Original Article

ASSOCIATION OF ALPHA PROTEIN LEVELS WITH THE SEVERITY OF VIRAL DISEASE IN PATIENTS WITH HBV AND HCV INFECTION

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ABSTRACT

Objective: To determine association of alpha-fetoprotein (AFP) levels with presence and severity of disease in patients with CLD due to HBV and HCV viral infection.

Material and Methods: This observational study was carried out in the Chemical and molecular Pathology Department, Sheikh Zayed Hospital and Medical College, Rahim Yar Khan from between 2016-2018. The study was conducted by collecting the clinical and laboratory data of patients with chronic liver disease as a part of routine management attending our hospital. We collected the data of 230 study subjects including eighty-five females and one hundred and forty-five males. All of them were having chronic liver disease which was diagnosed by ultrasonography and referred to pathology laboratory for their routine Liver Function Tests, serology for hepatitis B and C, follow up investigation along with Alpha-feto protein (AFP) levels and viral load. Twenty-five females and same number of males as disease free controls were also included.

Results: Serum levels of AST, ALT and alkaline phosphatase (ALP) were found to be significantly higher in hepatitis patients with Chronic Liver Disease (CLD) as compared to control subjects. Similarly, total and direct bilirubin were also observed to be elevated in study subjects as compared to controls. Total proteins and globulins were higher while serum albumin was lower in study group as compared to control subjects (p<0.05). All the laboratory parameters showed significant difference statistically (p<0.005) except for globulin.

Mean viral load in patients with HCV (n=130) was found to be $2.2x10^6$ IU/mL while in patients with HBV (n=100) it was $4.1x10^5$ IU/mL. A significant positive correlation (r=0.82) was found between viral load (estimated by quantitative PCR) and AFP in HBV positive subjects. No significant correlation was found between viral load and AFP in hepatitis C positive patients.

Conclusion: Serum AFP levels were high in CLD patients with HBsAg positive along with the high viral load. Serum AFP could be helpful in detecting progression in patients with HBV.

Key Words: Alpha-feto protein, HBV, Hepatocellular carcinoma, Viral load, Chronic Liver Disease

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INTRODUCTION

Alpha fetoprotein is a glycoprotein which is produced in the embryonic life by the yolk sack and fetal liver cells. It becomes undetectable gradually in about eight to twelve months after the birth. Elevated levels of AFP are usually found in liver disease, germ cell tumors and in hepatocellular carcinoma [1,2].

Viral hepatitis is one of the major causes for the development of hepatocellular carcinoma (HCC), it is observed that chronic hepatitis due to HCV affects more than 170 million people all over the world and among them 50 % of the individuals usually also have steatosis at this stage [3,4]. Globally it is well known that HBV infection is the major causative factor of mortality from end stage viral diseases and also carries risk for cirrhosis and HCC (30-70 years of age) almost as high as 41.5 % and 21.7 % respectively [5].

Almost 50 % of all cases of HCC and all cases of early age HCC are because of chronic hepatitis due to HBV viral infection [2]. Due to the increasing public awareness and following strategic scheme of hepatitis B vaccination program, there is a gradual decline in the prevalence of chronic HBV infection but still it is a serious public health issue all over the world [6]. Due to inadequate medical resources in Eastern Asia and Sub-Saharan African regions, the burden of disease is high. According to the updated statistics, about 240 million people worldwide are infected with chronic HBV [7].

The use of serum AFP is well documented for the diagnosis, screening and monitoring of heptocellular carcinoma but its level can also be

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increased in certain malignancies other than liver and also in hepatitis which may be acute or chronic in nature [8]. The serum levels of AFP have been reported previously to increase during regeneration of hepatocytes following resection or necrosis [9]. It has also been observed that ALT levels have direct correlation with serum AFP levels in patients suffering from recurrent exacerbation of chronic hepatitis with histological evidence of bridging necrosis on liver biopsy. The prevalence of high serum AFP levels has been observed from 10 % to 42 % in chronic hepatitis C patient [10].

The present study has been planned to study the correlation of the serum AFP levels with viral load. We also observed the relationship between the concentration of AFP levels with gender and severity of CLD in the study population.

MATERIAL AND METHODS

The study was conducted by collecting the clinical and laboratory data of patients with CLD as a part of routine management attending our hospital. The study was carried out after taking the ethical approval from the institutional review board. We collected the data of 230 study subjects including eighty-five females and one hundred and forty-five males. All of them were having CLD which was diagnosed by ultrasonography and referred to pathology laboratory for their routine LFTs, serology for hepatitis, follow up investigation along with Alpha protein levels and viral load. We also included age and gender matched twenty-five females and same number of males as disease free controls. Patients with diabetes mellitus, hypertension and any other infection (HIV) or coinfection (HDV) were excluded. A specific proforma was designed to keep the record of all the patients included in the study.

Hepatitis B surface Antigen (HBsAg) and anti-hepatitis C viral load was detected by RT-PCR, by using the prescribed protocol given in the kit. The amplified product was detected by using fluorescent dyes in RT-PCR, which were linked to the oligonucleotide probes to bind specifically with the amplified product and then the fluorescent probes were detected after each replication cycle. The lower detection limit of the assay used was 5.0×10^2 IU/ml while its higher detection limit was 5.0×10^8 IU/ml. Their health records were searched for diagnosis, age, sex and other medical history of the patients at the time of presentation. A specific laboratory code was assigned for each patient so that no name or identifier other than age, sex appears in our record. AFP level was assayed using ELECSYS e411 by Roche diagnostics, Switzerland. The principle of the technique is based on Electrochemiluminescence immunoassay. HBV and HCV viral load was detected using quantitative Real time RT-PCR. While the liver functions tests were evaluated using Beckman Coulter AU-680 USA. Twenty-five males and 25 females of similar ages were used as controls. They were recruited in 2017 from disease-free staff and students of the hospital, who had no evidence of CLD based on history and normal laboratory findings.

Statistical Analysis: Data was entered in SPSS version 22 and descriptive statistics was carried out like the frequency distribution etc. Inferential statistics like Independent sample-t-test for parametric data was used for statistical comparison of the results. Pearson`s Correlation was used to study the correlation between AFP levels and viral load.

RESULTS

There were 145 male (63.04 %) and 85 females (36.96 %) with age ranged from 30 to 70 with a mean of 43 ± 6.5 years. Hundred (43 %) out of 230 subjects were seropositive for HbsAg while 130 (57 %) were seropositive for Anti HCV. The control subjects were negative for both HBsAg and Anti HCV. The mean AFP level of the study patients was 229 ± 14 ng/mL while the mean of control was 10± 11 ng/mL ($p < 0.01$)

The mean (AST) activity was 147 ± 18 µ/L, while the mean control value was 10.8 ± 12 µ/L. The means (ALT) and alkaline phosphatase (ALP) activities were 85 ± 12 µ/L and 176 ± 15 µ/L, respectively, while the means activities in control subjects were 14 ± 10 µ/L and 60±12 µ/L, respectively. The means total bilirubin and direct bilirubin were 78.7 ± 6.8 µmol/L and 21.0 ± 4.1 µmol/L, while the means of the control subjects were 10.6 ± 1.2 µmol/L and 4.2 ± 1.0 µmol/L, respectively. The means total protein, albumin and globulin were 55.6 ± 3.6 g/L, 28.6 ± 1.9g/L and 20.6 ± 2.8 g/L, respectively, their mean control values were 65.2 ± 1.1 g/L, 42.1 ± 1.1 g/L and 21.9 ± 1.2 g/L. Statistically significant differences were observed in all the parameters ($p< 0.001$) except for globulins. Mean viral load in patients with HCV (n=130) was found to be $2.2x10^6$ IU/mL while in patients with HBV (n=100) it was $4.1x10^5$ IU/mL. A significant positive correlation ($r =0.82$) was found between viral load (estimated by quantitative PCR) and AFP in HBV positive subjects. No significant correlation was found between AFP levels and viral loads in HCV positive subjects.

Table-1: Clinical and Biochemical characteristics of study subjects with CLD and disease-free controls. (mean ± SEM).

Variable	Study subject	Control	
Age (years)	45±6.5	42±2.5	
Number of subjects	230	50	
HbsAg +ve	100 (43 %)	0(0%)	
Anti HCVAb +ve	130 (57%)	0(0%)	
AFP (ng/ml)	229±14	10±11	<0.001
AST(U/L)	147±18	10.8±12	p<0.001
ALT (U/L)	85±12	14±10	p<0.001
ALP(U/L)	176±15	60±12	p<0.001
Total Bilirubin(µmol/L)	78.7±6.8	10.6±1.2	p<0.001
Direct Bilirubin(µmol/L)	21±4.1	4.2±1.0	p<0.001
Total Protein	55.6±3.6	65.2±1.1	p<0.001
Albumin(g/L)	28.6±1.9	42.1± 1.1	p<0.001
Globulin(g/L)	20.6±2.8	21.9±1.2	p>0.001

Table-2: Gender distribution of HBV and HCV infection.

Gender	Total	HBV positive	HCV Positive
Male	145	59 %	55.3 %
Female	85	41 %	44.7 %

Table-3: Laboratory parameters of chronic liver disease patients (mean ± SD).

Variables	HBV positive CLD group	HCV positive CLD group	P-value
AFP(ng/ml)	205±34	155±15	<0.0001
AST(IU/L)	120±12	85±24	<0.005
ALT(IU/L)	80±20	75±13	0.07
ALP(IU/L)	130±28	1125±30	0.86
Total bilirubin (mg/dl)	2.3±1.1	2.0±2.0	0.85
Albumin (g/L)	23±10	21±15	0.05

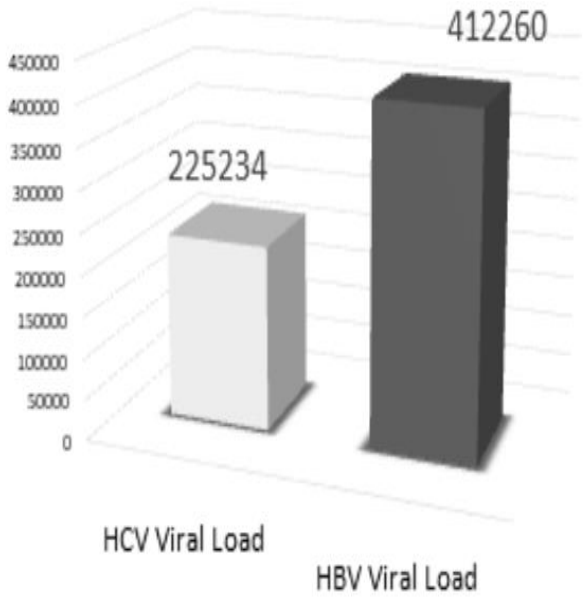

Figure-1: Viral load IU/mL in HCV and HBV cases

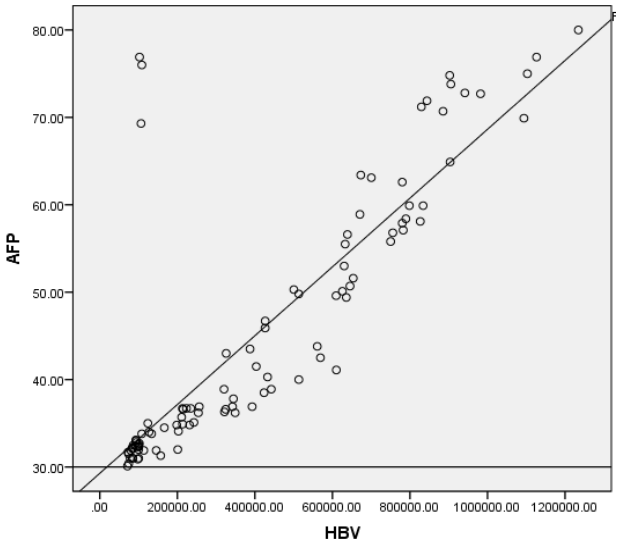

Figure-2: AFP and viral load correlation in HBV subjects (n=100)

DISCUSSION

Alpha fetoprotein is a 70 KDa-glycoprotein which is usually not found in disease free adults. It has role as tumor marker and used as a biomarker for hepatocellular carcinoma and other gastrointestinal tumors. It is normally produced in fetal life by liver and yolk sac. Increased AFP levels are also observed in patients with viral hepatitis, without hepatocellular carcinoma or cirrhosis. In western countries prevalence of AFP is found to be between 10 to 40 % in patients with chronic hepatitis [11]. The mean AFP level (Table-1) in the present study patients were found to be 159±9.9 ng/mL while the mean of control was 1.93±0.24 ng/mL ($p < 0.01$) as the study population were the patients with chronic liver disease. Though increased level of AFP may also be found in certain benign conditions like hepatic necrosis, cirrhosis, acute and chronic hepatitis, pregnancy and ataxia-telengectasia for which the underlying mechanism can be altered hepatocyte-hepatocyte interactions and loss of normal architecture arrangement following liver injury resulting in elevated AFP levels [12,13].

The result indicated that 57 % of the study subjects (Table-1) were positive for HCV antibody. Though the epidemiological data suggests that in contrast to the common belief of association of HCC with HBV infection as major etiological factor, HCV is the most common cause of HCC and CLD in countries with high prevalence rate of HCV such as Pakistan and Egypt [14].

HBV was found to be positive in 43 % of patients with CLD in our study. The prevalence of HBV observed in different regions of Punjab showed the highest rate in district Rahim Yar Khan (8 %) followed by Liaqat

Pur (6.99 %), Pak Patten (5.32 %) and Gujranwala (5 %). While lowest prevalence was observed in Lahore (2.3 %) and Depal Pur (3 %) [15].

This alarming rise in the prevalence of HBV in many districts of Punjab including Rahim Yar Khan is attributable to many factors like illiteracy, lack of awareness about communicable diseases, poor socioeconomic status, unsafe transfusions of blood and increases in intravenous drug abuse.[16]Data from another study reported the risk factors of HBV infection in people exposed to unsafe dental and general surgery, repeated use of syringes by the quacks and non-hygienic practices at the barber shops can be the possible factors for the transmission of infection [17].

In our study the levels of AFP were significantly high in patients with CLD due to HBV or HCV infections as compare to the control subjects which further signifies the utility of this biomarker for not only the diagnosis of chronic liver disease but also for its follow up. While it is considered as a predictive marker for the development of hepatocellular carcinoma in patients with cirrhosis [18,19].

Moreover, AFP levels in this study were found to be more elevated in patients with HBV infection as compare to HCV patients along with markers of hepatic inflammation (i.e. transaminases AST/ALT ratio and elevated AST, ALT) These findings can predict the aggressive behavior of the diseases process and its outcome in term of survival rate. These findings are in agreement with the studies carried out recently [20,21].

Mean AFP levels and viral load were also found to be high in patients with HBV infection in this study than in patients with HCV. These results are consistent with the study carried out by M. E. Mendy et al [22] showing that persistently elevated HBV-DNA levels may be used as a predictive marker for the development of chronic liver disease [23] or HCC and supporting the premise that these markers can be useful and durable for the estimation of the risk of the disease progression [24].

CONCLUSION

Since serum AFP levels were high in CLD patients with HBsAg positive along with the high viral load. Serum AFP could be helpful in detecting progression in patients with HBV viral infection. With this observation it can be suggested that chronic liver disease patients may shall be tested not only for the seromarkers but also for the HBV DNA viral load along with AFP levels for the early risk prediction of hepatocellular carcinoma.

AUTHORS CONTRIBUTION

S.Sabahat Haider: Design, data acquisition, analysis and final approval.

Farheen Aslam: Literature review & statistical analysis.

Muhammad Shahbaz Hussain: Sample analysis and literature review.

Muhammad Tariq Ghafoor: Final review and approval of manuscript.

Muhammad Abdul Rehman: Director & incharge of project.

Muhammad Ali Malik: Data collection & entry of Data in SPSS.

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