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Research Article

Hepatitis B Envelope Antigen (*HBeAg*) Antiginemia and the Development of Hepatocellular Diseases (HCDs): A Case Study of Kano-Nigeria

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ABSTRACT

The presence of hepatitis B envelope antigen (HBeAg) in serum indicates active viral replication in the Hepatocyte. HBeAg is thus a surrogate marker for the presence of hepatitis B virus (HBV). To determine the relationship between the positivity for HBsAg, HBeAg and development of hepatocellular diseases (HCDs). Two hundred (200) blood samples each of which were HBsAg-seropositive amongst patients attending Aminu Kano teaching hospital Kano, Nigeria, between April and November 2012, were tested for HBeAg using HBV combo kits (Cortez diagnostics). The status of HCDs was ascertained by some specific liver enzyme tests, which include alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP). The results of the study involved the screening of 200 sero-positives patients to HBsAg, out of which 34 (17.0%) were sero-positive to HBeAg. The relative risk of infection was found to be higher in males 31(15.5%) than in females 3(1.5%) for the surrogate marker. The most susceptible age group for HBeAg infection (6.0%) was found to be higher in the bracket ≤20 years. The occurrence of active, ongoing liver disease, indicated by the presence of HBeAg was significantly higher among males (P≤0.05). Analysis of the liver enzymes activity showed elevated serum level of ALT in 28(44.8%) of the HBeAg infected subjects indicating ongoing liver damage with 6(5%) perhaps developed hepatocellular diseases in relation to HBeAg. The percentage prevalence rate of HBeAg was higher in males than females and there was strong association between the positivity for HBeAg and the development of HCDs.

Keywords: Hepatitis, antigen, liver, hepatocellular, diseases, serum, blood.

INTRODUCTION

Hepatitis (plural hepatitidis), simply means injury to the liver which is characterized by the presence of inflammatory cells in tissue of the organ. The name was from ancient Greek word "heap" (ηπατ), meaning liver and suffix –"itis" meaning inflammation (Liaw *et al*., 1991). This condition can be self-limiting healing on its own, or can progress to scarring of liver. Hepatitis can be due to viral infection toxin (notably alcohol), chemical substances such as drugs especially Acetaminophene (paracetamol) which is commonly prescribe and found in over the counter or from autoimmune processes (Liaw *et al*., 1991). It might run a subclinical course when the affected person may not feel ill. The patient becomes sick and symptomatic when the disease impairs the liver functions, which include among other things, removal of harmful substances, regulation of blood composition and production of bile that helps in digestion process. Hepatitis may be acute when the hepatitis virus surface antigen last for less than six month in the blood, or chronic when it persist longer (Liaw *et al*., 1991). The condition is termed as hepatitis B when it is caused by hepatitis B virus; hepatitis A, C, D, E and F when caused by hepatitis A, C, D, E and F virus respectively (Levine *et al.,* 1994). These viruses with hepatitis B virus inclusive are collectively grouped as hepatitis viruses (Levine *et al.,* 1994). Hepatitis B is caused by hepatitis B virus (HBV) resulting from exposure to infectious blood or body fluids. Possible forms of transmission include (but not limited to) unprotected sexual contact, blood transfusion, reuse of contaminated needles and syringes and vertical transmission (i.e from mother to offspring) during child birth is also pronounced (Beasley *et al.,* 1977) . Hepatitis B can be transmitted between family members within household ,

possibly by contact of non-intact skin or mucous membrane with secretion or saliva containing hepatitis B virus or its part (Petersen *et al.,* 1976). However, at least 30% of reported hepatitis B among adults can not be associated with identifiable risk factor (Petersen *et al.,* 1976). In countries where HBV is highly endemic (hepatitis B surface antigen (HBsAg) prevalence rate of 8% or higher), most infections occur during infancy and early childhood. Infection occurs commonly in all age groups, although the high rate of chronic infection is primarily maintained by transmission during infancy and early childhood. Where endemicity is low (HBsAg prevalence rate of below 2%), infections occur in young adults, especially those belonging to known risk groups (ACIP 1990). In areas with high HBV endemicity, perinatal is the main route of transmission. Perinatal transmission is common; especially when HBV infected mothers are also HBeAg positive. HBeAg – positive mothers are more than 70% while from HBsAg – positive, HBeAg negative mothers; it is less than 10% (Nacos *et al*., 2000). Nigeria is highly endemic and the most common circumstance that leads to HBV infection in this population has not been fully elucidated. It has been reported that the exposure rate to HBV (frequency of HBeAg and anti-HBs) ranged from 59% in children aged less than 5 years to 72.5% in adults aged over 30 years, while the frequency of HBsAg alone was 40 and 10% respectively (Fakunle *et al*., 1981).

Acute hepatitis B causes liver inflammation, vomiting, jaundice and rarely death while chronic hepatitis B may eventually cause liver cirrhosis and cancer. This is mostly fatal with very poor response to current chemotherapy (Chang, 2007). A Hepatocellular disease (HCD) is a general term used in describing any case of hepatocytes (liver cells) abnormalities. HCD includes hepatocellular carcinoma (HCC), and liver cirrhosis (Chang *et al.,* 2009). Chronic hepatitis B usually result in the development of hepatocellular cirrhosis and/or carcinoma, a condition in which healthy liver tissue is replaced with death, malfunctional scar tissue (Chang *et al.,* 2009). Hepatocellular carcinoma consequently results in liver malfunction and production of pores on the liver as a result of which the increment of some specific liver enzymes level in the blood stream (Chang *et al.,* 2009).

MATERIALS AND METHODS

Study Area

The study was conducted in Kano state of the federal republic of Nigeria, which is located at north-west zone of Nigeria. Most of its area lies within Sudan savanna with its edges bordering guinea savanna in the south and Sahel savanna in the north. It is located on northern high plain of Hausa land at about latitude 12^0-$12^0$15' north and longitude $8^0$30'-$8^0$45'east, and has an elevation of 525 meters above sea level.

Study site

The study was carried out at Aminu Kano Teaching Hospital (AKTH), Kano, Nigeria from April – November 2012.

Sample Collection

The samples were collected from the subjects using 2ml syringes, which were labeled with the subjects' name/number in the general out-going patient of the laboratory. A tourniquet was tied on the arm of the subject at about three- quarter inch above the venipuncture site. The subject was asked to fist so that the veins appear more prominent, and the most prominent vein was cleaned with alcohol. The syringe's needle was then inserted into the vein with its graduation facing upward and the syringe's piston was drowning until the required amount of blood reached. The tourniquet was released and the subject's hand was then opened, cotton wool was placed over the vein puncture site and the needle was slowly removed, and the blood was properly dispensed into EDTA bottle immediately. The samples were centrifuged at 2000rpm (rpm= revolution per minute) for 5 minutes to separate the serum from the blood cells (Cheesbrough, 2005).

Rapid Detection of Hepatitis B Surface Antigen (HBsAg)

The rapid strip for HBsAg was removed from sealed package and then immersed vertically into the serum for 15 seconds, then the strip was removed from the serum and placed on flat tile surface, and the timer was started. The results were read and interpreted after 15 minutes. Positive samples were presented with two distinct red colour bands, one at the control region and the other at the test region. Negative results were presented with appearance of a single band at control region and invalid results were presented with no band appearance or with single band appearance at the test region.

Detection of Hepatitis B Envelope Antigen (HBeAg) using HBV Combo Kit

Using special kit known as HBV combo (Cortez diagnostics), which has wells where the serum are dropped and reading area. The kit has the ability to test for five different HBV antigens namely: HBsAg, anti-HBsAb, HBeAg, anti-HBeAb and anti- HBc. The kit was removed from the pouch and placed horizontally on the workbench, 3-4 drops of the serum were made in to the well of HBsAg and that of HBeAg and the results were read between 25-30 minutes.

Determination of Alanine Aminotransferase (ALT) Level

The reagent used was ALT reagent (Ramdox, laboratories, UK). It contains two reagents tagged as R_1 and R_2 which contain AL100 buffer and 2, 4- dinitrophenyl hydrazine respectively. Zero point five (0.5) ml of the sample was pipetted using micro-pipette into a sterile empty test tube, then 0.5ml of reagent I (R_1) was added to the sample, mixed and then incubated for 30 minutes at 27^0C together with reagent blank which is the mixture of 0.5ml of R_1 and 0.1ml of distilled water. Zero point five (0.5) ml of R_2 was then dispensed into each of the reagent blank and the sample mixtures, these were then mixed and allowed to stand for 20 minutes at temperature range of between 20-25^0C. zero point four (0.4) mol/L sodium hydroxide solution (0.4mol/L $NaOH_{aq}$) was prepared by dissolving 16g of $NaOH_s$ in 1L of distilled water. Then 0.5ml of 0.4mol/L $NaOH_{aq}$ was added to each of the reagent blank and the sample mixture and then mixed. The absorbance of the sample (A_{sample}) against reagent blank was read digitally using Spectrophotometer.

Determination of Aspartate Aminotransferase (AST) Level

The reagent used was AST reagent (Ramdox laboratories, UK). It also has two reagents R_1 and R_2, which consist of AS101 buffer and 2, 4- dinitrophenyl hydrazine respectively. The procedures are the same as that of ALT.

Determination of Alkaline Phosphatase (ALP) Level

The material used was alkaline phosphatase (ALP) reagent (Teco, diagnostics, USA). This consists of ALP substrate, ALP colour developer and standard. Sterile test tubes labeled with the specimens' number were used. Zero point five (0.5) ml ALP substrate was dispensed into each of the test tubes. In addition, 0.5ml of ALP substrate was dispensed into each of two different test tubes labeled reagent blank and standard. The temperatures of all the test tubes were equilibrated to 37^0C for 30 minutes. At regular intervals, 0.05ml (50µl) of the sample was added to each of the tube except that of the reagent blank and standard, instead 0.05ml of deionized water and 2-3 drops of ALP standard were added to them respectively. Following the same time intervals as above, 2.5ml of ALP colour developer was then added to all the test tubes including that of the reagent blank and the standard and then mixed gently well. The absorbance of the sample (A_{sample}) against reagent blank was read as that of the standard digitally using spectrophotometer.

RESULTS

Two hundred blood samples were used for the study, of which one hundred and forty eight (74%) were obtained from male subjects, while fifty-two (26%) were from female subjects (Table 1). Out of the 148 male samples, 31 (15.5%) were HBeAg- seropositive and 117 (58.50%) were HBeAg-seronegative while only 3 (1.5%) out of the 52 female samples were HBeAg- seropositive and 49 (24.5%) were HBeAg-seronegative (Table 1). When 166 (83%) which were HBsAg-seropositive but HBeAg-seronegative were subjected to liver enzymes test, 82, 120, and 50 were said to have high level of AL, TAST and ALP respectively (Table 3), while out of 34 (17%) that were seropositive for both HBsAg and HBeAg, 28, 30 and 18 have high level of ALT, AST and ALP respectively.

Table1: Sex related % prevalence of HBeAg

Sex	HBsAg-carriers	HBeAg (+)	%HBeAg (+)	HBeAg (-)	%HBeAg (-)	Total	%
Males	148	31	15.50	117	58.50	148	74.00
Females	52	3	1.50	49	24.50	52	26.60
Total	200	34	17.00	166	83.00	200	100.00

Key: HBeAg= Hepatitis B Envelope Antigen, HBsAg= Hepatitis B Surface Antigen

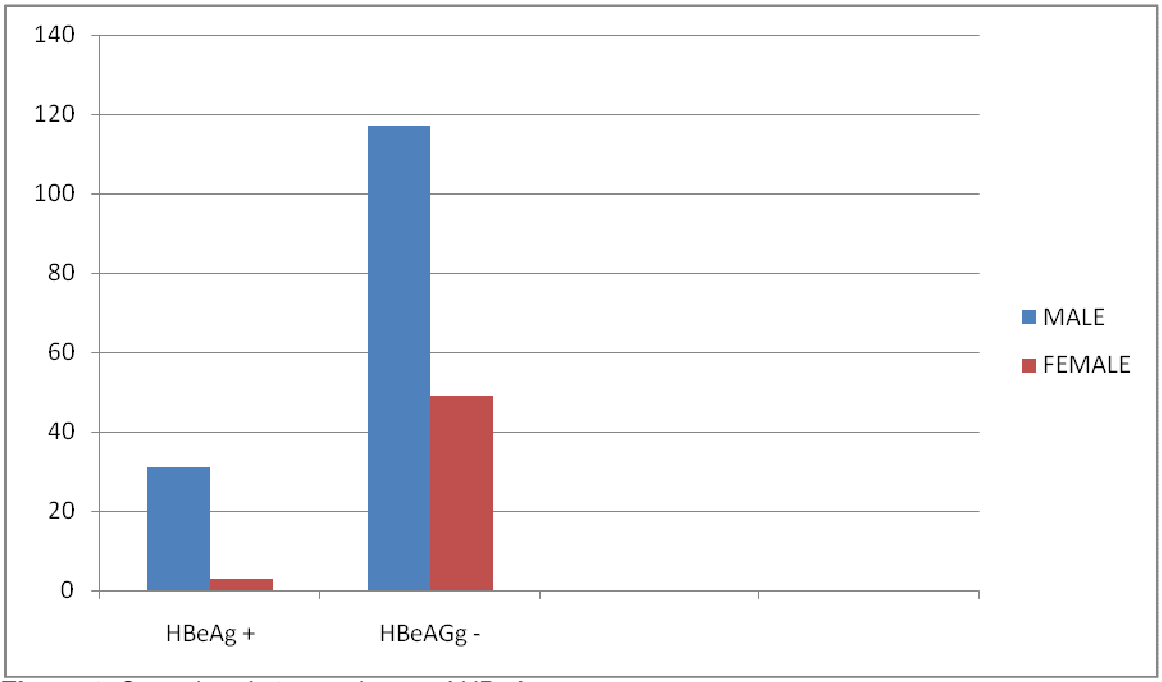

Figure 1: Sex related % prevalence of HBeAg.

Table 2: Age related % prevalence of HBeAg.

Age range	HBsAg-Carries	%	HBeAg (+)	%
≤ 20	34	17.00	12	6.00
21-30	61	30.50	6	3.00
31-40	54	27.00	3	5.00
41-50	37	18.50	4	2.00
51-60	9	4.50	6	3.00
60	5	2.50	3	1.50
Total	200	100.00	34	17.00

Key: ≤ = lessthan or equal

Table 3: Analysis of the liver enzyme test of HBeAg

Level	ALT%	AST%	ALP%
Normal	82 (49.40)	46 (27.71)	116 (69.88)
High	84 (50.60)	120 (72.29)	50 (30.12)
Total	166 (100.00)	166 (100.00)	166 (100.00)

Key: ALT= Alanine Aminotransferase, AST= Aspartate Aminotransferase, ALP= Alkaline Phosphatase

Table 4: ALT, AST and ALP levels of HBeAg seropositive samples.

LEVEL	ALT (%)	AST (%)	ALP (%)
NORMAL	6 (17.65)	4 (11.76)	16 (47.06)
HIGH	28 (82.35)	30 (88.24)	18 (52.94)
TOTAL	34 (100.00)	34 (100.00)	34 (100.00)

DISCUSSION

It has been reported that in most patients with hepatocellular carcinoma, the disease develops after the development of antibodies against HBeAg (Heyward *et al.* 1982). In this study, with all blood samples collected and assayed before the diagnosis of hepatocellular diseases the results showed that, the seroprevalence of hepatitis B envelope antigen (HBeAg) was higher among male sex with about 20.95% prevalence than that among female sex with about

5.77%. Though the reason is not yet known but it might probably be due to rapid exposure of male which render the virus become used to their physiology and anatomy (Liaw *et al.,* 1983).

Age related seroprevalence of HBeAg analysis, showed that age group ≤ 20 years with 6.00% prevalence has the highest prevalence rate. This could be due to weak or immature immune response of children. This also supports the findings of Chang (2007) who stated that in the endemic areas, Chronic infection occurs during childhood and mother to infant transmission which collectively accounts for approximately 50% of chronic infection cases.

Statistical analysis revealed that there was significant difference with regard to the development of hepatocellular diseases (HCD) among those who are seropositive for HBeAg and those that are seronegative for HBeAg, with higher prevalence among sero-positive subjects. This might be because of active replication of the virus in HBeAg-seropositive subject that increase the titer volume of the virus in blood and consequently make the virus available in sufficient amount to cause carcinoma of the liver or other liver related complications. Both indirect and direct carcinogenic mechanisms are involved in the pathogenesis of hepatocellular carcinoma induced by chronic HBV infection (Kew, 1998). HBV may induce hepatocellular carcinoma indirectly by causing chronic Necro-inflammatory hepatic disease (Chisari *et al.,* 1989). When HBV replication is sustained, as indicated by positivity for HBeAg, malignant transformation may occur as a result of continuous or recurrent cycles of Hepatocyte necrosis and regeneration. The accelerated rate of cell turnover may act as a tumor promoter through the accumulation of spontaneous mutations or DNA damage caused by exogenous factors, resulting in an increased selective growth advantage for transformed cells. The accelerated turnover rate may also result in cleavage of viral DNA at specific motifs; resulting in linear DNA that is inserted into chromosomal DNA through increased intracellular topoisomerase I activity (Wang, 1985). Active replication of HBV may also initiate malignant transformation through a direct carcinogenic mechanism by increasing the probability of insertion of viral DNA in or near proto-Oncogenic, tumor-suppressor genes, or their regulatory elements of cellular DNA23, 24. The integration of viral DNA may increase the production of transactivate protein hepatitis B X antigen, which may induce the malignant transformation of Hepatocyte, as well as bind to the p53 tumor-suppressor gene and disrupt its functions (Popper *et al*., 1987 and Kin *et al.,* 1991). High levels of ALP, AST and ALP were found in most patients who are positive for HBeAg, and in a substantial proportion of those with only HBsAg. Therefore, HBeAg is the most important predictor of the development of hepatocellular diseases.

CONCLUSION

From this study, HBeAg was more prevalent in males than females and age group of ≤20 years has the highest prevalence. This study also revealed that, there is association between the positivity of HBeAg and the development of hepatocellular diseases.

RECOMMENDATIONS

Based on the results obtained in this study, it is recommended that further studies should be carried out using larger number of specimens to determine whether the results obtained in this preliminary investigation are truly representative of the situation with regard to the potentiality of hepatitis B Viral infection as a major cause of mortality as well as morbidity world-wide. The public should be enlightened about viral hepatitis, its rate of increase and possible modes of transmission.

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