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Correlation of Liver Biopsy with Liver Enzymes and PCR among Egyptian Patients with Chronic Hepatitis C

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Abstract: Hepatitis C is a major health problem. In Egypt, the prevalence rate reaches 15% in rural areas. An important and striking feature of hepatitis C is its tendency towards chronicity that may lead to cirrhosis and hepatocellular carcinoma. Because of the morbidity and expense of liver biopsy, it is important to consider whether noninvasive testing such as ALT and HCV RNA quantification provides comparable information with respect to the level of liver disease activity. In this study we tried to correlate between the degree of necroinflammatory injury and stage of fibrosis with PCR and liver enzymes (ALT, AST & AP) in chronic hepatitis C. This study includes 90 untreated patients with a serological and histological diagnosis of chronic HCV infection. Serological evaluation was performed using Real-Time PCR. ALT, AST and AP levels were measured by automatic analyzer. Ishak scoring system was used for grading necroinflammatory injury and staging of fibrosis. Most of patients (76.7%) had mild degree of hepatitis. The majority of cases showed moderate degree of fibrosis. Serum Levels of both ALT and AST correlated with the grades of liver necroinflammatory activity but not the stage. Alkaline phosphatase levels as well as HCV-RNA titre correlated with neither the grades of liver necroinflammatory activity nor the stage of liver fibrosis. In conclusion, liver biopsy remains an important tool in the appropriate selection of patients for whom therapy can successfully treat.

Key words: Hepatitis C · Liver biopsy · Liver enzymes · PCR

INTRODUCTION

Hepatitis C is a major health problem. Global estimates indicate that three to four million persons are newly infected each year and 170 million people are chronically infected [1]. In Egypt, the prevalence rate reaches 15% in rural areas, with some age groups suffering from prevalence rates up to 50%. Incidence rates are estimated at 2-6 per 1,000 per year, a level that will maintain prevalence rates of 5-15% for the foreseeable future. The virus continues to be transmitted in medical and paramedical settings, as well as within communities and families. Approximately 5-7 million Egyptians carry antibodies for HCV [2]. Anti-HCV antibodies were found in 13.6% of volunteer blood donors, this high seropositive rate among Egyptians is about 35 folds higher than other countries [3]. They were also found in 54% of hospitalized, multi transfused children, 46.2% of adult on haemodialysis and 47.2% of adults with chronic liver disease or hepatoma [4]. An important and striking feature of hepatitis C is its tendency towards chronicity. In >70% of infected individuals, HCV establishes a persistent infection over decades that may lead to cirrhosis and hepatocellular carcinoma (HCC) [5]. HCV is an RNA virus which is highly genetically variable, with six different genotypes. Each genotype is divided into multiple subtypes with 80-85% similarity [6]. Each HCV genotype is unique with respect to its nucleotide sequence, geographic distribution and response to therapy [7]. HCV genotype 4 (HCV-4) is common in the Middle East and in Africa, where it is responsible for more than 80% of HCV infections and has recently spread to several European countries. Egypt has the highest prevalence of HCV-4, which is responsible for almost 90% of infections and is considered a major cause of chronic hepatitis, liver cirrhosis, HCC and liver transplantation in the country [8].

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An association between HCV-4 and the high rates of HCC in Egypt has been speculated. Currently, liver cancer constitutes 13% of all cancers in Egypt and is considered the second most frequent cancer in males after being the fourth in 1999. More than 65% of Egyptian patients with HCC are positive for HCV-4 and 11% had HCV/HBV co infection. Moreover, the distribution of HCC in Egypt closely parallels that of HCV-4, being more frequent among rural residents and farmers [9]. The role of liver biopsy in the management of chronic hepatitis C is the "gold standard" for assessing the grade of liver injury and stage of liver fibrosis in anticipation of antiviral therapy as stated by National Institutes of Health Consensus Development Conference in 1997 [10]. However, the relation of liver biopsy findings to standard interferon (IFN) and ribavirin (RBV) treatment outcome is heterogeneous [11]. Because of the morbidity and expense of liver biopsy, it is important to consider whether noninvasive testing such as liver enzymes and HCV RNA quantification provides comparable information with respect to the level of liver disease activity. A number of studies provide information related to this question. It is important to note that these studies focused on HCV antibody-positive blood donors rather than patients referred for disease symptoms [12].

In this study we tried to correlate between the degree of necroinflammatory injury and stage of fibrosis with PCR and liver enzymes [Alanine transaminase (ALT), Aspartate transaminase (AST) and Alkaline Phosphatase (AP)] in chronic hepatitis C.

MATERIALS AND METHODS

This study includes 90 untreated patients with a serological and histological diagnosis of chronic HCV infection, who enrolled from Al-Ahrar Zagazig Hospital, Egypt between February and May, 2011. Patients with decompensated liver disease, low hemoglobin (<13 g/dl for men and <12 g/dl for women), white blood cell count <3000/l, neutrophil count <1500/l, platelet count <100,000/l, HBsAg seropositivity, active schistosomiasis, elevated serum creatinine, poorly- controlled diabetes mellitus, hypertension, or psychiatric diseases were excluded.

Serological and Biochemical Evaluation: Serological evaluation was performed using Real-Time PCR Cobas TaqMan (Roche Diagnostics) that has a dynamic range between 10 IU/ml and 2 x 10^8IU/ml. ALT, AST and AP levels were measured by automatic analyzer (Vitros 250, Johnson & Johnson, USA). Serum levels of liver enzymes were determined at the time of liver biopsy.

Histological Evaluation: Percutaneous liver biopsy (baseline) with cores of at least 1-1.5 cm length or encompassing three portal areas in minimum were considered suitable for interpretation. The pathologist was unaware of clinical and biochemical data. Scoring system of Ishak *et al.* [13] (modified HAI grading and modified HAI staging) was used for the assessment of necroinflammatory injury and fibrosis stage. Histological activity was considered as minimal (score 1-4), mild (5-8), moderate (9-12) and severe (13-18). Fibrosis was staged separately on a scale 0-6, corresponding to no fibrosis (0), mild (1-2), moderate (3-4) and severe or cirrhosis (5-6) [13].

Biopsy Was Stained Using the Following Stains:

- Haematoxlyin & Eosin Stain: as a routine stain to search for lobular necrosis, portal inflammation and interface hepatitis.
- Masson Trichrome Stain: useful for determining the extent and pattern of fibrosis and evaluating portal tract structures in inflamed livers.
- Orcein Stains: to see ground glass appearance in hepatitis B virus and exclude possible hepatitis B virus occult infection and demonstrate elastic fibers and also evaluate for copper in Wilson disease.
- Prussian Blue Stain: to see possible hemochromatosis and evaluate ferric iron in portal tract macrophages which has been suggested as a marker for poor response to interferon.
- Periodic Acid-Schiff (PAS) Stain with Diastase: to evaluate possible α_1 antitrypsin deficiency.

Statistical Analysis: The data was analyzed by SPSS 10.0 software. Variables were presented as counts and percentages, mean ± SD. Correlations between histopathological grade and stage with serum HCV-RNA titer, ALT, AST and AP levels were assessed by means of Pearson correlation. $P<0.05$ was considered significant.

RESULTS

This study includes 90 patients whose age ranged from 18 up to 59 years with mean age (34.3). Most of the patients were males 69 (76.7%). Mean ALT, AST and AP

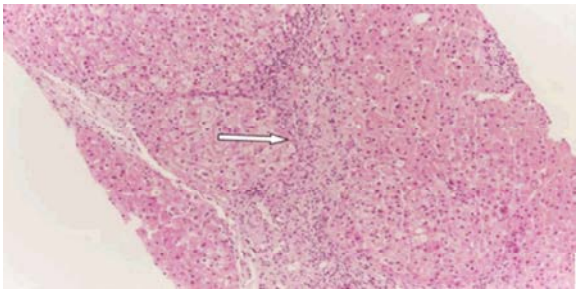

Fig. 1: Shows mild necroinflammatory activity with stage 5 Fibrosis - By Ishak system- (arrow shows marked bridging with occasional nodule) (H&E X200).

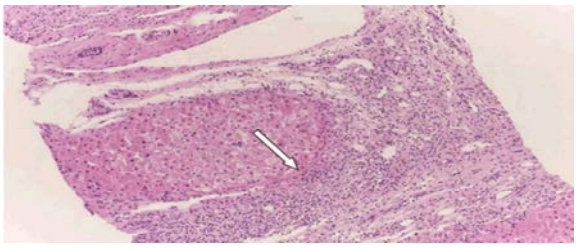

Fig. 2: Chronic hepatitis with moderate activity and stage 6 (complete cirrhosis) - By Ishak system - (arrow shows regenerating nodule & moderate inflammation) (H&E X200).

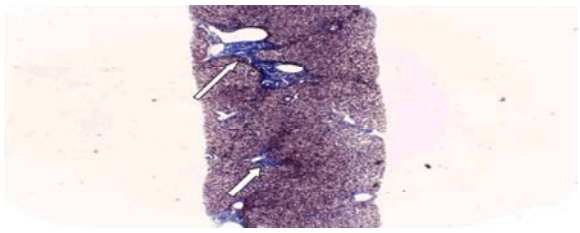

Fig. 3: Shows stage 3 fibrosis - By Ishak staging - (arrows show fibrous expansion of most portal areas with occasional portal to portal (P-P) bridging) (Masson Trichromex100).

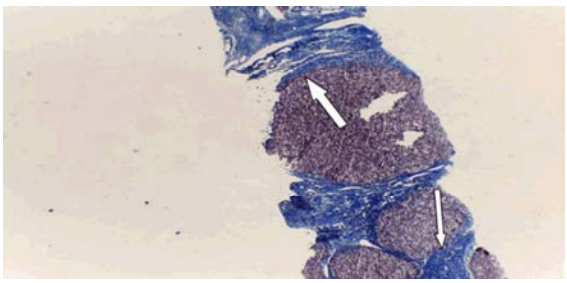

Fig. 4: Shows cirrhosis stage 6 - By Ishak staging - (arrows show thick fibrous septa with regenerating nodules) (Masson Trichrome X 100).

levels were 50 U/L, 42.5 U/l and 84.6 U/l, respectively. Mean PCR was 405104.4 IU/ ml. Most of patients (76.7%) had mild degree of hepatitis according to Ishak classification (Fig. 1), followed by moderate degree of hepatitis presented in 11.11% of cases (Fig. 2 and Table 1). The majority of cases showed moderate degree of fibrosis according to Ishak staging system (48.9%) (Fig. 3), while only 12.2% of cases showed marked fibrosis (Fig. 4 and Table 2). Most of the cases (41.11%) showed ALT level below 40 IU/L. The majority of cases (95.6%) showed AST level below 80 IU/L. AP level below 130 IU/L were detected in 94.4% of cases. PCR level below $2x10^5$ 10^8IU/ml was seen in 61.11% of cases (Table 3).

Serum ALT Level correlated with the grades of liver necroinflammatory activity, but not the stage (Table 4). Serum AST Level correlated with the grades of liver necroinflammatory activity but not the stage (Table 5). Alkaline phosphatase levels correlated with neither the grades of liver necroinflammatory activity nor the stage of liver fibrosis (Table 6). HCV-RNA titre correlated with neither the grades of liver necroinflammatory activity nor the stage of liver fibrosis (Table 7).

Table 1: Distribution of studied patients as regards Ishak Grade.

Total Ishak grade	No. of cases	Percentage %
Minimal (1-4)	5	5.56
Mild (5-8)	69	76.67
Moderate (9-12)	10	11.11
Marked (13-18)	6	6.67

Table 2: Distribution of studied patients as regards Ishak stage.

Ishak stage	No. of cases	Percentage %
(0-2)	35	38.9
(3-4)	44	48. 9
(5-6)	11	12.2

Table 3: Biochemical and serological parameters of chronic hepatitis C patients.

ALT	No. of cases	Percentage %
<40	37	41.11
40-80	34	37.78
>80	19	21.11
AST	No. of cases	Percentage %
<37	43	47.78
37-80	43	47.78
>80	4	4.44
AP	No. of cases	Percentage %
<130	85	94.44
>130	5	5.56
PCR	No. of cases	Percentage %
Undetected	2	2.22
$<2x10^5$	55	61.11
$2x10^5$-$2x10^6$	31	34.44
$>2x10^6$	2	2.22

Table 4: Correlations of ALT with Ishak parameters.

	ALT	
Ishak parameters	R	P- value
Ishak stage	0.158	0.138
Total Ishak grade	0.275	0.009*

Table 5: Correlations of AST with Ishak parameters.

	AST	
Ishak parameters	R	P-value
Ishak stage	0.165	0.120
Total Ishak grade	0.264	0.012*

Table 6: Correlations of Alkaline Phosphatase with Ishak parameters.

	AP	
Ishak parameters	R	P-value
Ishak stage	0.054	0.616
Total Ishak grade	0.009	0.934

Table 7: Correlations of PCR with Ishak parameters.

	PCR	
Ishak parameters	R	P-value
Ishak stage	0.052	0.628
Total Ishak grade	0.081	0.446

DISCUSSION

Our study showed that serum levels of ALT and AST correlated with Ishak grades of liver necroinflammatory activity, but not with the stage of liver fibrosis (Tables 4 and 5). In agreement with our results, Zechini *et al.* [14] suggested that a serum ALT and AST level, especially the AST level was associated with liver necroinflammatory activity. Likewise, Luo *et al.* [15] confirmed a statistically significant correlation between AST and ALT serum levels and the grading of necroinflammatory changes. Similar studies were done by Bacon [16] and Alberti *et al.* [17] demonstrated one-third of the patients with chronic hepatitis C showed normal ALT levels at the time of diagnosis and it remained normal during years of follow up, but the histological results indicate some of these patients presented with liver fibrosis that progressed into liver cirrhosis despite normal or nearly normal serum ALT levels. These findings suggested that serum ALT levels could not predict the histological damage of livers infected with HCV, which is in agreement with the results of our study in that the level of serum ALT was not markedly related to the stages of liver fibrosis. Thus, though a serum ALT level can correlate with the grades of liver necroinflammatory activity, it cannot serve as a parameter to assess the liver damage of patients with chronic hepatitis C. It is not easy to explain the reason for the phenomenon that patients present with severe liver damage though the ALT levels are normal or nearly normal. In general, ALT levels are released by direct virus-related cytopathic activity and/or by an immune-mediated process. Some studies suggested that the cellular immune response in patients of HCV infection with persistent normal ALT levels was less activated than those with abnormal ALT levels [18].

Another way to examine the relationship between ALT level and liver histopathology is to analyze the relationship between the specific level of ALT, histological diagnosis and the subcomponent histological features. When this was assessed retrospectively in a study done by Haber *et al.* [19] involving 90 biopsy specimens from patients with chronic hepatitis C, ALT levels were not predictive of severity of histological diagnosis.

As for Alkaline Phosphatase levels our study showed that they correlated with neither the grades of liver necroinflammatory activity nor the stage of liver fibrosis (Table 6).

Contradictory to our results, Luo *et al.* [15] confirmed a statistically significant correlation between AP serum levels and the grading of necroinflammatory changes, as well as a significant correlation between AP and the stage of liver fibrosis. Our results were also in contrast with another study done by Iwona [20] showed that AP levels correlated with the stage of liver fibrosis.

In this study, there was no significant correlation found between circulating HCV RNA titers and the grades of liver necroinflammatory activity or the stage of liver fibrosis (Table 7). HCV-RNA titer correlated with neither the grades of liver necroinflammatory activity nor the stage. Other study agreed with our findings, which was done by Puoti *et al.* [21], who stated that the severity of liver damage and the clinical features had no correlations with HCV load or HCV genotype. However on the contrary, Adinolfi *et al.* [22] concluded in their study that serum HCV-RNA titer correlated with the severity of liver damage, which could be accelerated by high HCV load and fat degeneration. Many factors may account for the discrepancies between these studies. *First* the test that

was used to quantitate HCV RNA was different from one study to another. *Second,* because serum HCV load fluctuates, thus being an unstable parameter, it cannot reflect the degree of liver damage in a given subject [23]. *Third,* HCV replicates in extra-hepatic sites as well as within the liver [24]. Thus a circulating HCV overload does not always imply an active state of viral replication in neither the liver, nor a severe degree of liver damage. *Last,* the discrepancy may result from the time interval between ALT and HCV RNA detection and liver biopsy performance. However, it is worth mentioning that in our study both ALT test and HCV RNA titer were drawn on the same day with the liver biopsy.

The almost universal finding of HCV RNA elevation in patients with liver disease and its absence in individuals with normal biopsy specimens have led some to question whether the level of HCV RNA might be predictive of the degree of liver injury. Most studies that have used branched DNA testing have not been able to detect any relationship between the level of HCV RNA and the grade or stage of hepatitis. In contrast, several studies using reverse-transcription polymerase chain reaction have found a correlation with liver histopathology. Thus, testing of the level of HCV RNA in serum is not clinically useful to distinguish between various grades of histological injury or to monitor disease progression. Although the reasons for the lack of correlation between liver histopathology and these blood test parameters remain unclear (further studies are needed to explain), one explanation may be that the former is shaped by dynamic events such as immunologic activity, frequency and intensity of exacerbations and the total duration of disease, as well as more static factors such as the route of transmission and viral genotype. Evaluation of histological activity by alternative test methods such as ALT and HCV RNA, which are often performed on a limited number of samples, is likely to give misleading information about overall disease intensity in a chronic and dynamically changing condition like chronic hepatitis C [25].

CONCLUSION

Though serum HCV-RNA titer and ALT, AST levels are common tests as part of a routine evaluation of patients with chronic hepatitis C, it can be used to assess the response during and after treatment, but cannot reflect the histological liver change accurately. Liver biopsy remains an important tool in the appropriate selection of patients for whom therapy can successfully treat.

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