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Relation of Alpha-Fetoprotein to HCV and HBV in patients with chronic liver disease

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ABSTRACT

Objectives: To determine the frequency of Hepatitis B and C patients, as well as to determine whether elevated serum alpha-fetoprotein (AFP) levels were associated with hepatitis B and C infection.

Methods: A cross-sectional study was conducted between March 19th and June 29th, 2021, in Kirkuk City. A total of 86 Hepatitis B and 24 Hepatitis C patients were included in the study. All patients were referred to the Hepatology and Gastroenterology centers in Kirkuk for treatment. Each patient had 5 milliliters of blood drawn via venipuncture to determine AFP levels, liver function, Hepatitis B Surface Antigen (HBsAg), and the presence of Hepatitis C antibodies. Tests were performed using these blood samples.

Findings: Serum alpha-fetoprotein (AFP) levels were found to be significantly associated with Hepatitis B and C infections. The study showed that AFP levels were highest in patients with chronic liver disease (CLD) who tested positive for Hepatitis C antibodies (849 ng/mL), followed by those with Hepatitis B surface antigen (HBsAg) positivity (279 ng/mL). AFP levels were much lower in those without hepatitis infections (16.3 ng/mL). This suggests that elevated AFP levels are strongly correlated with HCV infection, with a notable difference between HCV and HBV infections, and lower levels in hepatitis-negative patients.

Conclusions: In conclusion, serum AFP level was higher in CLD patients with HCV antibody positive compared to HBsAg positive CLD and hepatitis negative patients.

KEYWORDS: HCV; HBV; AFP; ALT

1. INTRODUCTION

Even though hepatitis B virus (HBV) is the most common cause of hepatitis, hepatitis C virus (HCV) is rapidly emerging as an infection requiring attention.1HCV is a major cause of acute and chronic liver diseases. Several authors have reported the prevalence of these viruses among chronic liver disease (CLD) patients in various centers.[1-6] These studies have shown wide variations in the prevalence rates of these viruses among chronic liver disease (CLD) and hepatocellular carcinoma (HCC) patients. Alpha-fetoprotein (AFP) is a clinical serum marker for the diagnosis of HCC. Elevated AFP levels were also found in patients with viral hepatitis without HCC, especially in those with chronic hepatitis C and liver cirrhosis. Although there is no accurate record of the yearly incidence of infection in this environment, an estimated 150,000-450,000 individuals are infected yearly in the United States of America. [7-10]

Serum Alpha-fetoprotein (AFP) is routinely used as a marker of HCC in patients with CLD. However, sserum AFP is also elevated in some non-hepatic malignancies and conditions such as acute and chronic hepatitis[8,9], and may also be elevated in patients with CLD due to hepatitis B and C infection. The frequency of elevated serum AFP in CLD patients may vary according to the hepatitis genotype infection.[11]

The objective of this study is to evaluate the frequency of hepatitis B and C patients and to correlate the levels of elevated serum AFP with hepatitis B and C infection.

2. METHODS

A cross-sectional study was carried out in Kirkuk City from March 19th to June 29th, 2021. The number of hepatitis patients under study was: 86 for hepatitis B and 24 for hepatitis C. These patients were admitted to the Hepatology and Gastroenterology centers of Kirkuk. Each patient enrolled in this study had 5 milliliters of blood drawn through a vein puncture with a 5-milliliter syringe. AFP, liver function tests, hepatitis B surface antigen (HBsAg), and hepatitis C antibody tests were performed using blood samples placed into sterile test tubes and left for 30 minutes at 37 °C. After centrifugation at 3000 rpm for 15 minutes, the obtained plasma from the first tube was aspirated and transferred to Eppendorf tubes and stored in deep freeze at -20°C for biochemical and serological tests, including serum AF and liver function tests. Patients' medical records were searched for information on diagnoses, ages, genders, and other medical histories at the time of presentation.

3. RESULTS

There were 62 males and 24 females aged 40 to 71 years, with a mean of 46 ± 6.5 years. Thirty-six (41.7%) out of 86 subjects were seropositive for HBsAg, while 24 (27.9%) were seropositive for HCV antibody. Control subjects were seronegative for both HBsAg and HCV antibodies. The mean AFP level of the study patients was 359 ± 9.9 ng/mL, while the mean of the controls was 1.93 ± 0.24 ng/mL. The study shows that the highest mean AFP level was recorded among patients with chronic hepatitis B with stage four fibrosis (cirrhosis) (33.72 µg/L) and the lowest mean was recorded among patients without fibrosis. There was a strong positive correlation between AFP level and the stage of fibrosis (R:0.902) (Figure 1).

Table 1. Relation of AFP level with stages of fibrosis in patients with chronic hepatitis B

AFP level (µg/L)	Stages of fibrosis*					*P. value*
	0	1	2	3	4	
No.	14	7	7	12	8	
Mean	8.35	15.7	21.3	27.19	33.72	0.0001
SD.	2.92	1.51	3.02	3.34	2.53	

*Note. * 0: No fibrosis; 1: grade 1. 2:grade 2. 3: grade 3. 4: cirrhosis*

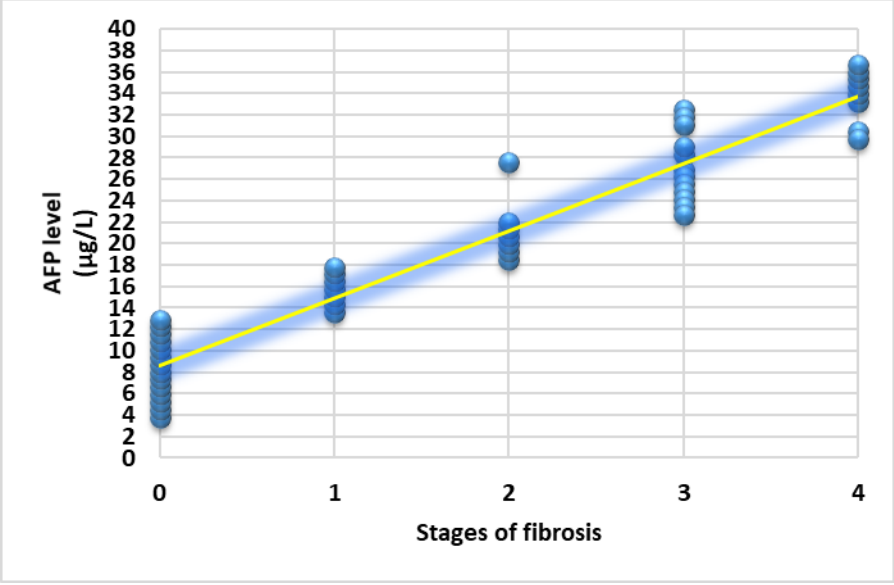

Figure 1. Correlation between AFP level and the stages of fibrosis in patients with chronic hepatitis B

The mean aspartate aminotransferase activity was 145 ± 1.8 u/L, while the mean control value was 10.8 ± 1.2 u/L. The means alanine aminotransferase and alkaline phosphatase activities were 75 ± 5.1 u/L and 176 ± 1.6 u/L, respectively, while the means activities in control subjects were 8.6 ± 1.2 u/L and 29±4.2u/L, respectively. The means of total bilirubin and direct bilirubin were 68.7 ± 6.8 µmol/L and 26.0 ± 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 50.6 ± 3.6 g/l, 29.6 ± 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 (Table 2).

Table 2. Compared variables between patients with CLD and Controls

Variables	Patients with CLD	Controls	P-value
Age (Years)	46 ± 6.5	41 ± 2.5	
Number of subjects	86	50	
No positive for HBsAg	36 (41.7%)	So (0%)	<0.0001
No positive for HCV antibody	24 (27.9%)	0(0%)	<0.0001
No negative for HBsAg and HCV	26 (18.6%)	0(0%)	<0.0001
AFP (ng/mL)	359 ± 9.9	1.93 ± 0.24	<0.0001
AST (u/L)	145 ± 1.8	10.8 ± 1.2	<0.0001
Alk. Phosphatase (u/L)	75 ± 5.1	8.6 ± 1.2	<0.0001
Total Bilirubin (µmol/L)	68.7 ± 6.8	10.6 ± 1.2	<0.0001
Direct Bilirubin (µmol/L)	26 ± 4.1	4.2 ± 1.0	<0.0001
Total Protein (g/L)	50.6 ± 3.6	65.2 ±1.1	<0.0001
Albumin (g/L)	29.6 ± 1.9	42.1 ± 1.1	<0.0001
Globulin	20.6 ± 2.8	21.9 ± 1.2	>0.05

Table 3 shows the clinical characteristics of chronic liver disease (CLD) patients by status of hepatitis B and C infection. The mean AFP level in the 36 subjects, who were seropositive for HBsAg, was 279 ± 20, while the mean level of AFP in CLD patients that were seropositive for HCV antibody was 849±23 ng/mL, and in those without hepatitis infection was 16.3 ± 3.6 ng/mL. The mean AFP level was highest ($P < 0.001$) in HCV-positive CLD patients followed by HBsAg-positive and those without hepatitis infections.

Table 3. Clinical findings of chronic liver disease patients by status of hepatitis B and C infection

Variables	Patients with HBsAg	Patients with HCV infection	Patients without Hepatitis infection
Number of subjects	36	24	26
AFP (ng/mL)	279 ± 20	849 ± 23	16.3 ± 3.6
AST (u/L)	131 ± 2.6	142 ± 2.0	109 ± 5.6
A(u/ L)	74 ± 6.2	73 ± 5.6	68 ± 4.8
Alk. Phosphatase (u/L)	168 ± 6.2	176 ± 5.2	146 ± 8.6
Total Bilirubin (µmol/L)	69 ± 5.1	66 ± 4.1	65 ± 6.0
Direct Bilirubin (µmol/L)	28 ± 6.1	26 ± 3.6	25 ± 4.1
Total Protein (g/L)	51 ± 4.1	50.2 ± 2.1	50.7 ± 3.1
Albumin (g/L)	29.1 ± 1.2	28.5 ± 2.1	29.1 ± 2.0
Globulin	20.1 ± 2.8	21 ± 3.1	20.4 ± 1.4

4. DISCUSSION

AFP is a clinical serum marker that can be used to diagnose head and neck cancer. However, patients with viral hepatitis who do not have HCC, particularly those with CHC and liver cirrhosis, may have elevated AFP levels as well. Previous studies reported that AFP levels increase during hepatocyte regeneration, especially after massive hepatic necrosis and chronic HCV infection. The differences in elevated AFP levels may be due to patient populations, sample sizes, and definitions of serum AFP elevation. [1,2,4] Failure to clear the virus after acute infection can lead to dormant carriage or chronic hepatitis B, which may progress to liver fibrosis, cirrhosis, and/or hepatocellular carcinoma. Several viral factors such as HBV genotype, viral load, HBV genome mutations, and serum HBeAg and HBsAg levels have been shown to predict clinical outcomes after persistent infection.[5] It is primarily produced by the fetal yolk sac, liver, and intestine. After birth, its production is almost completely shut down, with concentrations dropping to less than 10 ng/ml. In benign liver disease, such as acute and chronic hepatitis, or cirrhosis, low-grade elevations of AFP are common, but values above 200 ng/ml are used as a surrogate marker for liver cancer. A variety of factors, including different patient populations, sample sizes, and definitions of elevated serum AFP levels, could explain the prevalence and wide variation of elevated AFP levels found in different studies. As a result of this study, approximately 41.7 percent of patients with CLD tested positive for HBsAg, a percentage that was higher than that reported by some other authors. No clear explanation exists for the significantly higher incidence of HBV and HCV infections among study patients. However, this may be due to the fact that our research was conducted on CLD patients rather than the general public.

Although it was not found that co-infection with both HBV and HCV resulted in more severe liver disease than mono-infection, the study shows that co-infection with both HBV and HCV resulted in a higher risk of liver cancer than mono-infection.[9] A previous study by Fathima et al.[5] found that 0.4 percent of blood donors were infected with the Hepatitis C virus. In a similar study conducted in Vietnam, HBV-related CLD was found to be responsible for 47 percent of cases; however, only 23 percent of these cases were HCV seropositive. According to one report, liver disease is the third most common cause of death in African medical wards while hepatitis B remains to be the most common cause throughout the continent.[11,13] HBV and HCV infections are known to play a role in the etiology of liver diseases yet, the relative importance of these infections varies over time and from region to region.

Patients in the study had high levels of AFP, which was expected, and their levels were statistically different from those in the control group (P 0.001). When it comes to liver disease, serum AFP is an important tumor marker, and its determination is extremely useful in both diagnosing the disease and monitoring its progression after treatment. During follow-up, the presence of this protein in patients suffering from cirrhosis serves as a risk factor for the development of hepatocellular carcinoma. [14,15] When comparing CLD patients with HCV antibody positivity to those with HBsAg positivity and those without hepatitis infection, the serum AFP level was the highest in the HCV antibody-positive group. This conclusion was reached in line with other findings, like Chwla.[16] In a previous study, Abdoul et al.[39], discovered a link between serum AFP levels and sustained virological response in HCV-infected patients. An indication of it is the association of HCV infection with more severe liver diseases than hepatitis B or CLD. It has been shown that the HCV gene products (which include core, NS3/4B, and NS5A) can induce malignant transformation of hepatocytes in a cell model system and, in some cases, can result in hepatocellular carcinoma in humans.[17,18] Furthermore, it has been demonstrated that the expression of HCV protein alters several potentially oncogenic pathways, including cell signaling, transcription, modulation, apoptosis, transformation, translational regulation, and interaction with the translational machinery and post-translational modification system (PTM).[19] The presence of carcinoma in the liver, in addition to the potential direct effects of the HCV virus on the host genome, may result in a cycle of inflammation, necrosis, and regeneration as by-product of chronic hepatitis C-induced liver cell injury. An increase in cell turnover in the context of inflammation and oxidative DNA damage may facilitate the accumulation of genetic and epigenetic alterations, which may include the activation of cellular oncogenes and proliferative signaling pathways, telomerase activation, and the accumulation of chromosomal damage. The serum ALT levels of CHC patients have been shown to be mildly elevated in the past, and elevated serum AFP levels have been shown to be positively correlated with serum ALT levels in the past (p 0.001).[8,9]

5. CONCLUSIONS

In conclusion, serum AFP level was higher in CLD patients with HCV antibody positive compared to HBsAg positive CLD and hepatitis negative patients. Clinicians should maintain a high level of suspicion of HCV infection when high levels of AFP are recorded in patients with CLD.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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