



Correlation of serum alpha-fetoprotein (AFP) level with liver function parameters in hepatitis B virus (HBV) infected patients in Bangladeshi population

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Abstract

Hepatitis B attacks the liver and can cause both acute and chronic disease. Elevated ALT, AST and ALP are the parameter of liver function test. Along with this high alpha fetoprotein serum levels have also been found in 60–70% of patients with hepatocellular carcinoma; nevertheless, there are other causes that increase this particular protein. A prospective study was performed among 32 healthy control (HBsAg-) and 96 patients (HbsAg+) with their first attack of HBV-related acute hepatitis. In these study AFP, AST, ALT and ALP levels were measured. Among the 29 patients, 9 of them exhibited AFP level in between 10-20(IU/ml), 8 of them exhibited AFP level in between 21-70(IU/ml) and 12 of them exhibited AFP level in between 71-300(IU/ml) with a highest level of 297.90(IU/ml). Beside those 29 patients, other 67 patients of our 96 patient groups have also showed normal AFP level lower than 10(IU/ml). The AFP level of the control group was found normal and it was less than 10(IU/ml).The mean AFP level of patient group is significantly higher ($P < 0.001$) compared to the control group and this value is higher than the reference range. The mean AFP level of patient group above 40 years old was significantly higher ($P < 0.001$) compared to the patient group of less than 40 years old. The Serum AFP level has shown positive correlation with the serum ALT ($p=0.01; r=0.9330$), AST($p=0.01, r=0.927$) and ALP($p=0.01, 0.829$).

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Introduction

Hepatitis B virus (HBV) is a major human pathogen responsible for acute and chronic liver disease. Over 95% of acutely infected adults recover completely and spontaneously from the infection, whereas most individuals who were infected with neonatally transmitted infections develop persistent viral replication or chronic infection (Ganem D *et.al.*, 2004, Iannacone M *et.al.*, 2007). Chronic HBV infection can progress to life-threatening complications such as cirrhosis and hepatocellular carcinoma (HCC) (Anzola M, 2004). Worldwide, more than 350 million people are chronically infected with HBV. More than one-third of these individuals will die from serious liver diseases such as cirrhosis and HCC if their conditions are left untreated (Chisari FV *et. al.*, 1995). Therefore, it is important to improve our understanding about HBV infection and its relation with various liver function parameters particularly those that change during HBV infection. The understanding will lead to the development of better treatments for HBV infection.

The HBsAg however is the first marker to appear in serum. The presence of HBsAg indicates recent infection and if it persists for more than 6 months the patient may become a chronic carrier. Individuals who remain HBsAg positive for at least six months are considered to be hepatitis B carriers (Lok, A *et. al.*, 2007). Carriers of the virus may indicate the presence of chronic hepatitis B, which would be reflected by elevated serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) levels, generally liver disease is associated with some degree of hepatic necrosis such as cirrhosis, carcinoma, viral or toxic hepatitis, and obstructive jaundice.

Alpha-fetoprotein (AFP) is the major protein found in serum during fetal life (Crandall BF *et. al.*, 1981). The Serum levels begin to fall before birth and reach values of less than 10 ng/ml within the first few weeks of life. Serum AFP can be elevated in some

disease states, particularly hepatocellular carcinoma (HCC) (McMahon BJ *et. al.*, 1987; Bellet DH *et. al.*, 1985 & Kew MC, 1983).

A transient rise in serum AFP concentration in the patient with acute viral hepatitis has also been reported (Abelev, G. I. 1971; Akeyama, T *et.al.*, 1972; Ishii, M 1973; Purves, L. R *et. al.*, 1973; Ruoslahti, E *et. al.*, 1972 & Smith, J. B. 1971). The cause is not known but the possible mechanisms include liver cell retrodifferentiation during hepatocyte regeneration, viral alteration of alpha-fetoprotein synthesis, or an acute-phase reaction to liver injury (Purves, L. R *et.al.*, 1972).

Therefore, the aim of the study was to establish any correlations of Alpha-feto protein levels and selected tests of liver cell function and integrity measured serially in patients with acute viral hepatitis.

Methods and materials

Study population

A prospective study was performed among 96 HBV infected patients and 32 healthy individuals. Blood samples were collected from the 96 patients, from "Gastroliver Hospital and Research Institute". All of the collected specimens were HBsAg positive. Blood sample of 32 healthy people were also collected, they are considered as control group. The HBV marker (HBsAg), Alpha-feto protein levels and selected tests of liver cell function (AST, ALT, ALP) have been measured in the study subjects.

Diagnostic definition

HBV-related acute hepatitis was defined according to: (i) HBV marker: positive for serum hepatitis B surface antigen (HBsAg), (ii) biochemical parameters: levels of serum alanine aminotransferase (ALT) >10-fold the upper reference limit (URL), serum aspartate aminotransferase (AST) >10-fold the upper reference limit (URL) and serum alkaline phosphatase (ALP) <3 × URL. Serum alpha fetoprotein (AFP) level was measured to investigate whether or not there are

any correlation between those biochemical parameters and AFP level among the highly HBsAg positive HepatitisB patients. The AFP level greater than 10(IU/ml) is considered as an abnormal elevation.

Specimen collection

Each blood sample was Collected by venipuncture in a tiger-top or red top vacutainer tube, and was allowed to clot. Then it was centrifuged within 4 hours of collection. Serum was separated from blood and then it was kept in freezer (temp:-20°C). The frozen specimens were thawed, homogenized and centrifuged to eliminate any particulate matters before assay.

Laboratory methods

Alpha-feto protein (AFP) levels were estimated by the AFP EIA Kits (DRG). This estimation was based on the principle of a solid phase enzyme-linked immunosorbent assay. The qualitative confirmation test for the presence of HBsAg in the serum sample was carried out by using the Hepatitis B Surface Antigen (HBsAg) EIA Kit (Diasorin). It is an enzyme immunoassay kit on microstrip format. The selected tests of liver cell function (AST, ALT, ALP) were made by using AST, ALT, ALP Kits (Labkit, Germany) is based on the principle of kinetic method.

Study design

Based on the presence of HBsAg the study was divided into two groups: control group [HBsAg(-)VE] (n=32) and patient group [HBsAg(+)VE] (n=78). The patient groups were further divided into two groups in term of age: patients who were less than 40 years old (20-39, n=57) and who were more than 40 years old (40-65, n=39).

Results

AFP levels in different study group

The AFP level greater than 10(IU/ml) is considered as an abnormal elevation. Out of our 96 patient group, 29 (30.2%) of them was reported as elevated AFP level. Among the 29 patients, 9 of them

exhibited AFP level in between 10-20(IU/ml), 8 of them exhibited AFP level in between 21-70(IU/ml) and 12 of them exhibited AFP level in between 71-300(IU/ml) with a highest level of 297.90(IU/ml). Beside those 29 patients, other 67 patients of our 96 patient groups have showed normal AFP level lower than 10(IU/ml). The AFP level of the control group was found normal and it was less than 10(IU/ml). The mean AFP levels are (2.71± 0.56), (30.36 ± 4.21) in control group [HBsAg(-)VE] and patient group [HBsAg(+)VE] respectively. The mean AFP level of patient group is significantly higher (Z=62.70; P< 0.001) compared to control group and this value is higher than reference range. Fig.1[a] shows the average AFP level in patient [HBsAg(+)VE] and control [HBsAg(-)VE] serum sample.

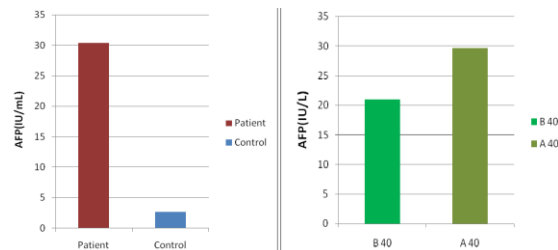


Fig. 1. [a] level of serum AFP in the study subject. [b] level of AFP before age 40 (B 40) and after age 40 (A 40).

In our investigation, the number of patients in the patient group under 40 years was 57 and the number of patients in the over 40 years was 39. The mean AFP levels are (21.01±4.12), (29.70±5.21) in patient group under 40 years and patient group over 40 years respectively. A significant correlation was found between the AFP level and the age of the patient group where the mean AFP level of patient group (after 40 year) was increased significantly (P< 0.001) compared to the patient group (before 40 years).

AST, ALT & ALP level in the study subjects

Table 1 shows that patients ALT, AST and ALP level are significantly higher than control group (p=0.01). The mean level of ALT (60.71±17.14), AST (54.63±10.54) and ALP (54.63±10.54) of the patient group (after 40 years) were higher

compared to the mean level of ALT(56.28±16.08), AST(50.86±9.62) and ALP(139.75±36.28) of the patient group who were less than 40 years (Table 2). But the changes were not at significant level.

Table 1. Level of ALT, AST and ALP in the study subjects.

Study Subject	ALT (IU/L)	AST (IU/L)	ALP(IU/L)
Control	15.28±5.18	21.65±5.64	39.25±13.87
Study subject	*62.36±17.1	*56.78±12.42	*143.73±21.96

Values are in mean ±SD. The vertical line for the same parameter followed by *are significantly different at $p=0.01$ level.

Table 2. Level of ALT,AST and ALP between two different age group patients.

Study Subject	ALT (IU/L)	AST (IU/L)	ALP(IU/L)
PB40	56.28±16.08	50.86±9.62	139.75±36.28
PA40	60.71±17.14	54.63±10.54	147.71±25.58

Values are in mean ±SD. PB40(patient less than 40years old) A; PA40(patient more than 40 years old)

Correlation of serum AFP level with HBsAg, ALT, AST, ALP levels among the patient group

Correlation was performed among different parameters. A significant positive correlation was found among AFP and ALT ($p=0.01$; $r=0.9330$), AST ($p=0.01$, $r=0.927$) and ALP($p=0.01$, 0.829) levels. ALT level is also significantly positively correlated with AFP ($p=0.01$; $r=0.933$),AST($p=0.01$; $r=0.987$), ALP($p=0.01$; $r=0.847$) levels. Other two parameters AST and ALT are also significantly positively correlated. In case of AST the significance was AFP ($p=0.01$; 0.927),ALT($p=0.01$; $r=0.987$) and ALP($p=0.01$; $r=0.842$).

Discussion

Several studies have been conducted to demonstrate the elevation of serum AFP level in a patient with Hepatitis B. One of the study (Di Bisceglie AM *et.al.*, 1989) showed that the major cause of AFP elevations in patients with chronic hepatitis B is an exacerbation of disease. In 86% of cases, AFP elevations occurred shortly after the onset of an exacerbation in the underlying chronic hepatitis. This was particularly common among patients with

severe disease who had liver cirrhosis. 27% of patients with AFP elevations died of a liver related death compared to only 0.7% among those with normal AFP. Similar findings have been reported from areas of the world where chronic HBV infection is endemic (Seeff LB *et.al.*, 1987)

The majority of patients in this study (91%) were initially positive in serum for HBeAg. Thus they would be classified as having chronic hepatitis B rather than being healthy carriers of HBsAg (Di Bisceglie A.M *et.al.*, 1987) Therefore this study emphasizes the necessity of AFP screening of patients with chronic state and active viral replication.

(Smith J.B 1971) found elevated level of alpha-fetoprotein (AFP) in patients with virus-B hepatitis (identified by finding HBsAg in the serum). Persistence of HBsAg after an attack of acute hepatitis has recently been incriminated in the etiology of primary liver cancer and it has been postulated that the presence of elevated alpha-fetoprotein levels allows the antigen to persist. In other investigation, the serum alpha-fetoprotein concentrations returned to normal after recovery from the attack of hepatitis (M.C.Kew *et.al.*, 1973). Another investigation was governed to find out the correlation between Serum AFP Level and HBsAg, Age and Sex in Subjects of the National Cancer Institute, Thailand (2009).They concluded that In HCC patients, no correlation of serum AFP level with HBsAg with age and sex was found.Yet another study found the elevated AFP levels correlated positively with age among the patient with hepatitis C (Wei-Chen Tai *et.al.*,2009).

In our Study, the number of patients in the patient group under 40 years was 57 and the number of patients in the over 40 years was 39. A significant correlation was found between the AFP level and the age of the patient group. The mean AFP level of patient group (after 40 year) was increased significantly ($P < 0.001$) compared to the patient group (before 40 years). On the other hand the level

of AST,ALT and AFP was not significantly different between two age groups.

The normal serum levels of alpha-fetoprotein reflect the normal rate of liver cell turnover. Studies in which the liver of experimental animals was subjected to partial resection or toxic injury have suggested that, increased production of alpha-fetoprotein occurs during regeneration of hepatocytes (Purves L.R *et.al.*, 1972; 1973). The finding of Akeyama.T. *et. al.*, 1972 and Ruoslahti. E *et. al.*, 1972b showed that maximal levels of alpha-fetoprotein in their patients with acute hepatitis occurred during the recovery phase of the illness. Increased synthesis may be due to retrodifferentiation of mature liver cells during regeneration or, as suggested by the latter authors, proliferation or activation of relatively undifferentiated adult hepatocytes that have the alpha-fetoprotein genome in an unrepressed state. In another study (M.C.Kew *et.al.*, 1973) eight patients showed an increase in alpha-fetoprotein level as the ALT level, used as an index of hepatocyte damage, was returning to normal, a pattern which would fit a hypothesis of increased synthesis during hepatocellular regeneration. In the remaining patients alpha-fetoprotein paralleled ALT, suggesting rather an acute-phase reaction to liver injury or perhaps a direct consequence of the presence of the virus. This latter pattern was also found by Ishii .M (1973) in five patients with acute hepatitis.

Fulminant hepatitis with coma was present in the only patient with normal serum levels of alpha-fetoprotein. An acute-phase response might not be anticipated with massive liver necrosis, and the failure of the serum alpha-fetoprotein concentration to rise in such a patient might possibly be used as an index of irreversibility of the liver damage. However, in another patient having liver necrosis has a very high level of alpha-fetoprotein.

The cause of elevated AFP in patients with non-tumor liver disease is unclear. One of the study (

Yongnian H. *et.al.*, 2008) showed that the peak production of a great majority of the two groups of patients with elevated AFP levels was observed at early phase of the disease, when liver damage was excessively severe, but not at the convalescent phase. When hepatocytic regeneration occurred, suggest that elevations of serum AFP in acute and chronic liver diseases may not be due to subsequent hepatocyte regeneration induced by hepatic inflammation. Longitudinal studies showed that elevations of serum AFP levels at baseline level in chronic hepatitis B(CHB) patients confirmed by liver biopsy had been proven to be associated with a higher risk of decompensated cirrhosis, hepatocellular carcinoma (HCC) (Di Bisceglie AM *et. al.*, 1989 & Xu B *et.al.*, 2003) implying that patients with elevated serum AFP had more advanced liver disease than did those with normal levels. Very high levels of serum AFP suggestive of the possibility of HCC were occasionally found in patients with chronic HBV infection, especially those with cirrhosis, but no occurrence of HCC (Bae JS *et.al.*, 2005 & Yao FY *et.al.*, 2003) All these observations put forward the hypothesis that marked fibrosis or cirrhosis, a state of significant altered hepatocyte architecture, may be the underlying cause of increased serum AFP and, just at the presence of fibrosis or cirrhosis, hepatocyte necroinflammation can trigger elevations of AFP. This can explain why AFP elevations have frequently been found in CHB patients, with remarkable AFP elevations being associated with exacerbations of the underlying liver disease (Lok AS *et.al.*, 1989) whereas normal AFP levels are found in a great majority of patients with acute self-limited hepatitis B (ASL-HB) and only low levels of AFP in a minority of them, although they had more severe liver necroinflammation.

If the cause of the elevation of serum AFP in the patient with acute viral hepatitis is due to an acute-phase reaction to liver injury, alpha-fetoprotein levels would rise as tests of liver function (ALT,AST,ALP) and concentrations would be expected to follow the pattern of these tests. So it

would be expected that serum AFP level correlates positively with the liver function parameters like ALT, AST, ALP. One of the studies (J.F.Tsai J.F *et.al.*, 1994) have shown that serum AFP level correlated positively with AST($r=0.201, P=0.007$), ALT ($r=0.178, P=0.017$), ALP ($r=0.152, P=0.042$). The result of conventional liver function tests in patient with raised AFP were worse than in patients with normal AFP.

In our study we also get a significant positive correlation among AFP protein and AST, ALT and AFP protein.

Conclusion

The study revealed the following outcomes:

1. Alpha feto protein level of is significantly higher ($P < 0.001$) in patients compared to control group .
2. AFP shows significant correlation with age. The mean AFP level of patient group after 40 years is increased significantly ($P < 0.001$) compared to the patient group before 40 years.
3. The Serum AFP level is correlated positively with the serum ALT level($r=.933^{**}$; $\rho=.793^{**}$, $P < .001$) among the patient group.
4. The Serum AFP level is correlated positively with the serum AST level($r=.927^{**}$; $\rho=.799^{**}$, $P < .001$) among the patient group.
5. The Serum AFP level is correlated positively with the serum ALP level($r=.829^{**}$; $\rho=.786^{**}$, $P < .001$) among the patient group.

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