0.35 0.2-1.5	0.640.3 0.1-1.6	0.04	0.96*
Protein /gm% $\overline{X}$ ±SD Range	7.8±0.5 7-9	7.9±0.5 7-9.2	0.85	0.39*
Albumin/gm% $\overline{X}$ ±SD Range	4.3±0.3 3.5-4.8	4.2±0.3 3.4-4.8	1.46	0.14*
ALT x IU $\overline{X}$ ±SD Range	46.8±10.7 22-70	46.6±16.7 20-93	0.06	0.95*
AST x IU $\overline{X}$ ±SD Range	47.6±13.6 20-75	47.7±13.1 20-77	0.01	0.98*
ALK x IU $\overline{X}$ ±SD Range	159.5±34 110-222	169.1±36.9 120-240	1.04	0.29*
PT/ Seconds $\overline{X}$ ±SD Range	12.7±1.3 11-16	13.3±1.8 11-17	1.6	0.11*
PTH /*pg/ml* $\overline{X}$ ±SD Range	45.1±10 22.6-73	42.3±14 21-72	0.87	0.38*
Calcium $\overline{X}$ ±SD Range	9.1±0.5 8.2-10	8.2±0.7 7.1-10	1.06	0.29*
Phosphate $\overline{X}$ ±SD Range	3.9±0.4 2.7-4.5	3.8±0.4 2.8-4.5	0.83	0.4*
Total testosterone x ng/ml $\overline{X}$ ±SD Range.	4.5±3.1 0.05-9.5	**5.7**±2.6 0.2-10.1	1.237	0.221*

DISCUSSION

The aim of this study was to test if HCV infection alone can be a risk factor for bone disease before causing severe liver damage that intervenes with the patient's nutritional status and bone metabolism. Studying HCV as risk factor for bone disease depended on the fact that HCV is actually a systemic illness that has so many extrahepatic manifestations.

In our study, there were no significant differences between the three studied groups as regards age and gender distribution as well as common risk factors of low BMD. This neutralization of risk factors and exclusion of decompensated cirrhosis,

cholestasis and postmenopausal females will help focus on HCV alone and avoid a confounding factor.

In patients with chronic hepatitis C (group II) the frequency of osteopenia was 11 (36.7%), osteoporosis 2 (6.7 %), total patients with low BMD was 13 (43.3%). In cirrhotic patients (group III), the frequency of osteopenia was 13 (43.3%), osteoporosis was 3 (10.0%), and total patients with low BMD was 16(53.3%) vs 1(5.0%) in the control group (group I). The previous studies showed wide variability in their results. Tsuneoka et al. [29] found osteoporosis in 20% of patients with chronic hepatitis and in 40% of patients with cirrhosis. Duarte et al. [15] found low BMD values in about 20–25% of HCV affected patients. Sokhi et al. [30] found that osteopenia in the cirrhotic patients awaiting liver transplantation was 34.6% and that of osteoporosis was 11.5%. Schiefke et al. [12] found that osteoporosis affected 26% in viral hepatitis B and C. Bunchorntavakul et al. [31] found that osteoporosis and osteopenia were 3.5% and 22.8%, respectively in chronic hepatitis C in Thai patients. Turkeli et al. [32] estimated the prevalence of osteopenia and osteoporosis in cirrhotic patients to be 45% and 42.5%, respectively. Javed et al. [33] found osteoporosis in 26% and osteopenia in 42% of patients with cirrhosis due to viral hepatitis. George et al. [34] found decreased BMD in 68% in cirrhosis patients. Goral et al. [35] found osteoporosis in 37% of patients with cirrhosis. Another study by Goubraim et al. [36] found a high prevalence of BMD abnormalities in cirrhotic patients (post viral B, C) with total rate of 80.4%, osteoporosis was found in 28.2% of cases and osteopenia in 52.2% of cases. Some Egyptian studies have also found high rates of bone loss in patients with cirrhosis: 87.8% in Salama et al. [37] and 86.6% in Ahmed et al. [38]. These variable percentages of bone disease in chronic HCV in comparison to this study may be related to age, gender, stage of liver disease as well as nutritional state and most studies which reported high percentage were decompensated cirrhosis.

In the present study testosterone was mild decreased in cirrhotic group in comparison to chronic hepatitis C and control group but all within normal range with no effect on bone disease (no significant difference between patients with low BMD and patients with normal BMD. This was in agreement with Gallego-Rojo et al. [39], Wariaghli et al. [19] and Pelazas-González et al. [40] who found that Patients with HCV infection had significantly lower testosterone levels than controls, but no effect on bone disease. This finding in our study disagrees with George et al. [34] who found high incidence of hypogonadism in patients with cirrhosis with no relation to bone disease and Diamond et al. [41] who reported that the two major risk factors for the development of osteoporosis were cirrhosis itself and hypogonadism. The explanation of that debate may be that most patients in our study were middle-aged, well-nourished and had good general condition, good liver function and those who had cirrhosis were compensated. Hypogonadism in most studies was mostly related to the severity of liver disease, alcohol intake and nutritional state.

In this study, there was no significant difference between serum calcium, phosphorus, or parathyroid hormone (PTH) with low BMD patients and patients with normal BMD. Although, PTH mild increase in cirrhotic group. This is in agreement with Karan et al. [42], Schiefke et al. [12] Goral et al. [35], Goubraim et al. [36] who found high PTH level in cirrhotic patients but with no difference between patients with normal BMD and those with osteoporosis or osteopenia. Our study also agrees with Duarte et al. [15], Bunchorntavakul et al. [31], George et al. [34] and El Karmouty et al. [43] who found serum calcium, phosphorus and PTH levels were insignificantly different in patients with normal BMD and patients with low BMD.

Our study disagrees with that of Bai et al. [44] who found that an increased level of PTH was an independent risk factor associated with low BMD. Also, Younes et al. [45] who recorded a significant increase in serum PTH level in patients with cirrhosis but serum PTH was inversely correlated with BMD. This could be explained by the fact that the role of calcium-parathyroid hormone-vitamin D axis in hepatic osteodystrophy is controversial and still unclear [46,47]

There was no difference in age and gender between patients with low BMD and patients with normal BMD. This finding disagrees with Tsuneoka et al. [29] and Ormarsdottir et al. [48] who found that the advanced age of patients was an independent risk factor of osteoporosis in patients with chronic liver disease including chronic hepatitis and cirrhosis. Also Figueiredo et al. [49] who found that there was a significantly

lower BMD in post-menopausal female patients compared to male and pre-menopausal patients. And Sokhi et al. [30], Mahmoudi et al. [50] who found that female gender is a predictive factor of the occurrence of metabolic bone disorders particularly osteoporosis in patients with cirrhosis. This conflict can be due to the fact that most patients in this study were males in middle age, small percentage were female in child bearing period.

Sambrook and Cooper [51] explained the role of age and gender in their study as follows, patients with liver cirrhosis older than 50 years had lower BMD and Peak adult bone mass is achieved early in life, with a gradual, progressive decline in BMD beginning at about age 40 to 45 years but more rapidly in women, for whom the decline accelerates after menopause [51].

There was no difference as regard smoking and body mass index (BMI) as common risk factors in diseased and control groups. Also no difference between patients with normal BMD and patients with low BMD. This is in agreement with Ormarsdottir et al. [48]; Salama et al. [37]; George et al. [34]; Goral et al. [35] who found that there were no significant differences as regard BMI, between the patients with normal and low BMD. This was in disagreement with Goubraim et al. [36] who found that patients with low BMD have a lower BMI compared with those with normal BMD. This could be explained by that BMI was in average range and smoker was small percentage (13.3% in HCV, 5% in control group) in diseased and healthy control groups respectively.

In the present study there were no significant differences as regards liver biochemistry (ALT, AST, ALP, bilirubin, serum protein, serum albumin, prothrombin time) between patients with low BMD and patients with normal BMD. This is in agreement with Smith et al. [26] who found no correlation between serum bilirubin and reduced BMD in patients with end-stage liver disease [26].

Finding in this study that disagrees with Menon et al. [52], Karan et al. [42], Uretmen et al. [53] and Goral et al. [35] who found that there is a negative correlation between serum bilirubin level and BMD. This study also disagrees with El Karmouty et al. [43] who found significant negative correlation between serum bilirubin, prothrombin time and BMD, also a highly significant positive correlation between BMD and serum albumin in patients with chronic liver disease. Our study also disagrees with Corazza et al. [9] and Figueiredo et al. [49] who found a highly significant positive correlation between BMD measurements and serum albumin levels. Also, Duarte et al. [15] who reported significant negative correlation between BMD measurements and prothrombin time. This could be explained by that BMD values were affected with the deterioration in liver functions and patients in this present study were with good liver function and the cirrhotic patients were compensated liver disease (child A).

CONCLUSION

Osteoporosis and its milder form osteopenia are an important extrahepatic manifestation of chronic HCV. Reduced BMD is common in chronic HCV-infected patients. HCV infection is a risk factor of osteoporosis.

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Transforming Growth Factor Beta One and Non Alcoholic Fatty Liver Disease

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Background and study aim: Hepatic steatosis reflects an imbalance between the uptake and synthesis of fatty acids by the liver and their oxidation and export. The mechanism of cell injury remains unclear. Transforming growth factor – beta 1 (TGF-β1) as a proinflammatory cytokine has become an important issue in the context of pathogenesis and progression of non alcoholic fatty liver disease (NAFLD). This study was planned to assess the value of TGF-β1 in different forms of NAFLD

Patients and Methods: This study included 62 patients; 20 patients with benign steatosis (group 1), 20 patients with non alcoholic steatohepatitis (NASH) (group 2) and 22 patients with cirrhosis (group 3), as well as 7 healthy subjects who served as a control group. Each group was subclassified according to the presence of obesity, type 2 diabetes mellitus and hypertriglyceridemia. All participants were subjected to abdominal ultrasound, ultrasound guided needle liver biopsy and routine laboratory investigations e.g. complete blood picture, liver function tests, fasting and 2 hours postprandial blood glucose and serum triglycerides.

Results: Serum TGF-β1 in the benign steatosis group was insignificantly different from the control group, while NASH and cirrhosis groups had significantly higher levels compared to control and benign steatosis groups (P<0.001). TGF-β1 in NASH group was significantly higher than in cirrhosis group (428.78 ± 117.15 vs 260.42 ± 110.22 ng/ml, P=0.032). In benign steatosis group, TGF-β1 was insignificantly different among subgroups. In NASH and cirrhotic patients, TGF-β1 was significantly higher in dyslipidemic subgroups.

Conclusion: Serum level of TGF-β1 was higher in patients with severe forms of NAFLD (NASH and cirrhosis) than in patients with benign steatosis.

INTRODUCTION

Non alcoholic fatty liver disease (NAFLD) consists of a spectrum of diseases including simple steatosis, non alcoholic steatohepatitis (NASH) and cirrhosis. NASH consists of steatosis plus inflammation, necrosis and fibrosis [1]. NAFLD affects 14-30% of the general population in United States [2]. NAFLD has been associated with multiple metabolic risk factors including, central obesity, dyslipidemia, hyper-tension, insulin resistance and type 2 diabetes mellitus [3-6]. The mechanism of cell injury in NAFLD entails that excess fatty acids in the liver induces formation of free radicals, which cause lipid peroxidation and induce proinflammatory cytokines [1]. Of the cytokines secreted as a response to cell injury, transforming growth factor – beta 1 (TGF-β1) plays the dominant role in mediating fibrosis, through its contribution to the activation of stellate cells and their production of extracellular matrix proteins [7].

Serum level of TGF-β and tissue level of TGF-β mRNA can be measured and used as diagnostic and prognostic markers for human diseases [8]. In the liver, TGF-β1 is the most abundant isoform of this family. It has many actions in the liver including: fibrogenesis, growth inhibition (of normal hepatocytes and stellate cells), mitogenesis, pro-apoptosis and chemo-attraction [9]. TGF-β1 stimulates extracellular matrix production not only by hepatic stellate cells but also by sinusoidal endothelial cells, however, its effect varies from one condition to another. In the context of hepatic regeneration, TGF-β1 is antiproliferative rather than pro-fibrogenic [10].

Different reports have linked TGF-β1 to different pathological hepatic states like cirrhosis and tumors [11-12]. Yet, to our knowledge, only scanty reports have assessed the link between TGF-β1 and different pathological spectrum of NAFLD. This study was designed to assess the value of TGF-β1 in different forms of NAFLD.

PATIENTSAND METHODS

This study had been carried out in the Departments of Internal Medicine and Clinical Pathology, Faculty of Medicine, Zagazig University, Egypt. Patients suspected to have NAFLD were included with the following criteria:

1- Those who are diabetic, obese or hyper-lipidemic with or without unexplained hepatomegaly.
2- Ultrasound-evidence of fatty liver.

Patients were excluded from the study if they had a disease process that might induce steatosis, inflammation or fibrosis **[13]**, as viral hepatitis, autoimmune hepatitis, Wilson's disease, hereditary hemochromatosis and those who have drug induced liver disease (e.g. methotrexate, tamoxifen, corticosteroids, amiodarone and synthetic estrogen). Pregnant females and patients with known history of alcohol consumption were also excluded. Out of the 295 patients who were primarily studied for being obese, diabetic or hyperlipidemic, 62 patients were included in this study, being free from exclusion criteria and fit to our inclusion criteria. The included patients were 35 females and 27 males with mean age 40.86±10.09 years (range 19-70 years). In addition, seven apparently healthy individuals, selected from the relatives of our patients, served as a control group, they were three males and four females, with mean age 49.29±6.16 years (range 44-60 years). The study was approved by our hospital ethical committee and after being informed about the purpose and procedures of the study, all participants signed an informed consent form. All patients and controls were subjected to thorough clinical evaluation. Obesity was defined as body mass index (BMI) ≥ 30.

Laboratory evaluation to all patients and controls included:

- Complete blood counts (CBC), fasting and 2 hours postprandial blood glucose, serum triglyceride level by colorimetric method, serum cholesterol by direct spectrophotometry, as well as routine liver function tests and enzymes including alanine aminotransferase (ALT) and aspartate aminotransferase (AST).
- Blood tests to fulfill exclusion criteria e.g. viral hepatitis serological markers (HCV Ab and HBsAg) by enzyme linked immunosorbent assay (ELISA) and auto-antibodies for autoimmune hepatitis including smooth muscle antibody (SMA) and liver kidney microsomal antibody (LKM).
- Serum level of TGF-β1 by ELISA.

Principle of the assay of serum TGF-β1 by ELISA [13]:

- A solid phase ELISA was used which is based on the sandwich principle. Standards and samples from patients and controls were diluted in assay buffer, acidified with HCL and then neutralized with NaOH. Afterwards, the neutralized standards and samples were added to the antibody coated microtiter wells.
- Then a monoclonal mouse anti TGF-β1 antibody, a biotinilated anti mouse IgG antibody and the streptavidin-HRP enzyme complex were incubated in succession.

Abdominal ultrasound and liver biopsy:

On ultrasonography, fatty infiltration of the liver produces a diffuse increase in echogenicity as compared with that of the kidneys. The cirrhosis was diagnosed on clinical, laboratory, ultrasound and histopathologic bases. Ultrasound guided percutaneous true-cut needle liver biopsy was performed to all patients.

Histopathological examination of the specimens:

Biopsy specimens were fixed in buffered formalin and embedded in paraffin wax. Sections were stained with Hematoxylin and Eosin for morphological evaluation, Perls' Prussian blue stain for assessment of iron loading and Masson's trichrome, for assessment of fibrosis. The specimens were analyzed by one pathologist with experience in liver pathology, with grading of steatosis, lobular inflammation, hepatocyte ballooning, NAFLD activity scoring, and fibrosis staging according to internationally agreed parameters [14]. Based on histopathologic diagnosis and classification, the included patients were assigned into three groups (Table1); 20 patients with benign steatosis (group 1), 20 patients with non alcoholic steatohepatitis (NASH) (group 2) and 22 patients with cirrhosis (group 3). Each group was further classified into three subgroups a, b and c according to the

presence of obesity, type 2 DM and hypertriglyceridemia respectively.

Statistical evaluation

Statistical analysis was done by using SPSS (Statistical Package for Social Science) version 19. The data were presented in the form of means and standard deviation, or in the form of numbers and percentages. Data were tested using the proper tests, including student's t-test, one way ANOVA and Chi-Square. Level of significance is P<0.05.

RESULTS

There were no significant differences among the studied groups as regard their sex and age distribution (Table 1).

Table (1): Demographic data of all patients and control groups.

Groups \ Demographic data		Sex				Age / year		
		Female		Male		Mean	SD	Range
		N	%	N	%			
Control group n=7		4	57.1	3	42.9	49.29	6.16	(44-60)
Group 1 (Steatosis) n=20	Obese	5	65.1	2	34.9	43.43	11.49	(30-64)
	Diabetic	4	57.1	3	42.9	50.14	9.67	(39-70)
	Hypertriglyceridemic	3	50	3	50	52.67	7.97	(37-60)
Group 2 (Steatohepatitis) n=20	Obese	6	85.7	1	14.3	44.43	14.15	(27-70)
	Diabetic	3	42.9	4	57.1	47.86	11.57	(38-70)
	Hypertriglyceridemic	3	50	3	50	41.67	13.26	(19-57)
Group 3 (Cirrhosis) n=22	Obese	6	75	2	25	45.38	10.39	(34-58)
	Diabetic	2	33.3	4	66.7	48.67	3.44	(44-52)
	Hypertriglyceridemic	3	37.5	5	62.5	47.00	4.60	(42-55)
P value		NS				NS		

Level of significance of P value: < 0.05 NS= non significant

Regarding Liver function tests and enzymes in all groups (shown in Table 2): the significantly highest bilirubin level was found in those with cirrhosis (P<0.001), the lowest albumin levels were reported among those with cirrhosis (P<0.01), the significantly highest levels of AST and ALT were seen among all patients with NASH (P<0.001) and prothrombin concentration was significantly worse among patients with cirrhosis (P< 0.001).

Table (2): Liver functions and enzymes in all patients and control groups.

Group \ Test		N	Serum bilirubin (mg/dl)		Serum albumin (gm/dl)		Serum AST (u/l)		Serum ALT (u/l)		Prothrombin concentration(%)	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control		7	0.400**	0.129	4.386*	0.522	16.00*	2.65	25.57*	6.35	107.14**	18.22
Benign steatosis	Obese	7	0.729	0.125	4.186	0.398	24.29	4.07	25.57	4.08	100.00	3.02
	Diabetic	7	0.771	0.512	4.057	0.447	20.14	6.36	20.71	5.28	97.86	5.67
	↑TG	6	0.850	3.367	4.417	0.366	19.33	1.86	22.00	3.58	96.50	6.44
Steato-hepatitis	Obese	7	0.857	0.321	4.029	0.382	43.66*	13.78	85.80*	20.89	90.43	9.31
	Diabetic	7	0.814	0.353	3.900	0.245	37.43*	7.89	76.14*	12.92	91.43	8.08
	↑TG	6	0.717	0.117	4.350	0.399	38.33*	6.25	74.17*	27.30	94.17	7.36
Cirrhosis	Obese	8	1.750**	1.281	2.800*	0.338	22.63	3.11	27.25	3.06	56.13**	10.75
	Diabetic	6	1.517**	0.733	2.633*	0.585	24.50	3.62	28.33	2.73	48.33**	14.28
	↑TG	8	2.412**	1.309	2.750*	0.342	23.50	3.93	26.50	3.42	55.38**	9.58

One way ANOVA $P < 0.05^{*}$, $P < 0.001^{**}$

In Table (3) we compared CBC components in all studied groups, and found that red blood cells count (RBCs), hemoglobin concentration and platelets count were significantly lowest among patients with cirrhosis (P<0.001).

Table 3: CBC in all patients and control groups.

Group \ Test		*N*	RBCs count (Million/cmm)		HB concentration (gm%)		WBCs (x103/cmm)		Platelet count (x103/cmm)	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control		7	5.3286**	0.3352	15.143**	1.069	7.33	2.14	183.86**	29.35
Benign steatosis	Obese	7	3.9014	0.3973	11.757	1.359	5.93	1.39	241.00	37.62
	Diabetic	7	4.0757	0.3534	11.400	2.012	5.16	1.36	244.43	15.78
	hypertriglyceridemia	6	3.8983	0.5329	11.033	1.343	4.32	0.79	219.50	67.20
Steato-hepatitis	Obese	7	4.0686	0.8291	11.443	2.501	6.72	2.56	209.29	58.27
	Diabetic	7	3.9729	0.4567	12.043	2.403	7.26	2.18	228.71	50.19
	hypertriglyceridemia	6	4.1700	0.3450	12.017	1.123	7.28	2.19	238.83	56.80
Cirrhosis	Obese	8	3.1638**	0.3497	9.775**	0.838	5.03	1.14	128.00**	68.29
	Diabetic	6	3.1133**	0.7188	8.917**	1.996	5.84	0.97	91.50**	48.85
	hypertriglyceridemia	8	3.0800**	0.5236	10.850**	1.122	6.03	1.23	140.25**	23.82

One way ANOVA P < 0.001**

In Table (4) we compared serum TGF-β1 values among NAFLD patients and controls. There were a highly significant difference among all groups (p<0.001). While, the mean serum value of TGF-β1 of the benign steatosis group was insignificantly different from the control group, the NASH and cirrhosis groups had significantly higher levels than both the control group (P<0.001) and the benign steatosis group (P<0.001).

Table (4): Serum TGF-β_1 values (ng/ml) among NAFLD patients and control groups.

Groups	N	Mean	SD	Range
Control	7	134.8	63.21	(47-198.3)
Benign steatosis	20	156.31	17.51	(120-179.2)
NASH	20	428.78	117.15	(238-1041.6)
Cirrhosis	22	260.42	110.22	(46-982.2)
One way ANOVA	P < 0.001			

Student t-test:

Control	vs	Benign steatosis	P = 0.32
Control	vs	NASH	P < 0.001
Control	vs	Cirrhosis	P = 0.009
Benign steatosis	vs	NASH	P < 0.001
Benign steatosis	vs	Cirrhosis	P = 0.031
NASH	vs	Cirrhosis	P = 0.032

Table (5) shows Serum TGF-β1 values (ng/ml) among all subgroups of different groups. Although the levels of TGF-β1 were higher in benign steatosis than the control and in obese and dyslipidemic cases more than diabetic cases of benign steatosis group, the difference doesn't reach significant level. All three subgroups of both NASH and cirrhotic patients had significantly higher TGF-β1 compared to control group (P<0.001) and the dyslipidemic group showed the highest values.

Table (5): Serum TGF-β_1 values (ng/ml) among different subgroups.

Group		N	Mean	SD	Range	One way ANOVA	Student t-test
Control		7	134.8	63.21	(47-198.3)	P>0.05	P>0.05 between any two subgroups, or between control and any subgroup.
Benign steatosis	Obese	7	160.85	13.21	(138-174)		
	Diabetic	7	145.02	19.95	(120-179.2)		
	Hypertriglyceridemic	6	164.19	14.03	(80.0-738.2)		
Control		7	134.8	63.21	(47-198.3)	P<0.001	P<0.001 (between control and any subgroups). P>0.05 (between any two subgroups).
Steato-hepatitis	Obese	7	357	146.7	(40.6-593)		
	Diabetic	7	397.1	148.2	(173.6-944)		
	Hypertriglyceridemic	6	400.1	145.1	(110-1041.6)		
Control		7	134.8	63.21	(47-198.3)	P<0.001	P<0.01 (between control and ↑TG). P<0.05 (between control vs obese or diabetics).
Cirrhosis	Obese	8	236.84	90.12	(41.4-490.9)		
	Diabetic	6	238.25	95.17	(78.7-782.2)		
	Hypertriglyceridemic	8	290.71	101.71	(70.7-982.2)		

DISCUSSION

Non alcoholic fatty liver disease is characterized by fat accumulation in the liver, which may progress to non-alcoholic steatohepatitis and cirrhosis [4]. It is becoming increasingly recognized worldwide due to its prevalence in obesity, diabetes and insulin resistance syndrome [15]. We investigated the status of TGF-β1 in NAFLD in all its pathological forms in a cross sectional way. We also evaluated it in respect to the common etiological factors for NAFLD; obesity, type 2 DM and hypertriglyceridemia. The preponderance of female sex over male was specifically higher in the obese group. Within the obese group, females tended to have benign steatosis and NASH (Table 1). This was in agreement with Luyckx et al., who identified female sex as a risk factor for NASH [16]. In our study, NASH occurred in a nearly similar frequency in both sexes in the diabetic and hypertriglyceridemic groups (Table 1). This trend was also found in another study on NASH patients by Becon and his colleagues [17]. Although NAFLD occurs in all age groups including children, we conducted our work only on adult patients. In this category, the mean age of NAFLD occurred mostly in the forties (Table 1). This was in agreement with several studies that showed the highest prevalence of NAFLD in those between 40 and 49 years of age [17-18].

Our results showed values of serum bilirubin, serum albumin and prothrombin time within normal range among different groups, except in cirrhotic patients (Table 2). Similar results were obtained by multiple studies on NAFLD patients which demonstrated that no hepatic dysfunction occurs until cirrhosis and liver failure start [18-20]. On the other hand, and going with multiple previous reports [17,19-20], ALT level was significantly higher in NASH patients than other groups (Table 2). Hence, we used high ALT level as a laboratory criteria for identifying NASH patients. RBCs count, hemoglobin concentration and platelet count were significantly lowest among patients with cirrhosis (Table 3). This was in agreement with technical review by American gastroenterological association on NAFLD that stated that hematological parameters are usually normal unless cirrhosis develop [21].

Our results showed that plasma TGF-β1 was significantly higher in NAFLD patients when compared to the control individuals. Although the TGF-β1 of the benign steatosis group was insignificantly different from the control, the NASH and cirrhosis groups had significantly higher levels when compared with either control group or benign steatosis group. Moreover, the TGF-β1 level in NASH group was higher than in cirrhosis group (Table 4). The above findings were in agreement with Yokohama et al., whose work demonstrated that blood markers of fibrosis including TGF-β1 and type IV collagen were significantly elevated in patients with NASH [22]. Our results were also supported by that obtained by Hasegawa et al., who concluded that the plasma TGF-β1 level in NASH patient was significantly elevated as compared to healthy controls and benign steatosis patients [23]. Hence, TGF-β1 could be a useful marker for diagnosis of NASH.

In our study, the dyslipidemic subgroup had significantly highest level of TGF-β1 when compared to obese and diabetic subgroups, within each patient group (Table 5). Comparative data in that field was not sufficiently tested before; however, some retrospective studies

showed high prevalence of obesity, diabetes and dyslipidemia in cryptogenic cirrhosis with or without associated hepatocellular carcinoma [24-25]. This supports the hypothesis that NASH may be an etiological factor in some of these patients. Moreover, in agreement with our results, Bugianesi et al. had identified hypertriglyceridemia (by logistic regression analysis) as the most significant independent factor in that field [25].

Zhe et al., reported that blockade of TGF-β signaling prevents liver fibrosis and dysfunction in the rat, but they recommended further future studies to avoid the unfavorable consequences, such as the inflammation and tissue necrosis observed in TGF-β1 gene-disrupted mice [26]. It was also suggested that insulin sensitizing agents like metformin, and other agents like angiotensin II receptor antagonists, ursodeoxycholic acid, gemfibrozil, N-acetyl-cysteine and α-tocopherol may have a beneficial effect in patients with NASH by lowering the serum levels of TGF-β1[27-29].

CONCLUSION

This study revealed that TGF-β1 was significantly higher in severe forms of NAFLD (NASH and cirrhosis) versus benign steatosis suggesting its role in the progression of NAFLD.

TGF-β1 assessment can be recommended as a future non invasive method for evaluation of NAFLD severity. Also, further studies are needed to evaluate the beneficial effect of TGF-β1 signaling blockade as a new therapy for NAFLD.

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Urinary Neutrophil Gelatinase-Associated Lipocalin as Predictor for Development of Hepatorenal Syndrome in Patients with Hepatic Cirrhosis

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Background and study aim: Liver cirrhosis has many complications, hepatorenal syndrome (HRS) is one of the most serious complications of it. Neutrophil gelatinase-associated lipocalin (NGAL) is a protein expressed by injured kidney tubular epithelia, urinary NGAL levels rise early in cases of acute kidney impairment before elevation of serum creatinine. The aim of this study is to evaluate NGAL as a biomarker for early detection of HRS in patients with hepatic cirrhosis.

Patients and Methods: Seventy five patients were studied and divided into two groups, group (I) 50 patients with liver cirrhosis without impairment of kidney functions and group (II) 25 patients with cirrhosis and impaired kidney functions. Urinary NGAL level were measured by ELISA.

Results: The mean value of urinary NGAL level in patients with liver cirrhosis was 50 ± 33 ng/ml while in patients with cirrhosis and HRS was 750 ± 250 ng/ml which showed highly statistically significant difference between both groups of patients.

Conclusion: Urinary NGAL increases significantly in patients with liver cirrhosis associated with impairment of kidney function than in those with stable cirrhosis and normal kidney function, so it can be used as a marker for prediction of development of HRS in cirrhotic patients.

INTRODUCTION

Acute kidney injury (AKI) is a common complication in patients admitted to hospital with advanced liver cirrhosis, it is observed in approximately 20% [1]. In cirrhosis, AKI include pre-renal azotemia, acute tubular necrosis (ATN), and hepatorenal syndrome (HRS) [2,3]. HRS is a dangerous complication, it is functional impairment of kidney function as a result of abnormal hemodynamics leading to splanchnic and systemic vasodilatation associated with renal vasoconstriction [4]. Serum creatinine has been the most commonly used marker of AKI, but it is non-specific marker of kidney dysfunction, as it may be normal or near normal in patients with advanced liver cirrhosis despite of diminished kidney function which may be due to muscle cachexy which they suffer from [5], so AKI can develop before change in serum creatinine, therefore investigations of a biomarker for early identification of patients at risk is very important for early diagnosis, because delay in diagnosis not only affect AKI treatment outcome, but it worsens portal pressure elevation in patients with HRS [6,7].

Neutrophil gelatinase-associated lipocalin (NGAL) is a protein expressed by injured tubular epithelia of the kidney [8,9]. Urinary NGAL (NGAL) rises early in AKI, prior to elevation of serum creatinine [8-11], urinary NGAL is not affected by volume status, diuretic use or pre-renal azotemia [12]. Chronic kidney disease (CKD) does not induce NGAL expression [12]. Urinary NGAL has been shown to be useful marker in number of clinical settings as predictor of development of AKI and mortality [13,14], but the information on the role of urinary NGAL in cirrhosis and HRS is very few [15,16].

On this background, the aim of this study was to investigate urinary NGAL as early predictor for development of HRS in patients with advanced liver cirrhosis.

PATIENTSAND METHODS

Seventy five patients with liver cirrhosis; 50 patients (group I) with liver cirrhosis without kidney affection serum (creatinine <1.5 mg/dl) and 25 patients (group II) with liver cirrhosis and impaired kidney functions (serum creatinine≥1.5mg/dl),this value was chosen as it has been selected in several conferences and publications as cut off for impairment of kidney function in cirrhosis, admitted to Tropical Medicine Department and Intensive Care Unit from January 2012 to June 2013, patients were divided into two groups.

Exclusion criteria:

1- Urinary tract infection.
2- Pre-renal azotemia.
3- Chronic parenchymal kidney disease or patients on chronic hemodialysis.
4- Obstructive uropathy.
5- Malignancy.

All patients were subjected to the following:

- Full history taking.
- Thorough clinical examination.
- Complete blood picture.
- Liver and kidney function tests.
- Urine analysis.
- Glomerular filtration rate (GFR)was calculated according to Cockcroft-Gault formula [17].
- Urine NGAL level by ELISA (BioPorto Diagnostics Hellerup, Denmark).
- 24-h urine amount was measured.10ml urine sample was stored at -80ºC until measuring urine NGAL.

Definitions

Cirrhosis: The diagnosis of cirrhosis was based on a combination of clinical, biochemical and ultrasonographic findings.

Impairment of Kidney Function: Impairment of kidney function was diagnosed when serum creatinine concentration was greater than 1.5mg/dL. This value of serum creatinine was chosen because it has been selected in several consensus conferences as cut-off to define impairment of kidney function in cirrhosis; (1) **prerenal azotemia** due to volume depletion was considered when patients had a history of fluid losses in the preceding days (due to either bleeding, diuretic overdose, or other causes), together with compatible findings, absence of other causes of impairment of kidney function, and reversibility of kidney impairment as indicated by decrease of serum creatinine below 1.5mg/dL after fluid resuscitation; (2) **chronic kidney disease** (CKD) was defined by evidence of structural kidney abnormalities, proteinuria, and/or abnormal urine analysis, with a glomerular filtration rate of less than 60 mL/min per 1.73 m^2.; (3) **HRS** was defined using the current definition of the International Ascites Club. HRS is a functional renal failure due to renal vasoconstriction and low renal perfusion. Kidney histology is normal or shows lesions that do not justify the decrease in the glomerular filtration rate (GFR). It is characterized by impaired renal function and marked abnormalities in the arterial circulation and activity of endogenous vasoactive systems. The traditional concept is that HRS is due to deterioration in circulatory function secondary to an intense vasodilation in the splanchnic circulation (peripheral arterial vasodilation hypothesis). During the last decade, however, several features suggest a much more complex pathogens [24-27]; (4) **ATN** was diagnosed in patients who had at least three of the following: hypovolemic and/or septic shock or treatment with potentially nephrotoxic agents, urine sodium greater than 40mEq/L, urine osmolality lower than400mOsm/kg, and fractional excretion of sodium greater than 2% without diuretics [28,29].

Statistical analysis:

Analysis of data was performed with Statistical Package for Social Science computer program (SPSS Inc., version 16.0, Chicago, IL). Numerical data were expressed as mean± standard deviation and range. Qualitative data were expressed as frequency and percentage. Fisher's exact test was used to examine the relation between qualitative variables. For quantitative data (normally distributed) comparison between two groups was done using student *t*-test and Man Whitney for nonparametric ($P < 0.05$; significant).

RESULTS

Seventy five patients were included in this study, they were divided into two groups:

Group (I): 50 patients with liver cirrhosis without kidney affection (Normal kidney function tests).

Group (II): 25 patients with liver cirrhosis and impaired kidney functions.

Table (1): Patients characteristics at time of hospital admission

	Group I N = 50	Group II N = 25	P
Age	40 ± 5	38 ± 8	0.5*
Sex (Male / Female)	30/20	15/10	0.43$
Liver function tests :			
S. bilirubin (mg/dl)	3.2 ± 2.1	5.2 ± 3.1	0.04*
Direct Bil (mg/dl)	1.5 ± 1	3.1 ± 1.2	0.05*
ALT (U/L)	57.3 ± 8	79.5 ± 10	0.13+
AST (U/L)	73.5 ± 25	102.5 ± 71	0.03*
S. Albumin (g/dl)	3 ± 0.6	2.8 ± 0.8	0.001+
Kidney function tests			
S. creatinine (mg/dl)	0.9 ± 0.2	2.1 ± 0.9	<0.001*
BUN (mg/dl)	20 ± 10	50 ± 18	<0.001*
GFR (mil/min)	80 ± 23	30 ± 15	<0.001*
S. Na (mEq/L)	137 ± 5	130 ± 6	<0.001+
S. Ka (mEq/L)	4.2 ± 0.6	4.3 ± 0.7	0.03+
Urine Na (mEq/L)	45 ± 30	29 ± 21	<0.001*
GFR (ml/min)	78 ±30	31 ±10	<0.001*
Child Pugh Turcot Score	8.6 ± 2.2	12.1 ± 1.8	<0.001+
MELD-Na Score	14 ± 4	31 ± 6	<0.0001+

* Man Whitney +test student *t* test $Fisher's exact test

The demographic characteristics of patients are shown in Table 1. Highly significant impairment of kidney function in group II and significant increase in child score and MELD-Na score.

Table (2): Urinary NGAL levels in studied groups

	Group I N = 50	Group II N = 25	P
Urinary NGAL Mean ± SD	50 ± 30	750 ± 250	<0.0001*

Highly significant increase in urinary NGAL in patients with impaired kidney function than those with normal kidney function (Table 2).

Table (3): correlation of different laboratory parameters with urinary NGAL levels:

	Urinary NGAL	
	r	p
Serum creatinine	**0.335**	**0.001**
Child-pugh-Turcot score	**0.386**	**0.001**
MELD-Na score	**0.615**	**<0.001**

Significant positive correlation between urinary NGAL levels and serum creatinine (r=0.335,p=0.001), also there was positive correlation between urinary NGAL levels with Child score and MELD-Na score (r=0.386,p=0.001,r=0.615, p<0.001 respectively, Table 3).

Table (4): Sensitivity, specificity, positive predictive value, negative predictive value and accuracy of urinary NGAL in diagnosis of HRS

Sensitivity	Specificity	Ppv	Npv	Accuracy
89 %	75 %	70 %	88 %	87 %

Ppv=positive predictive value Npv=negative predictive value

At cut off 110 ng/ml uNGAL has 89%sensitivity, 75% specificity, 70% positive predictive value, 88% negative predictive value and 87% accuracy for diagnosis of HRS(Table 4, Figure 1).

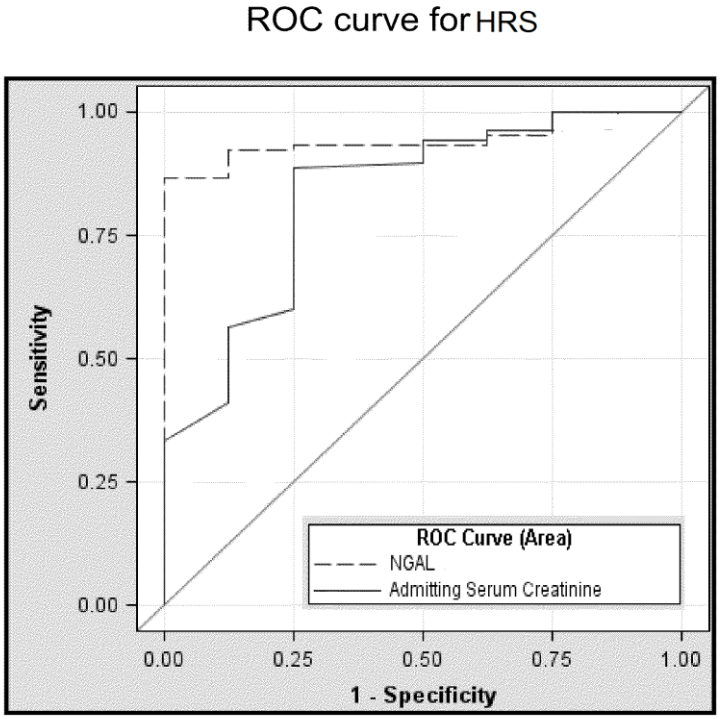

Figure 1: ROC curve for diagnosis of HRS using urinary NAGL

DISCUSSION

The result of this study showed that urinary NGAL was significantly higher in patients with liver cirrhosis and impaired kidney functions than in those without impaired kidney function, this result is in agreement with Gungor et al. [18] who found that patients with HRS had significantly higher plasma and urinary NGAL levels compared with stable cirrhosis patients and control subjects.

Also the result in agreement of Verna et al. [16] who evaluated the role of urinary NGAL in determining development of HRS and in patient mortality in patients with liver cirrhosis. Overall, 118 patients (44%) of patients had normal kidney functions while the rest had impaired kidney functions. The result of that study showed that the elevation of urinary NGAL in HRS was intermediate between that of pre-renal azotemia and intrinsic AKI.

The result of current study is in agreement with Nickolas et al. [19] who found urinary NGAL in patients with AKI (416 mg/g creatinine) was significantly higher than in patients with pre-renal azotemia, chronic kidney disease or normal kidney function. But, the result of this study differ than that of Fagundes et al. [20] who found urinary NGAL levels are increased in patients with cirrhosis and ATN compared to those of several other causes as HRS this difference may be due to in the current study patients with impaired kidney functions were divided to HRS and ATN

because no kidney biopsies were taken because of short life expectancy and potential bleeding complications due to coagulopathy.

The result of this study also is going with that of Singer et al. [21] who found urinary NGAL was significantly higher (257 ug/L) in patients with AKI compared to patients with pre-renal azotemia and unclassifiable causes (31 and 49 ug/L respectively).

Singer et al. [21] found that a urinary NGAL cut-off level greater than 104 ug/L provided specificity of 0.88 for diagnosis of AKI with high positive likelihood ratio, whereas urinary NGAL cut-off level of 47 ug/L provided a sensitivity of 0.89 and a low negative likelihood ratio for exclusion of AKI.

In this study urinary NGAL sensitivity 89% and specificity 75% ,ppv70%,npv 88% near that of Qasem[22] and El-Bassat [23] who-found(95.5% ,90.2%sensitivity,76.1%,67.9%specificity,65.5% ,79%ppv,and99.2%,91%npv respectively.

So, urinary NGAL can be used for early detection of kidney injury in patients with liver cirrhosis for rapid initiation of management and improvement of patient outcomes, which is our primary objective.

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Doxycycline Induced Extensive Esophageal Ulcerations: Case Report and Review of the Literature

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ABSTRACT

Drug induced esophageal disease is common. Doxycycline is one of the commonest causes of drug induced esophageal disease. The diagnosis is usually clinical but endoscopy is the gold standard diagnostic modality. Treatment is mainly depending on discontinuation of the offending medication. If left untreated it can have serious consequences like delayed esophageal stricture. A 22-year-old male had been prescribed doxycycline capsules for acne and developed odynophagia. Endoscopy revealed extensive esophageal ulcerations. He was managed symptomatically with proton pump inhibitors and his odynophagia improved over a period of five days. He was discharged with proper advice regarding medication ingestion and proton pump inhibitor for four weeks.

INTRODUCTION

Drug induced esophageal disease (DIOD) was first described in a patient who ingested potassium chloride tablets [1]. Since this initial report, the frequency of pill-induced esophageal injury has continued to grow and more than 1000 cases of drug induced esophageal injury have been reported [2]. The incidence of pill esophagitis has been estimated as approximately four cases per 100,000 per year [3]. Drug induced esophageal diseases are usually under estimated, as many cases usually resolve rapidly after cessation of the medication [4].

Doxycycline is one of the commonest causes of drug induced oesophageal ulcers, it is commonly prescribed for pelvic inflammatory disease and acne, hence doxycycline induced oesophageal injury is more common in females [5].

Pathogenesis

The mechanism of DIOD is believed to be due to prolonged contact of the caustic contents of the medication with the esophageal mucosa [6]. The typical esophageal lesion showing a small punched out ulcer in the area that was in contact with a high concentration of medication. The site of injury is frequently found in areas in which the esophageal lumen is compromised by the aortic arch, the esophago-gastric junction, or an enlarged left atrium. Thus, medication-induced injury requires that the pill or capsule remain in the esophagus for a prolonged interval, and that its contents be caustic to the esophageal mucosa [5].

The situations that enhance pill retention, thereby increasing the likelihood of esophageal injury may include: lack of an adequate fluid intake and ingestion of a pill immediately prior to sleep, since salivation and swallowing frequency are markedly reduced during sleep [7].

Patients with cardiac disease, particularly following thoracotomy, also appear to be at increased risk. Although this may be due to anatomical changes in the mediastinum, these patients are often more elderly and likely to be taking commonly implicated drugs, such as quinidine or potassium compounds. In contrast, pill-induced esophageal injury has not been commonly reported in patients with known esophageal motility disorders. These patients are often more attentive to swallowing function, which may provide protection when pills are ingested [6].

Clinical presentation

Patients with DIOD will often present with the sudden onset of odynophagia and retrosternal pain with no history of prior esophageal disease; the pain may be so severe that swallowing saliva is difficult. Patients often relate the onset of symptoms to the swallowing of a pill without water, commonly at bedtime. Typical scenarios include the teenage patient with acne who takes tetracycline at bedtime without water, and the elderly patient in a nursing care facility given a number of medications with a small amount of water while recumbent prior to sleep [5].

Diagnosis

Medication-induced esophagitis is often suspected when typical symptoms appear abruptly after

improper ingestion of a pill known to cause esophageal injury. Confirmatory endoscopy or barium radiography is more important in patients with particularly sever or atypical symptoms

Upper endoscopy is the most sensitive procedure; findings are abnormal in virtually 100 percent of cases [3]. Endoscopy is also helpful to rule out alternate diagnoses such as reflux esophagitis, infectious esophagitis, or malignancy. The typical endoscopic appearance of pill-induced esophageal injury is a discrete esophageal ulcer with relatively normal surrounding mucosa [1]. However, others reported doxycycline induced both esophageal and gastric ulcerations [8].

Here we present a severe, unusual endoscopic appearance of a case of doxycycline-induced esophageal ulcers at mid-esophageal segment.

CASE REPORT

The patient was a 22 years old male who had been prescribed doxycycline 100 mg capsule b.i.d. for acne by pharmacist. He took the first dose of doxycycline 100 mg capsule at 4 pm and 2 hours later he develops mild midsternal pain, one hour after the second dose which was taken at 4 am with no water and went to bed immediately, he wake up with sever retrosternal chest pain and odynophagia. The pain was burning in character and was located midsternal. It was constant in nature with fluctuations in severity. Swallowing solids and liquids significantly aggravated his pain. There was no history of dysphagia, abdominal pain, fever, melena, rectal bleeding or weight loss. His past medical history was irrelevant, he was a nonsmoker and denied using alcohol, aspirin or non-steroidal anti inflammatory drugs (NSAIDs). His physical examination was normal.

Initial investigations revealed a normal complete blood count, normal liver and kidney function tests and a negative troponin. His chest x ray was normal. An upper endoscopy revealed circumferential extensive ulcerations with irregular, hyperemic mucosa which bleeds easily on touch and covered with whitish fibrin at the level of the mid-esophagus (Figure 1).

According to the previous data he was diagnosed to have doxycycline induced esophageal ulcerations, so doxycyclin was discontinued and proton pump inhibitor was given intravenously. The patient returned after 5 days and experienced improvement of his symptoms and told that he starts oral feeding normally.

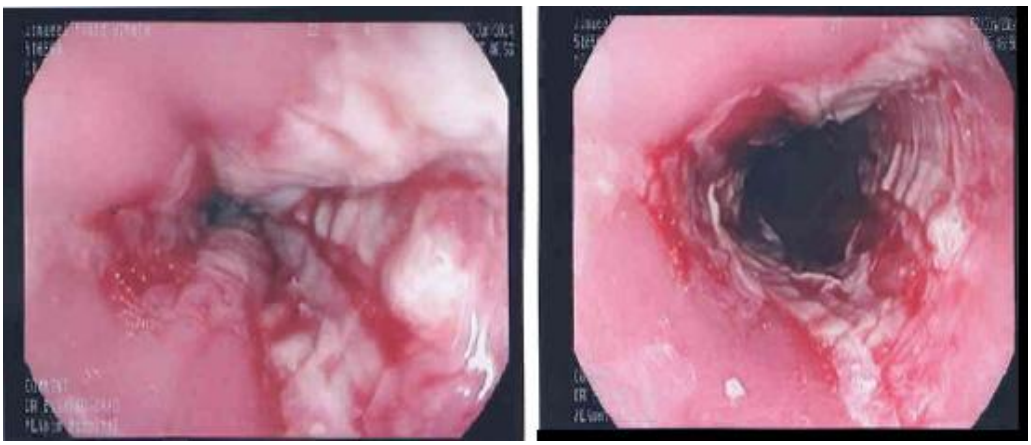

Figure (1): Extensive ulcerations of the mid-esophagus

DISCUSSION

Drug induced esophageal disease is a common condition that is under reported, it was first reported in the 1970 [9]. Antibiotics account for 50-60% of drug related esophageal toxicity and tetracyclines, in particular doxycycline, are commonly implicated [10].

Patient factors have been implicated in the causation of oesophageal injury; an elderly patient who takes multiple medications is at high risk for oesophageal injury. Such patients may not remember the advice given by pharmacists. Patient with pre-existing oesophageal diseases such as reflux oesophagitis, scleroderma or oesophageal motility disorders also fall into this high-risk group.

Our case, demonstrates that even young and healthy individuals are not immune to drug induced injury and there are other factors that are equally important. However, among the reported cases of pill-induced injury, the proportion of the patients having a motility disorder such as achalasia and scleroderma or an anatomical narrowing such as tumor or stricture is low [11].

Medication factors play an important role in drug induced esophageal disease. Medicine taken with less or no water or taken at bed time may remain in contact with the oesophageal mucosa for a long duration and can cause direct mucosal injury [12]. There is decreased salivation and swallowing during sleep and this may increase the transit time through the esophagus. Liquid formulations are less likely to cause oesophageal injury compared to tablets. Sustained release formulations may cause oesophageal injury as they tend to be large in size and hence difficult to swallow. Gelatin based capsules may stick in the oesophageal lumen at the sites of anatomical narrowing (e.g. aortic arch) or pathological narrowing (e.g. enlarged left atrium) and can cause local mucosal injury. The chemical nature of the formulation is also important in the causation of damage. Most of the esophagus damaging medicines are acidic and cause direct toxic effects when they remain in contact with the mucosa for a long duration. Doxycycline can produce a pH of less than three when dissolved in 10 ml of water or saliva [13]. It is also shown that doxycycline capsules remain in oesophagus for three times long as doxycycline tablets [14].

Our patient was taking doxycycline capsules at bed time with minimal water. In a reported series of eight patients with tetracycline induced oesophagitis, seven patients were taking the drug in capsule form **[**15]. It is recommended that such medicines be taken in the sitting or standing position and with at least 100 ml of water. It has been reported that a shorter oesophageal transit time occurs when medication is swallowed with more liquids [16]. Also, it is advisable to remain upright for at least 15 minutes after the ingestion of medicine [4].

A detailed history and high index of suspicion is the key to an accurate diagnosis. The common symptoms of drug induced esophageal disease are dysphagia, odynophagia and retrosternal chest pain. In one case series that examined 36 patients with doxycycline related esophageal toxicity, 94% of patients had odynophagia, 80% had retrosternal chest pain and 54% had dysphagia [5]. The symptoms start within hours or days after ingestion of medicine and typically get better within a few days of discontinuing the drug [11].

Endoscopy is the gold standard diagnostic modality. The common endoscopic finding is one to several discrete shallow, small ulcers in the mid-esophagus [5]. Particles of medicine can also be found at the site or ulcer formation. Our patient had circumferential extensive ulcerations with irregular, hyperemic mucosa which bleeds easily on touch and covered with whitish fibrin at the level of the mid-esophagus (Figure 1).

Drug induced esophageal disease is self-limiting and symptoms usually improve on discontinuation of the medicine. Our patient already stopped taking doxycycline after the first day. Proton pump inhibitors or H2 receptor antagonists have no proven role in the absence of reflux oesophagitis. Topical protective agents and local anaesthetics such as liquid sucralfate or lignocaine may be of benefit for ulcer healing and pain relief. Delayed oesophageal stricture formation may require endoscopic dilatation [3]. The simple advice of swallowing medication with plenty of water in an upright position can prevent the consequence of erosive oesophagitis.

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