ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Role of Biomarkers, Immune Complements C3 and C4 and Alfa Fetoprotein Levels in Chronic Liver Disease in Egypt.

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ABSTRACT

Some of the population in Egypt has a heavy burden of liver diseases, mostly due to chronic infection with hepatitis C and B viruses and Bilharzias that is considered as important environmental risk factor, which can go unnoticed until decades after infection, when liver damage becomes evident. The aim of the present study was to investigate the impact of liver biomarkers, Alfa fetoprotein (AFP) and complements C3 and C4 as immune biomarkers in sera of patients suffering from chronic hepatitis C and B and bilharzias. The study population included patients attending the Armed Forces Medical Research Laboratories and Blood Bank. Individuals under investigation were divided into four groups comprising 30 individuals each. Group 1: included control healthy individuals, group 2: included patients with chronic HCV, group 3: included patients with chronic HBV and group 4: included patients with Bilharzias (BILZ) infected by *Schistosomiasis mansoni*. All patient groups were characterized by increase in all biochemical markers of liver function tests (AST, ALT and GGT) and reduction in albumin level in their sera as compared to the control group. The results also illustrated that in HCV and HBV groups, there were significant reduction in serum levels of complement system C3 and C4, these effects were associated with significant increase in AFP level compared to control group. In patients suffering from HCV, the RNA viral load as measured by quantitative PCR showed no statistically significant correlation with AST, ALT, GGT, AFP, C3, C4 and albumin. In conclusion the results of this study indicate it is advisable to estimate these biomarkers for the hope of early screening for population who are at risk for developing liver cancer.

Keywords: HCV; HBV; Bilharzias; Complement C3; Complement C4; AFP.

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ISSN: 0975-8585

INTRODUCTION

Schistosomiasis is a significant environmental health problem in more than 70 countries distributed between Africa, Asia and South America, with an infection rate of one in 30 individuals. Data on Schistosomiasis, hepatitis B virus (HBV) and hepatitis C virus (HCV) co-infection are scarce. However, there is a high prevalence in countries where schistosomiasis is endemic [1].

It is known that the major cause of death is primarily associated with cirrhosis in the liver as well as other conditions including liver failure, hematemesis from esophageal varices, hepatic encephalopathy and hepatocellular carcinoma. It was regarded that HCV may complicate the course of schistosomiasis and vice versa with a perhaps synergistic effect [2].

Waterborne diseases have been estimated to cause more than two million deaths and four billion cases of diarrhea annually. Waterborne pathogenic organisms include bacteria, protozoa and viruses. Heavy metal contamination of water is also a potential threat to human health [3]. Domestic activities such as washing clothes and fetching water in infected water expose women and children to infection. Recreational activities like swimming and poor hygiene also make children vulnerable to Schistosomiasis [4].

According to the World Health Organization there are 130 – 150 million people chronically infected with the hepatitis C virus (HCV), corresponding to 2-2.5% of the world's total population. There are considerable regional differences. The prevalence in Egypt is >10% [5]. Approximately 20-30% of chronically infected individuals develop cirrhosis over a 20 to 30 year period of time [6].

Alfa fetoprotein (AFP) has been regarded as the most useful serum protein thus far for patients at risk for hepatocellular carcinoma (HCC) [7]. However, its sensitivity for detecting HCC ranges between 25%-60% and its specificity is also low because serum AFP can also be detected in patients with cirrhosis (11%-47%) and chronic hepatitis (15%-58%) [8]. An increase in the serum concentration of AFP is primarily used as a tumor marker for HCC evaluation and liver cirrhosis [9].

The complement system is also involved in the pathogenesis of a variety of liver disorders, including viral hepatitis, liver injury and repair, fibrosis, alcoholic liver disease, and liver ischemic/reperfusion injury [10]. Complement components contribute to clearance of virus infections both directly and indirectly, contributing to lysis of enveloped virions [11] and virus infected cells, through the action of the C5-C9 membrane attack complex [12]. However, suppression of humoral innate immunity by HCV, and the role of these proteins in HCV pathogenesis are also considered [13].

SUBJECTS AND METHODS

The study was carried out on 120 male individuals who were divided into the following groups:

Group 1 (Control group): Including 30 clinically healthy individuals, they were healthy volunteers.
Group 2 (HCV): Including 30 patients infected with hepatitis C virus. They had HCV RNA in their sera as evidenced by polymerase chain reaction (PCR) (QIAsymphony DSP Virus/Pathogen kit, Sarstedt AG and Co.).
Group 3 (HBV): Including 30 patients infected with hepatitis B virus. HBV patients were diagnosed by positive hepatitis B surface antigen using qualitative detection by the chemiluminescent microparticle immunoassay (CMIA) technology [14] using the instructions provided by Abbot Ireland Diagnostics.
Group 4 (BILZ): Including 30 patients suffering from *Schistosoma mansoni* infestation. Bilharziasis patients were diagnosed by positive *Schistosoma mansoni* antibodies using the indirect hemagglutination test (IHA) [15]. The kit provided by the Siemens Healthcare Diagnostics, Germany.

Venous blood samples were withdrawn from patients and healthy persons. The samples were collected in dry sterile 5 ml tubes and were allowed to clot at 37°C for 15 minutes and centrifuged at 4000 rpm for 10 minutes, then, sera were separated. Sera of subjects of the controls and patients were subjected to quantitative determination of the following biochemical parameters according to kits instruction manual.

ISSN: 0975-8585

1- Liver function tests: Including AST, ALT, GGT and albumin were performed as follows: Alanine and aspartate aminotransferases and gamma glutamyltransferase [16] and serum albumin [17] were measured using the kit supplied by Roche Diagnostics /LTD, USA using Hitachi modular.

2-Alfa fetoprotein was determined by using a chemiluminescent microparticle immunoassay (CMIA) technology [18] using instructions provided by Abbott Ireland Diagnostics using vitrous 5600.

3-Serum complement C3 and C4 concentrations were estimated by immunonephelometry according to **Thomas** [19] using commercially available kit provided by Siemens Healthcare Diagnostics, Germany using Behring nephlometry prospect.

Statistical analysis

Results were expressed as mean ± standard error (SE). One way analysis of variance followed by *post hoc*-least significant difference analysis (LSD) was performed using the statistical package for social science (SPSS) version 16 to compare all the studied groups. Pearson's correlation coefficient (r) was used to find the degree of correlation between RNA viral load and either of biochemical parameters estimated in serum of HCV patients. The values of $P<0.05$ were considered significant.

RESULTS

There was no significant difference between ages of control and patient groups, as their mean ages were as follows: group 1 (48.9 ± 0.69), group 2 (50.1 ± 0.82), group 3 (48.8 ± 0.62) and group 4 (48.7 ± 0.35).

Table (1), illustrates that transaminases AST and ALT levels were significantly elevated in the serum of patient groups as compared to normal control (group 1). The increase of AST in HCV group was more evident and amounted to 160.93% of the normal control group. However, the mean ± SE recorded (38.85 ±2.27 U/L for AST) and (33.54 ± 1.92 U/L for ALT) in patients with bilharzias, does not exceed the accepted upper level of reference range which is up to 40 U/L.

Table (1): Levels of serum aspartate and alanine aminotransferases (AST and ALT), gamma glutamyltransferase (GGT) and concentration of serum albumin in different experimental groups

Groups	Parameters			
	AST (U/L)	ALT (U/L)	GGT (U/L)	Albumin (g/dl)
Group 1 Mean ±SE.	25.83±1.00	25.90±1.32	35.90±1.58	4.48±0.1
Group 2 (HCV) Mean ±SE. % change from control	67.40±4.27[a] 160.93%	48.71±2.47[a] 88.06%	72.25±6.98[a] 101.3%	3.29±0.11[a] -26.56%
Group 3 (HBV) Mean ±SE. % change from control	55.96±1.73[ab] 116.6%	51.78±2.00[a] 99.92%	40.48±2.07[b] 12.76%	4.40±0.13[b] -1.78%
Group 4 (BILZ) Mean ±SE. % change from control	38.85±2.27[abc] 50.4%	33.54±1.92[abc] 29.49%	62.93±3.86[ac] 75.29%	3.90±0.17[abc] -12.95%

Letters a, b & c represent statistical differences with groups 1, 2 & 3 respectively at $P< 0.05$.
Group 1, normal control; Group 2, patients with hepatitis C virus; Group 3, patients with hepatitis B virus & Group 4, patients with Bilharzias.
The reference ranges are up to 41 U/L, up to 40 U/L, 8-61 U/L and 3.97-4.94 g/dl for ALT, AST, GGT & albumin concentration respectively.

ISSN: 0975-8585

It was found that HCV caused significant increase and decrease in the AST level and albumin concentration respectively as compared to HBV and bilharzias patients. Furthermore, HBV patients had higher value of ALT than bilharzias patients (51.78 ± 2 vs. 33.54 ± 1.92 U/L).

The results also indicates that there are significant decrease of serum albumin and increase of serum GGT level in HCV (group 2) and bilharzias (group 4) as compared to normal control. Albumin concentration and GGT levels estimated in serum of HBV patients (group 3) are found to be within reference range (3.97- 4.94 g/dl) and (8-61 U/L) respectively with mean values ± SE (4.40 ± 0.13 g/dl) and (40.48 ± 2.07 U/L) respectively.

It was found that when HBV compared to bilharzias group, there was a significant increase in the levels of serum AST and ALT and serum albumin concentration. Furthermore, the mean value of ALT which recorded in patients with HCV tended to have non significant decrease as compared to HBV patients and significant increase as compared to patients with bilharzias.

Table (2) represents that the concentration of Alfa fetoprotein was significantly increased in the sera of all disease groups compared to control, and the increase was evident in the HCV group reaching to 883.88% of the control. With the knowledge that the mean ± SE of AFP (6.09 ± 0.67 ng/ml) estimated in group 4 was near the upper clinically reference range (0.0 -7.0 ng/ml). AFP was significantly higher in HCV patients and HBV patients compared with bilharzias group and also in HCV group compared with HBV group.

The percentage decrease in the levels of C3 and C4 complements was -52.46% and -57.89% respectively in the serum of HCV patients compared to control (group 1). The mean ± SE for C3 and C4 measured in the sera of HBV and bilharzias patients showed normal reference range. The sera from patients infected with HCV and HBV displayed significantly lower C3 complement than sera from bilharzias patients. Also levels are decreased in HCV patients compared with HBV patients.

Table (2): Concentration of serum Alpha fetoprotein (AFP) and levels of complements 3 and 4(C3 and C4) in different experimental groups:

Groups	Parameters		
	AFP (ng/ml)	**C3 (mg/dl)**	**C4 (mg/dl)**
Group 1 Mean ±SE.	2.42±0.19	139.71±2.92	20.33±1.11
Group 2 (HCV) Mean ±SE. % change from control	23.81±1.45[a] 883.88%	66.41±3.61[a] -52.46%	8.56±0.57[a] -57.89%
Group 3 (HBV) Mean ±SE. % change from control	18.53±0.92[ab] 665.7%	101.94±3.14[ab] -27.03%	9.90±0.48[a] -51.3%
Group 4 (BILZ) Mean ±SE. % change from control	6.09 ±0.67[abc] 210.3%	138.93±5.12[bc] -0.56%	27.14±1.48[abc] 33.5%

Letters a, b & c represent statistical differences with groups 1, 2 & 3 respectively at $P< 0.05$.
Group 1, normal control; Group 2, patients with hepatitis C virus; Group 3, patients with hepatitis B virus &Group 4, patients with Bilharzias.
The reference ranges are 0.00 -7.00 ng/ml, 90- 180 mg/dl and 10-40 mg/dl for AFP, C3& C4 respectively.

When the HCV Ab-positive patients (group 2) were subjected to testing of HCV RNA viral load by quantitative PCR technique, it was found that 30% of the patients had low titer (HCV-RNA up to 200,000 IU/ml), 60% had moderate titer (HCV-RNA 200,000 to 1,000,000 IU/ml) and 10% had higher titer (HCV-RNA greater than 1,000,000 IU/ml). The study revealed also that there were no statistically significant correlation between RNA viral load as measured by quantitative PCR with either AST (P = 0.762, r = 0.076); ALT(P = 0.932, r = 0.253); GGT (P =

0.793, r = 0.117); AFP (P = 0.394, r = -0.072); C3(P = 0.716, r = 0.122) ; C4 (P = 0.194, r = 0.008) or albumin (P = -0.80, r = 0.079).

DISCUSSION

From the current study, table (1), it was found that there is a significant increase in the levels of aminotransferases (AST and ALT) in the serum of patients with hepatitis C (group 2) and hepatitis B (group 3) viruses. The awareness of hepatic intracellular enzymes that have leaked into the circulation was indicated by significant elevation of ALT and AST. These are the markers for hepatocellular damage [20].

In this study, the levels of aminotransferases (AST and ALT) in the serum of patients with bilharizias recorded (33.54 ± 1.92 U/L and 38.85 ± 2.27 U/L respectively) were at the upper normal reference range, but still significantly higher than control. This is accordance with Leite et al. [21] who reported that the patients with hepatosplenic schistosomiasis showed abnormal liver function tests compared to the healthy controls with significantly increased levels of serum AST and ALT. It was reported that infection with *S.mansoni* may be a possible risk factor for HCC which is considered the fifth most common cancer in the world [22].

The present study revealed that, there is no significant correlation at (P<0.05) when comparing RNA viral load detected by the quantitative PCR technique with either AST, ALT, GGT ,C3,C4, AFP or albumin which was measured in the serum of patients suffering from HCV (group 2). The study of Abraham et al. [23] showed that the viral load was independent of ALT level in HCV. Also at the same time they observed no significant correlation between HCV RNA viral load and AST levels. The present data agreed with the results obtained by Noreldin et al. [24] who found that high results of liver function tests may be indicator for the severity of liver damage in chronic HCV patients but also PCR should be done as some cases show normal results while their RNA viral load were high.

Moreover, result in table (1) indicates that HCV, HBV and bilharizias led to decrease in the concentration of serum albumin when compared to healthy people. In disease states, hypoalbuinaemia is secondary to decreased human serum albumin production or transcapillary leakage into the interstitial space. A previous study [21] demonstrated a lower concentration of albumin in serum of patients with hepatosplenic schistosomiasis. Also, the function of human serum albumin is impaired in patients with cirrhosis [25].

In the present data, and in relation to normal control, although, there is significant elevation in serum GGT level, the non significant elevation was only in HBV patients. Previous studies identified GGT as a prognostic marker of fibrosis in chronic liver diseases [26] and schistosomiasis [27]. The current results were matched with results of Hui et al. [28] who reported that, the chronic hepatitis which is caused by the hepatitis B and C viruses is associated with high GGT levels, which can be used as a noninvasive diagnostic marker and as a predictor of fibrosis.

It is obvious that HCV and HBV patients demonstrated pronounced elevation in the AFP concentration compared to bilharizias patients, table (2). Zakhary et al. [29] revealed that benign liver diseases such as schistosomiasis, hepatitis and cirrhosis showed significant increase in AFP, but it was still significantly lower than that of HCC.

Beside to the elevation of AFP in patients with HCV and HBV than bilharizias patients, ALT elevation was seen in the current study. The present results are in line with the findings of Li et al. [30] who suggested that, higher serum levels of AFP and ALT were risk factors associated with the development of HCC, regular monitoring of these serum markers in hepatic cirrhosis patients is necessary.

However, when comparing patient groups with normal persons in the present study, it was found that patients with HCV showed significant reduction in serum C3and C4. They reached -52.46 % and -57.89% respectively as compared to control. Although, HBV significantly reduced C3 and C4 relative to normal control but still at low normal reference range, The complement C4 levels were observed to be increased in the bilharizias patients relative to control where the increase still within normal reference range (10- 40 ng/dl).

The complement system is one of the most important weapons of innate immunity and is involved in all infectious processes. It is not only a mechanism for direct protection against an invading pathogen but it also interacts with the adaptive immunity to optimize the pathogen-specific humoral and cellular defense cascade in the body [31]. Lower complement levels were closely correlated with liver injury and impaired liver aggregation [32].

A previous study found that C3and C4 complements decreased significantly in cirrhotic patients. The authors suggested that the reduction by alternative pathway can be the result of an increased consumption or a decreased production due to liver insufficiency [33]. Furthermore, Pasha et al. [34] reported that, the levels of complements C3 and C4 in liver of cirrhotic patients were grossly decreased when compared with controls.

CONCLUSION

The measurement of serum albumin, immune complements (C3 and C4) and AFP may be used to increase the overall diagnostic accuracy in hepatitis, as the early and accurate diagnosis of hepatitis is necessary for the proper management of the disease.

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