ISRG Journal of Multidisciplinary Studies (ISRGJMS)

ISRG PUBLISHERS
Abbreviated Key Title: isrg j. multidiscip. Stud.
ISSN: 2584-0452 (Online)
Journal homepage: https://isrgpublishers.com/isrgjms/
Volume – II Issue - VI (June) 2024
Frequency: Monthly

PREVALENCE OF HEPATITIS B VIRUS INFECTION AMONG TUBERCULOSIS PATIENTS IN EBONYI STATE.

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| **Received:** 19.04.2024 | **Accepted:** 23.04.2024 | **Published:** 02.06.2024

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Abstract

Hepatitis B virus (HBV) and tuberculosis (TB) represent major public health problems. There are limited data on HBV infection among TB patients without Human Immunodeficiency Virus (HIV). The goal of this study was to assess the prevalence of Hepatitis B virus infection among tuberculosis patients. This was a cross-sectional study conducted among TB patients attending clinic at TB centres in Ebonyi State (Mile 4 Hospital and Mater Misericordiae Hospital). Questionnaire was used in obtaining information on the demographic and social life pattern of the patients. Blood samples were collected from the patients, EDTA – anticoagulated blood samples were used for CD4 T-cells estimation, while the serum extracted from the clotted samples was used for HBsAg, HBeAg and ALT estimation. A total of 145 TB patients were studied with 12 (8.3%) and 1(0.7%) being seropositive to HBsAg and HBeAg respectively. The only patient that was seropositive to HBeAg had an elevated Alanine transaminase (ALT) of 147mg/dl and CD4 T-cells count of 584cells/µl. Of the 145 subjects, 83(57.2%) were on Directly Observed Treatment Scheme (p=0.449) while 62(42.8%) were not (p=0.597). 6(4.2%) of the patients that were on DOTS were seropositive to HBsAg. A univariate analysis of risk factors showed that none of the risks were associated with HBV. The ALT values for those on DOTS showed that elevated ALT values were higher than the normal values with 6(4.2%) and 2(1.4%) seropositive to HBsAg. Considering the ALT values of patients yet to initiate DOTS, those with normal values showed higher seropositivity of 4(2.8%). For patients that had not started DOTS, also those with CD4 T-cells count of ≥500cells/µl demonstrated the highest seropositivity of 3.5% to HBsAg with mean value of 703.4±313.3. This study demonstrated a low prevalence of Hepatitis B virus infection among tuberculosis patients. HBV co-infection on tuberculosis patients on DOTS poses synergistic increase in hepatotoxicity among patients.

Keywords: *HBV, Tuberculosis, ALT, DOTS*

DOI: 10.5281/zenodo.11423081

INTRODUCTION

Hepatitis B virus and tuberculosis co-infections are common in developing countries like Nigeria. Both Hepatitis B virus infection and tuberculosis are common globally, half of all individuals infected with HIV are also infected with tuberculosis. Also, HIV and HBV co-infection is also common, since these diseases share similar potential routes of transmission (Puoti *et al.,* 2002). Most importantly, they cause severe morbidities and have a high propensity to cause increased mortality especially among immunocompromised individuals. It has been estimated that over 350 million people are chronically infected and 1-2 million deaths per year are directly or indirectly caused by the infection (Madrey, 2000).

Twin studies of Hepatitis B virus infection and tuberculosis showed a strong host genetic component to individual variability in disease susceptibility (Bellamy and Hill, 1998). Co-infection of TB and HBV is an important public health issue.

Mycobacterium tuberculosis has been estimated to infect almost one third of the world's population, especially those living in developing countries and in developed countries. The resurgence of tuberculosis worldwide is due mainly because of two reasons; there is an increased transglobal migration and travel which has led to easy dispersion of the bacterium and there is an increase of immunocompromised populations due to the pandemic nature of human immunodeficiency virus infection (Bellamy *et al.,* 1999).

Worldwide, about 350 million persons are chronic carriers of hepatitis B virus (WHO, 2000).It causes a serious public health malady globally and is the major aetiology of cirrhosis, chronic hepatitis and hepatocellular carcinoma. Approximately 2 billion people have serologic evidence of past or present HBV infection. The global disease burden is high because of the high HBV-related morbidity and mortality (Hou *et al.,* 2005).

Tuberculosis is among the major global threats to public health and has been implicated as an agent which causes disease that rapidly progress to death when not properly and effectively treated. It is competing with human immunodeficiency virus as a leading cause of death globally. Unfortunately, tuberculosis is associated with poverty as it marginally and disproportionately affects the poorest and most vulnerable population groups wherever it occurs. It would not be possible to control and completely eradicate tuberculosis without an effective surveillance system to evaluate the epidemiology and assess the impact of control measures (Sulis *et al.,* 2014). This study aimed at determining the epidemiology of Hepatitis B virus infection among selected tuberculosis patients and assessing the morbidity caused by hepatitis B virus and tuberculosis.

MATERIALS AND METHODS

Study Area

This study was conducted in patients admitted into the two major missionary hospitals in Ebonyi State that are dedicated centers for treating tuberculosis. They are Mile Four hospital Abakaliki and Mater Misericordiae hospital, Afikpo. These centres receive tuberculosis patients from South – East Nigeria for diagnosis, therapy and follow-up therapy. A total of 145 subjects were enlisted for this study.

Inclusion Criteria:

This study was a cross-sectional study that was conducted among Tuberculosis patients. The inclusion criteria were patients in whom tuberculosis had been confirmed using Acid Fast Bacilli staining and/or Gene Xpert technologies. Subjects were categorized into two; those that were newly diagnosed of tuberculosis and those that were on DOTS. The exclusion criteria are those that had co-infection with HIV.

Ethical Consideration: Ethical clearance was sought for and obtained from the clinical ethics committee of the two hospitals that were studied.

Specimen Collection

A total of 5ml of blood was collected from each subject. Then 2ml was dispensed into EDTA containers while 3ml was dispensed into plain tubes for serum extraction.

LABORATORY ANALYSIS

ESTIMATION OF $CD4^+$T CELLS

Whole blood specimens dispensed into EDTA containers were analyzed immediately for the population of $CD4^+$ T cells using automated method. 20µL of whole blood was added into a Partec test tube and mixed gently with 20µL of CD4 mAb. The mixture was incubated for 15minutes at room temperature in the dark. Thereafter, 800µL of No-lyse buffer was added to the mixture then it was vortexed gently for 5minutes. Then the specimen was analyzed for CD4 T-cells using Partec cyflow machine.

Normal value of CD4 – 301 – 20,000µL

Dilution factor for CD4 count – 42

Check count beads – 20000µL/whole blood.

Detection of HBsAg

Serum specimens were used to determine the presence of HBsAg using the HBsAg test kit.

Detection of HBeAg

The presence of HBeAg was determined using sandwich ELISA technique. Polystyrene microwell strips pre-coated with monoclonal antibodies specific for HBeAg. Serum specimen was added to the microwell together with a second monoclonal antibody conjugated with Horseradish peroxidase (HRP-Conjugate). The specific immunocomplex formed in the presence of HBeAg in the specimen was captured on the solid phase during incubation. After washing to remove the sample and unbound HRP-conjugate, chromogen solutions containing Tetramet hylbenzidine (TMB) and urea peroxidase was added into the wells. In the presence of antibody-antigen-antibody (HRP Sandwich complex) the chromogens which are colourless were hydrolysed by the bound HRP conjugate to a blue coloured product. After stopping the reaction with sulfuric acid the blue colour turned yellow. The coour intensity was measured using an ELISA reader. The colour intensity was proportional to the concentration of the antigen captured in the wells and to the specimen. Wells which remained colourless indicated that the specimens added to them were negative for HBeAg.

ESTIMATION OF ALANINE TRANSAMINASE

The concentration of Alanine transaminase in the serum of the subjects were estimated using Roche Reflotron machine. 20µL of the serum was dropped onto the sample chamber on the Reflotron strip and inserted into the machine. The specimens were analyzed electronically and the results were displayed on the screen of the machine. The results were compared against the normal values.

DOI: 10.5281/zenodo.11423081

The normal values of ALT is <14mg/dl. Elevated values ≥14mg/dl are indicators of liver damage.

Table 1: Characteristics and HBV Status of Subjects

Variable	Number	HBsAg Positive (%)	HBeAg Positive (%)
SEX			
Male	82	9 (6.2)	1 (0.7)
Female	63	3 (2.1)	0 (0.00)
P-Value		**0.178**	**1.000**
AGE			
≤20	9	0	0
21 – 30	23	2 (1.4)	0
31 – 40	40	4 (2.8)	0
41 – 50	35	4 (2.8)	1 (0.7)
≥51	38	2 (1.4)	0
P-Value		**0.796**	**0.467**
DOTS			
YES	83	6 (4.1)	0
NO	62	6 (4.1)	1 (0.7)
P-Value		**0.597**	**0.446**
Previous HBV Infection			
YES	21	1 (0.7)	0
NO	124	11 (7.6)	1 (1.3)
P-Value		**1.000**	**1.000**

Table 1: Assessment of Risk Factors for HBV-Infection

Variable	Number	HBsAg Positive (%)	HBeAg Positive (%)
Multiple sex partner			
YES	58	6 (4.1)	1 (0.7)
NO	87	6 (4.1)	0 (0.0)
P-Value		**0.544**	**1.000**
Previous blood transfusion			
YES	25	1 (0.7)	0 (0.0)
NO	120	11 (7.6)	1 (0.7)
P-Value		**0.692**	**1.000**
Hospitalized for past six months			
YES	14	0	0
NO	131	12 (8.3)	1 (0.7)
P-Value		**0.607**	**1.000**
Sharing of sharp objects			
YES	24	1 (0.7)	0
NO	121	11 (7.6)	1 (0.7)
P-Value		**0.691**	**1.000**

RESULTS

A total of 145 tuberculosis patients were enlisted for this study, comprising of 56.6% males and 43.4% females. They had a mean age of 42±15.9years.

Among the subjects studied, 8.3% were seropositive for HBsAg while 0.7% were seropositive for HBeAg. The patients with positive HBeAg had a CD4 count of 584cells/µL of blood and an elevated serum alanine transaminase concentration (147mg/dl). The patient was not on DOTS as at the time of the study.

There was no difference in the prevalence of HBsAg seropositivity among the different age groups. When the occurrence of HBV among the age groups was compared, it was not statistically significant.

Among the TB patients studied, 57.2% were on DOTS while 42.8% had not commenced the therapy. The subjects with no evidence of previous HBV infection had high seropositivity of HBsAg (7.6%). Among the population that had positive HBsAg only one was seropositive for HBeAg.

Among the patients on DOTS, those that had CD4 values of 500 cell/µL and above with mean value of 85.8%±28.5 had the highest HBsAg seropositivity of 2.8%, while those with values below between 499.9 -300 cells/µL none was seropositive for HBsAg. But those with values below 300cells/µL, 1.4% were seropositive to HBsAg. 7.6% of the subjects on anti-TB therapy who were co-infected with HBV had reduced CD4 T cell values below 300cells/µL.

For patients not on DOTS, a different scenario was observed. None of those with CD4 values less than 300 reacted to HBsAg while those with values between 499.9 – 300 cells/µL only one was seropositive to HBsAg with a mean of 385. However, 5 of the subjects with higher CD4 count of 500 cells/µL and above with mean value of 703.4±313.3 reacted to HBsAg.

Among the subjects on DOTS with HBV co-infection, 4.2% had elevated ALT value while 1.4% of the subjects without HBV had elevated ALT concentration.

The risk factors assessed indicated that none were statistically significant. Individuals that had multiple sexual partners and those who did not have multiple sexual partners both had HBsAg seropositivity of 4.1% respectively. Only 0.7% of those that had multiple sexual partners were seropositive to both HBsAg and HBeAg.

A total of 25 subjects had received blood transfusion. Among these, 0.7% were seropositive to HBsAg. Among the 12o subjects that had not received blood transfusion, 7.6% were seropositive to HBsAg, and among these 0.7% were reactive to HBeAg. None of the subjects had been hospitalized for the past six months were positive to HBsAg and HBeAg. However, among the 131 that had not been hospitalized, 8.3% were seropositive to HBsAg. Among

DOI: 10.5281/zenodo.11423081

the subjects that share sharp objects with other people, 0.7% were reactive to HBsAg while none was reactive to HBeAg.

DISCUSSION

The findings of our study (8.3% seroprevalence of HBsAg) agrees with that of previous studies such as 14.6% in Goiania city, Brazil (Aires *et al.,* 2012), 15.3% in Kassala, Eastern Sudan (Abdallah *et al.,* 2015). Likewise Abdul *et al.* (2013) reported a prevalence of 5.5% amongst tuberculosis patients in Pakistan while a seroprevalence of 15.5% was reported in Sokoto, Nigeria by Alo *et al.,* (2013).

The higher prevalence observed among males in this study has also been reported byprevious studies (Aires *et al.,* 2012; Abdallah *at al.,* 2013). Thishigher prevalence may be attributed to the social lifestyles of males and the higher predisposition they have of indulging in multiple sexual partners because of societal culture which makes it much easier for men to approach different potential sexual partners.

Tuberculosis and Hepatitis B virus co-infection can enhance development of hepatotoxicity and liver cirrhosis arising from either the hepatitis B virus or drug induced hepatotoxicity due to anti TB drugs. These easily produces dysfunction of the liver (Wong *et al.,* 1999; Saukkonen *et al.,* 2006).

This study observed that 4.2% of the subjects who had HBV and Tb co-infection and were on DOTS had elevated ALT concentrations. This observation may be due to the synergy in the toxicity of HBV and anti TB drugs and accentuates the thesis that HBV and tuberculosis co-infection results in rapid damage of the hepatocytes resulting to higher morbidities and mortality. This finding is in line with report from Mo *et al.,* (2014), who also identified that HBV is a risk factor for the development of abnormal liver function and mortality during anti-TB treatment.

This study strongly advocates that tuberculosis patients be informed and encouraged to have behavior modification. This is because changes in sexual practice have reduced the risk of hepatitis. Behavior modification is beneficial in developing countries such as Nigeria and hence would be effective in reducing to the barest minimum the probability of acquiring other sexual transmitted infections such as Hepatitis B virus and would aid in reducing the prevalence of tuberculosis and the chances of spreading the disease.

Tuberculosis contributes to subnormal CD4 cell levels in peripheral blood. This could be attributed to the high level of CD4 cells found in this study especially among subjects on DOTS. The continuous increase of CD4 cell count during the course of anti TB therapy suggests a reversible impact of active TB on CD4 cell homoestasis and this should be considered in the interpretation of CD4 cell counts among TB patients co-infected with either HBV or HIV (Skogmar *et al.,* 2013).

The major problem with TB and HBV co-infection is that they are both risk factors for the development of severe hepatotoxicity during treatment of both of them. The hepatotoxicity may necessitate discontinuation of treatment (Patel and Voigt 2002; Sulkowski *et al.,* 2002). HBV vaccination has been adjudged as the only effective method for preventing primary hepatic cancer, cirrhosis related to chronic HBV infection and severe hepatotoxicity during DOTS. This study therefore advocates that all TB patients upon diagnosis be mandated to take HBV vaccines to guarantee the prevention of HBV co-infection with its attendant maladies.

Conclusion

This study observed a low prevalence of HBV among TB patients. Hence, it should be mandatory that all individuals infected with tuberculosis are screened for HBV. Those who are non-reactive should be mandated to take HBV vaccines to guarantee that the infection is prevented. Further study on the interaction between CD4 T-cells in TB patients is advocated.

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DOI: 10.5281/zenodo.11423081