

# Assessment of Alpha Fetoprotein Levels and Gamma Glutamyl Transferase Activity in Hepatitis B and Hepatitis C Seropositive Subjects in Nnewi, Nigeria

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## Abstract

Hepatitis B and hepatitis C viral infections are the leading cause of liver cirrhosis and hepatocellular carcinoma worldwide. These conditions, which mar the hepatic functional integrity, are characterized by alterations in the liver function markers such as alpha fetoprotein (AFP) and gamma glutamyl transferase (GGT). In the present study, a total of 90 subjects were recruited. Out of this number, 30 were hepatitis B seropositive subjects, 30 hepatitis C seropositive individuals and the remaining 30 were apparently healthy individuals. The last group served as the control. Serum alpha fetoprotein levels were estimated by the Enzyme Linked Immunosorbent Assay (ELISA) technique and the method adopted for the determination of gamma glutamyl transferase activity was the kinetic-spectrophotometric procedure. The mean serum level of alpha fetoprotein was significantly higher in hepatitis B seropositive subjects compared with the control ( $P < 0.05$ ). The same pattern was observed when the mean serum activity of GGT of the hepatitis B seropositive subjects was compared with that of the control ( $P < 0.05$ ). Furthermore, the mean serum level of AFP and the mean serum GGT activity were significantly higher in hepatitis C seropositive individuals compared with the control ( $P < 0.05$ ). In contrast, no significant difference was observed in the mean serum levels of alpha fetoprotein in hepatitis B seropositive individuals compared with that of hepatitis C seropositive subjects ( $P > 0.05$ ). A positive correlation existed between AFP levels and GGT activity in hepatitis B seropositive subjects ( $r = 0.31$ ) and between AFP levels and GGT activity in hepatitis C seropositive subjects ( $r = 0.25$ ). These findings suggest that evaluation of serum alpha fetoprotein levels and gamma glutamyl transferase activity may be a valuable adjunct in the assessment of disease progression in hepatitis B and hepatitis C seropositive individuals.

**Keywords:** Hepatitis, alpha fetoprotein, gamma glutamyl transferase, disease progression.

## Introduction

Hepatitis B is an infectious disease of the liver caused by the hepatitis B virus (HBV) that affects hominoids, including humans; originally known as "serum hepatitis" (Barker et al., 1996). The disease has caused epidemics in parts of Asia and Africa, and it is endemic in China (Williams, 2006). Hepatitis B virus is a hepadnavirus and although its replication takes place in the liver, the virus spreads to the blood where viral proteins and antibodies against them are found in infected individuals. Hepatitis C virus (HCV) infection with its associated sequelae is a disease of major public health importance worldwide (Alao et al., 2008). HCV infection occurs frequently and is highly endemic in Nigeria (Halim and Ajayi, 2000; Erhabor et al., 2006). The high prevalence of HCV infection and its associated complications, such as liver cirrhosis and diabetes mellitus make HCV infection a disease of major public health importance worldwide and in Nigeria in particular (Araj et al., 1995; Tamim et al., 2001).

Hepatitis B and hepatitis C infections could be acute or chronic depending on how long the virus has been incubated in the body (El-Serag, 2011). Chronic forms of the HBV and HCV infections either may be asymptomatic or may be associated with a chronic inflammation of the liver (chronic hepatitis), leading to cirrhosis incidence of hepatocellular carcinoma (liver cancer) (Chang, 2007). Across Europe, hepatitis B and hepatitis C cause approximately 50% hepatocellular carcinomas (El-Serag and Rudolph, 2007).

Alpha fetoprotein (AFP) is the most abundant plasma protein found in the human fetus and plasma levels decrease rapidly after birth and has been shown to begin to decrease prenatally starting at the end of the first trimester (Chang, 2007). The levels of AFP increase beyond the normal range in pregnancy, hepatocellular carcinoma/hepatoma and yolk sac tumor, neural tube defects, Ataxia telangiectasia (Tamim et al., 2001). Gamma glutamyl transferase is a microsomal enzyme present in the cell membranes of hepatocytes, biliary epithelial cells, renal tubules, pancreas, prostate, intestine, gallbladder, spleen, heart and brain. Its activity is increased most notably in the liver, and has been used as a diagnostic marker (Crook, 2012).

The levels of alpha fetoprotein (AFP) and the activity of gamma glutamyl transferase (GGT) have been shown to be helpful as hepatic function markers in the assessment of the hepatocellular carcinoma, a common complication of hepatitis B and hepatitis C infections (Barker et al., 1996). Although the correlation of alpha

fetoprotein levels and the activity of gamma glutamyl transferase with hepatocellular carcinoma is not absolute (Coopstead, 2010), the results from these hepatic biomarkers could be relevant in the assessment of the hepatic functional status.

Hepatitis B and Hepatitis C infections have marked effect on liver function. Assessment of hepatic markers such as alpha fetoprotein levels and gamma glutamyl transferase activity could be relevant in evaluation of hepatic functional integrity. Thus, this study was carried out to assess the effect of hepatitis B and hepatitis C infections on the levels of AFP and ALP, to evaluate the activity of gammaglutamyl transferase (GGT) in hepatitis seropositive and compare these to values obtained in apparently healthy controls.

## **Materials and Methods**

### **Subjects**

A total of One hundred and fifteen (115) subjects were initially recruited for the study comprising of thirty-nine (39) hepatitis B positive and thirty-six (36) hepatitis C positive subjects and thirty (30) apparently healthy controls over a period of six months. These subjects comprising males and females were recruited from Nnamdi Azikiwe University Teaching Hospital Nnewi, Anambra state, Nigeria, a tertiary health care facility. After screening tests fifteen of these were excluded from the study. The final study population was ninety (90) subjects made up of 30 hepatitis B seropositive subjects (16 males and 14 females), 30 hepatitis C seropositive individuals (18 males and 12 females) and 30 control subjects (15 males and 15 females).

### **Exclusion and inclusion criteria**

Hepatitis B seropositive Subjects, hepatitis C seropositive individuals and normal control subjects were included in this study.

Chronic alcohol consumers, pregnant women, HIV seropositive subjects, individuals with hepatic pathologic conditions and diabetics were excluded.

### **Ethical consideration**

The ethical approval for this research was obtained from the Nnamdi Azikiwe University Teaching Hospital Ethical Committee (NAUTHEC). Informed consent was sought and obtained from the subjects.

### **Methods**

Five milliliters (5 ml) of venous blood was collected aseptically from each of the subjects. The samples were then centrifuged at 4,000 rpm for 5 minutes and the serum separated.

### **Hepatitis B Virus Detection**

Hepatitis B virus detection was performed using the method described by Swenson (1991). This is an immunochromatographic method.

### **Detection of Hepatitis C Virus**

The method as described by Wilber (1993) was used for hepatitis C virus detection.

### **Estimation of Alpha Fetoprotein**

Alpha fetoprotein level was estimated using the method as described by Chan and Miao (1986). This is essentially an Enzyme Linked Immunosorbent Assay (ELISA) technique. AFP ELISA Kit obtained from BioCheck, Foster City, California, USA. The ELISA machine (Mindray MR-96A) was used.

### **Determination of Gamma Glutamyl transferase (GGT) Activity**

The method as described by Mauro and Renze (2013) was used for the determination of gamma glutamyl transferase activity. This method is essentially a spectrophotometric procedure. Randox GGT kit obtained from Randox Laboratories Limited, Northern Ireland, United Kingdom.

### **Statistical analysis**

The statistical analysis was performed on the data generated using the mean, standard deviation, analysis of variance (ANOVA) and Pearson correlation (SPSS version 21). Values were deemed significant at  $P < 0.05$ .

## **Results**

As shown in table 1, there was a significantly higher increase in the mean serum levels of alpha fetoprotein in the hepatitis B seropositive subjects compared with control subjects ( $P < 0.05$ ).

**Table 1: Comparison of alpha fetoprotein (AFP) and gamma glutamyl transferase (GGT) activity in hepatitis B seropositive subjects and controls.**

SUBJECTS	Alpha fetoprotein (ng/ml) Mean $\pm$ S.D	Gamma glutamyl transferase (U/L) Mean $\pm$ S.D
Hepatitis B positive (n=30)	14.64 $\pm$ 11.60	60.10 $\pm$ 59.20
Hepatitis B negative (control group) (n=30)	3.07 $\pm$ 1.75	16.27 $\pm$ 7.34
<b>P value</b>	0.00	0.00

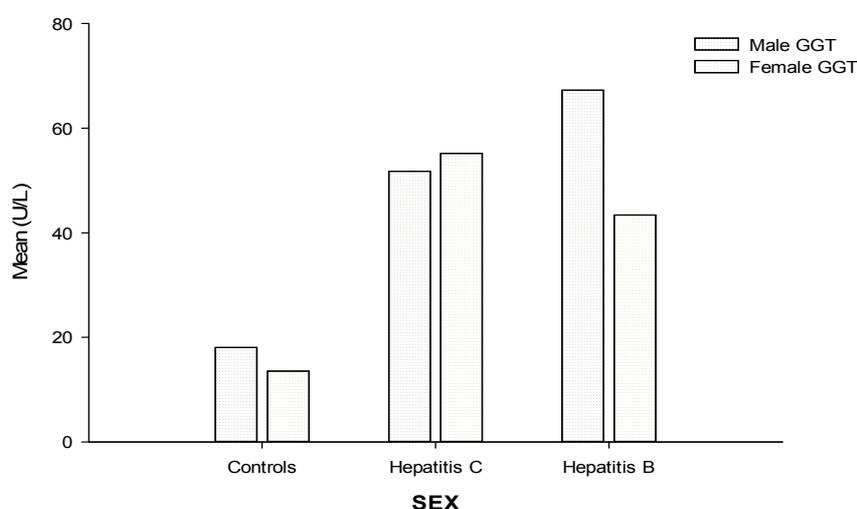
The mean serum activity of gamma glutamyl transferase was significantly higher in hepatitis B seropositive subjects compared to control subjects ( $P < 0.05$ ) (table 1).

**Table 2: Comparison of alpha fetoprotein (AFP) and gamma glutamyl transferase (GGT) activity in hepatitis C seropositive subjects and controls.**

SUBJECTS	Alpha fetoprotein (ng/ml) Mean $\pm$ S.D	Gamma glutamyl transferase (U/L) Mean $\pm$ S.D
Hepatitis C positive (n=30)	13.64 $\pm$ 11.23	52.76 $\pm$ 32.52
Hepatitis C negative (control group) (n=30)	3.07 $\pm$ 1.75	16.27 $\pm$ 7.34
<b>P value</b>	0.00	0.00

Results revealed significantly higher increase in the mean serum levels of alpha fetoprotein in hepatitis C seropositive subjects compared to control subjects was observed ( $P < 0.05$ ). This is shown in table 2. Also, findings show that there was significantly higher mean serum activity of gamma glutamyl transferase when comparing the hepatitis C seropositive subjects and control subjects ( $P < 0.05$ ). See table 2. There was no significant difference in the mean serum levels of alpha fetoprotein in hepatitis B and hepatitis C seropositive subjects ( $P > 0.05$ ).

There was an increase in alpha fetoprotein levels in males in control and hepatitis B seropositive subjects more than female subjects while the increase in alpha fetoprotein levels in hepatitis C seropositive female subjects is higher than those of the male subjects.



**Fig 1: Relationship between activity of gamma glutamyl transferase and the sex of control, hepatitis B seropositive and hepatitis C seropositive subjects.**

The increase in the mean serum activity of gamma glutamyl transferase (GGT) in males in control, and hepatitis B seropositive subjects was greater than those of females whereas the increase in gamma glutamyl transferase activities in hepatitis C seropositive female subjects is higher than those of male subjects.

There was a positive correlation between alpha fetoprotein levels and gamma glutamyl transferase activity in hepatitis B seropositive subjects ( $r= 0.305$ ). Also, a positive correlation was shown to exist between alpha fetoprotein levels and gamma glutamyl transferase activity in hepatitis C seropositive subjects ( $r= 0.245$ ).

## Discussion

Hepatitis B virus (HBV) and Hepatitis C virus (HCV) infections are among the greatest infectious disease problems among world's population. About 350 million people have chronic hepatitis B virus infection, and about 150 million people have been infected with hepatitis C virus (Russman *et al.*, 2007). However, these diseases are important candidates for the public health measures aimed at prevention, early diagnosis and treatment. Also, individuals with chronic hepatitis especially through HBV and HCV are at highest risk of progression to cirrhotic hepatocellular carcinoma (Di Bisceglie, 2004).

In the present study, the mean serum level of alpha fetoprotein (AFP) was significantly higher in hepatitis B seropositive subjects compared with the control ( $P<0.05$ ). Similarly, a significant increase was observed in the mean serum activity of GGT of the hepatitis B seropositive subjects when compared with that of the control ( $P<0.05$ ). Furthermore, the mean serum level of AFP and the mean serum GGT activity were significantly higher in hepatitis C seropositive individuals compared with the control ( $P<0.05$ ). However, no significant difference was observed in the mean serum level of alpha fetoprotein in hepatitis B seropositive individuals compared with that of hepatitis C seropositive subjects ( $P>0.05$ ). (Karaman *et al.* (2010) and Abd El Samiee *et al.* (2011) had earlier reported increases in the levels of AFP in viral hepatitis (HBV and HCV) and liver cirrhosis without evidence of hepatocellular carcinoma (HCC). The elevations may be as a result of the activation of the AFP gene in conditions such as viral hepatitis B and hepatitis C infections (Mizejewski, 2001). Hepatocarcinogenesis had been linked to serum AFP elevation (Shi-Ying *et al.*, 2007). AFP is a protein encoded by AFP gene in humans and the gene is located on the q arm of chromosome 4 (Harper and Dugaiczky, 1983). The gene is inactivated after birth, thus leading to the fall in AFP levels and subsequently is reactivated in conditions such as hepatitis B and hepatitis C viral infections (Hulbert *et al.*, 1974).

It has been suggested that AFP is synthesized by the regenerating liver cells (Shafritz *et al.*, 1981) and the mechanism for such an association is unknown (Imazek *et al.*, 1986). Reactivation of the AFP gene in these conditions is one of the primary mechanisms for hepatocarcinogenic process (Sato *et al.*, 1993; Mizejewski, 2001; Johnson, 2001). The effect of AFP on the growth of liver tumor in chronic hepatitis B and hepatitis C has been confirmed (Shi-Ying *et al.*, 2007) and the functional mechanism of AFP on the growth of tumors may also be attributed, at least in part, to receptor mediated cAMP pathway and/or calcium signaling resulting in over expression of certain genes (Sato *et al.*, 1993; Shi-Ying *et al.*, 2007). It is possible that hepatotoxic stimuli during, or shortly after, period of hepatitis B and hepatitis C infections may readily reactivate AFP synthesis in those cells that are actively synthesizing or still retain a latent capacity to produce AFP molecule (Hulbert *et al.*, 1974).

The elevations in GGT activity in hepatitis B and hepatitis C seropositive individuals observed in the present study are similar to that reported by Plotr *et al.* (2010). Furthermore, Silva *et al.* (2004) documented an increase in the GGT activity in chronic hepatitis C seropositive patients. In a separate study by Souza *et al.* (2008), an alteration in mean serum GGT values in HCV patients which were frequently maintained at persistently elevated levels was observed. However, this increase could be attributed to the destruction or blockage of bile ducts in the face of liver damage caused by hepatitis B and hepatitis C viruses in these patients thus leading to increase in the levels of GGT (Giannini *et al.*, 2001).

Serum gamma glutamyltransferase (GGT) is thought to be derived from the liver, but its high values predict morbidity and mortality for several diseases, such as cardiac infarction, stroke, diabetes, renal failure, hepatitis B virus infection, hepatitis C virus infection and hepatic cancer (Laker, 1990). GGT is usually the first liver enzyme to rise in the blood when any of the bile ducts that carry bile from the liver to the intestines becomes obstructed, for example, by tumors or stones, and this makes it the most sensitive liver enzyme test for detecting bile duct problems (Karaman *et al.*, 2010). Moreover, in patients free from renal damage, elevated gamma glutamyl transferase values have been associated with acute or chronic viral hepatitis (Giannini *et al.*, 2001). Myers *et al.* (2003) observed that, in patients with chronic hepatitis B, the GGT level can be a significant predictor of hepatic fibrosis due to its consistent elevation in the serum of patients for prolonged periods of time. Giannini *et al.* 1999 reported that chronic hepatitis C patients would suffer damage to the bile duct in hepatic fibrosis with subsequent elevation in GGT activity. Chronic hepatitis caused by 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 (Imbert-Bismut *et al.*, 2001; Hui *et al.*, 2005).

Furukawa *et al.* (1984) reported a growing number of evidence that AFP levels were significantly raised in anti-HCV positive compared with HBsAg positive individuals. However, in a recent study by Israa *et al.*

(2013), no significant difference was observed in the mean serum levels of AFP in hepatitis B seropositive individuals compared with those of hepatitis C seropositive subjects. It was hypothesized that hepatitis B and hepatitis C infections present with similar disease severity in these individuals resulting in concomitant reactivation of AFP gene.

In this study, no significant difference was observed in the mean serum values of AFP and GGT in males compared with females in hepatitis B seropositive individuals, hepatitis C seropositive subjects and the control group. This was similar to the report of Junaid *et al* (2009). However, Chuang *et al* (2009) noted that sex and age were essential factors for the development of hepatocellular carcinoma from hepatitis B and hepatitis C infections. In a recent study by Krishnamurthy (2013), an increase in the serum GGT activity in male individuals compared with female subjects was documented.

This study showed a positive correlation between AFP levels and GGT activity in hepatitis B seropositive subjects ( $r=0.305$ ). A similar correlation pattern was observed in hepatitis C seropositive individuals ( $r=0.245$ ). In a previous study by Rajagopal and Mohammed (2005) a positive correlation of GGT values in hepatitis B and hepatitis C seropositive subjects was reported. Furthermore, Shi-Ying *et al* (2007) found a positive correlation in AFP values in patients with HBV and HCV infections. However, some researchers in a separate study observed no correlation between AFP and GGT in hepatitis B and hepatitis C seropositive subjects (Plotr *et al.*, 2010).

### Conclusion

Viral hepatitis B and hepatitis C infections are associated with alterations in liver function markers. Consequently, infected subjects are at higher risks of progression to chronic liver diseases. In this study, we have been able to show that alpha fetoprotein levels and gamma glutamyl transferase activity are significantly increased in hepatitis B and hepatitis C seropositive subjects compared with the control group. We have also shown that a positive correlation existed between AFP and GGT values in hepatitis B and hepatitis C seropositive subjects.

### Recommendations

It is recommended that base-line and frequent evaluations of alpha fetoprotein levels and gamma glutamyl transferase activity be included in the protocol for the management of hepatitis B and hepatitis C seropositive individuals.

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