Studies on the Serological Markers of Hepatitis B Virus Infection among Children in Riyom LGA, North Central Nigeria

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ABSTRACT

Background: Globally, hepatitis B virus (HBV) infection has been identified as one of the most common infectious diseases of major health concern. This study was conducted to assess the prevalence of Hepatitis B virus infection among Children in selected communities at Riyom L.G.A. of Plateau State Nigeria.

Methodology: Two hundred (200) sera samples were collected from Pupils attending Primary Schools at three locations of study and analyzed using the HBsAg Monolisa ELISA kit and the HBV-5 panel test for the qualitative assessment of the markers of hepatitis B virus infection in human serum, plasma and whole blood.

Result: Overall result from the total samples assayed showed that, 58(29.0%) were seropositive, [{P value of 0.020}: P< 0.05] which indicates statistical significance. considering age of infection, children aged 5-9 years recorded a high prevalence of 15.0 %,[{ P value of 0.460}: P > 0.05]. Gender consideration of subjects screened showed that male subjects had a prevalence of 19.0% compared to 10.0% for Females [{P value of 0.0435}: P< 0.05]. Risk factors such as blood transfusion accounted for 1.5 %,[{ P value of 0.6138}:P > 0.05]. while subjects with traditional method of circumcision recorded a higher prevalence of 9.5% [{P value of 0.3120}:P< 0.05].Considering markers for HBV infection, findings showed that the highest rate of positivity recorded with the HBsAg showed 25% among children screened, HBeAg recorded 4.0%. Anti-HBs which indicate antibody to the HBsAg showed 35(17.5%) positivity while, Anti-HBe positivity recorded 15.0%.Similarly, Anti-HBc Positivity showed a record of 13.5% positivity.

Conclusion: The result obtained from this study showed a higher prevalence of the Hepatitis B Virus at our locations of study compared to similar studies conducted earlier within our location of study. It is strongly suggested that accurate diagnosis with effective screening of pregnant mothers be intensified, while the need for timely vaccination of children at risk be promptly embarked upon.

Key words: HBV Infection, Serological markers, Children.

INTRODUCTION

Hepatitis B virus (HBV) is a double stranded circular DNA virus; belonging to the family hepadnaviridae. [¹] The virus primarily interferes with the functions of the liver by replicating in liver cells called the hepatocytes. [²] Globally it causes about 1.2 million deaths per year.
due to its various complications including chronic hepatitis, liver cirrhosis, and liver cancer. An estimated 2 billion people have been infected with HBV, with 350-400 million of them remaining chronic carriers worldwide. [3] It has also been estimated that 25 - 30% of these chronically infected persons are at high risk of death from liver cirrhosis and cancer (WHO, 2004). [4] In Africa, the number of HBV carriers is estimated to be about 50 million representing about 10-20% of the general population and as many as 12.5 million will eventually die due to complications from hepatitis B - chronic hepatitis, cirrhosis and hepatocellular carcinoma (HCC). [5]

Hepatitis B infection is hyper-endemic in Nigeria. [6,7] In children, the infection occurs early in life and studies report hepatitis B surface antigen (HBsAg) prevalence rates of 20% while in adult population the rate varies from 10-38%. Younger age at acquisition of infection continues to be the most important predictor of chronic carriage and those who develop chronic hepatitis B have a 15 - 40% risk of developing the complications. [8,9] This chronicity is being due to their immature immune system. More than 95% of adults spontaneously recovers from an acute HBV infection as defined by clearance of the HBsAg from the blood, an effect that reflects the host’s degree of immune response. [8,10]

Most primary infections are self-limited with clearance of virus and development of immunity. However, an estimated 3% to 5% of adults and up to 95% of children develop chronic HBV infection. [11] In endemic areas, most individuals are infected by vertical transmission. [12] In Africa, more than half of the population becomes HBV infected during their life time and about 8% of inhabitants become chronic carriers; most of the infections take place during delivery or infancy. [13] Transmission occurs when infected blood or body fluid from an infected person enters the body of another who is not immune. [14,15]

Africans who are carriers of HBV are infected in early childhood, predominantly by horizontal transmission while Vertical transmission contributes 5-15% - (occurring more in those with high viral load and actively replicating virus). Prevention of vertical transmission is extremely important since HBV infection in early life usually results in a chronic carrier state. However, for probable genetic reasons, HBeAg positivity rates are much lower in African women of childbearing age. [8,16]

The global burden of the disease attributable to hepatitis B remains enormous, and this is largely due to lack of universal vaccination. Although high screening rates have been achieved among pregnant women, current efforts to identify and track infants born to HBsAg-positive mothers are inadequate. Advances in the prevention of perinatal HBV transmission will depend on improved health department identification, tracking, and case management of infants born to HBsAg-positive mothers. [17] Hepatitis B is preventable through vaccination and studies have confirmed protection following vaccination in both industrialized and non-industrialized communities. [18] Hence mass vaccination of the population should become paramount on a global scale, as this will decrease the reservoir of chronic carriers able to spread the virus.

The infectious virus consists of an outer envelope - HBsAg, the first Seromarker and one of the most useful markers of active or chronic hepatitis B infection, and an inner core made up of Hepatitis B core Antigen (HBcAg), found in acute or chronic infections and the e-antigen (HBeAg), which serves as a marker of active viral replication. [19,20] Serologic markers for the diagnosis of Hepatitis B Virus (HBV) infection involves measurement of a panel of distinct HBV specific antigens and host antibodies that react to these antigens.
In general, the panel of responses can determine whether a patient is susceptible to infection, immune as a result of resolved infection, immune as a result of vaccination, acutely infected or chronically infected. [23]

**MATERIALS AND METHODS**

**Study area**

The study was carried out at selected communities in Riyom Local Government areas of Plateau state, Nigeria.

**Ethical consideration**

Ethical approval for this research work was sought and obtained from the National Blood Transfusion Services (NBTS), North central zone Center-Plateau State Specialist Hospital-Jos Nigeria.

**Informed consent**

Informed consent - both verbal and written, were obtained from the child and/or the parent (s). They were however duly educated on the need for and benefits of the study.

**Study Population**

Subjects studied include two hundred (200) children aged 0-11 years. A well-structured questionnaire was designed and administered for the study. This was used to obtain social and demographic information of consenting participants.

**Inclusion and Exclusion criteria**

Subjects who gave informed consent through their parents or guardians and are asymptomatic by routine screening, were included in the study, subjects who had once been vaccinated with the required three doses of the vaccine and those who declined to offer consent were excluded from the study.

**Collection and processing of specimens**

Three millilitre (3ml) of blood samples were collected aseptically by venipuncture. Each blood sample obtained was transferred into a carefully labeled plastic microtitre tube containing ethylene diaminetetraacetic acid (EDTA) and stored in the refrigerator at 4°C. Each resultant supernatant (Plasma) was carefully decanted into a new labeled tube and stored at -20°C prior use.

**Laboratory Analysis**

Assay of collected sample was carried out by HBsAg ELISA test reagent manufactured by Biorad Laboratories. Monolisa HBsAg ULTRA assay is a one-step enzyme Immunoassay technique of the “Sandwich” type for the detection of the surface antigen of the Hepatitis B virus (HBsAg) in the serum or plasma.

**Screening using the 5-panel test kits for Serological markers**

HBV-5 panel test for the qualitative assessment of the markers of hepatitis B virus infection in human serum, plasma and whole blood. The HBV Panel Test is an Immunochromatographic assay method to quickly detect five major markers of HBV infections, HBsAg, Anti-HBs (HBsAb), Anti-HBc (HBcAb), HBeAg and Anti-HBe (HBeAb) in human blood specimens.

**Statistical analysis**

Data obtained were subjected to statistical analysis. Comparison of numerical variables between the study groups was done using Regression analysis to show relationship of the risk factors, the confidence intervals and the odd ratios. For independent and categorical data, X² tests was performed-value of less than 0.005 (P<0.005) was considered significant. All statistical calculations were done using the Graph pad prism statistical software and the statistical package for social sciences (SPSS) to determine any significant relationship between infection rate, gender, age and risk factors.

**RESULT**

Prevalence of HBV among the Children screened showed that Male subjects recorded a prevalence of (38)19.0% out of the 110(55.0%) subjects screened in this category. Compared to Females subjects with a prevalence of 20(10.0%) out of the 90(45.0%) screened, showing a statistical significance < 0.005.
Based on Age, Children aged 5-9 years recorded the highest prevalence of 30(15.0%). This prevalence on age of subjects in this category was found to be statistically insignificant, (Table 2). Risk factors among the Children screened showed that 19 (9.5%) prevalent was recorded among children that had history of circumcision with traditional birth attendance, this is closely followed by those who had their circumcision at Local Health Centers with a prevalence of 15(7.5%). (Table 3). Prevalence of 3(1.5%) was recorded among children with history of blood transfusion and those with family history of HBV Infection. All the risk factors are statistically insignificant, P>0.005. (Table 4). Considering markers for HBV infection, findings showed that the highest rate of positivity recorded with the HBsAg showed 25% among children screened, HBeAg recorded 4.0%. Anti-HBs which indicate antibody to the HBsAg showed 35(17.5%) positivity while, Anti-HBe positivity recorded 15.0%. Similarly, Anti-HBc Positivity showed a record of 13.5% positivity. (Table 5).

### Table 1: Distributions of Children screened based on Sex

<table>
<thead>
<tr>
<th>Sex</th>
<th>Total Number Examined (%)</th>
<th>No. Positive (%)</th>
<th>No. Negative (%)</th>
<th>p-value</th>
<th>OR</th>
<th>95% C.I.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>110 (55.0)</td>
<td>38 (19.0)</td>
<td>72 (36.0)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>90 (45.0)</td>
<td>20 (10.0)</td>
<td>70 (35.0)</td>
<td>0.0435</td>
<td>1.842</td>
<td>0.98-3.48</td>
</tr>
</tbody>
</table>

### Table 2: Distributions of Children screened based on Age

<table>
<thead>
<tr>
<th>Age</th>
<th>Total Number Examined (%)</th>
<th>No. Positive (%)</th>
<th>No. Negative (%)</th>
<th>p-value</th>
<th>OR</th>
<th>95% C.I.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-4</td>
<td>41 (20.5)</td>
<td>9 (4.5)</td>
<td>32 (16.0)</td>
<td>0.83</td>
<td>0.34-2.05</td>
<td></td>
</tr>
<tr>
<td>5-9</td>
<td>84 (42.0)</td>
<td>30 (15.0)</td>
<td>54 (27.0)</td>
<td>1.64</td>
<td>0.83-3.25</td>
<td></td>
</tr>
<tr>
<td>10-14</td>
<td>75 (37.5)</td>
<td>19 (9.5)</td>
<td>56 (28.0)</td>
<td>0.460</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

### Table 3: Distributions of Children screened based on risk Factors

<table>
<thead>
<tr>
<th>Exposure to risky lifestyles/behaviors</th>
<th>Total Number Examined (%)</th>
<th>No. Positive (%)</th>
<th>No. Negative (%)</th>
<th>p-value</th>
<th>OR</th>
<th>95% C.I.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>16 (8.0)</td>
<td>3 (1.5)</td>
<td>13 (6.5)</td>
<td>0.5767</td>
<td>0.54</td>
<td>0.15-1.98</td>
</tr>
<tr>
<td>No</td>
<td>184 (92.0)</td>
<td>55 (27.5)</td>
<td>129 (64.5)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 4: Distributions of Children screened based on Clinical History

<table>
<thead>
<tr>
<th>Clinical history</th>
<th>Total Number Examined (%)</th>
<th>No. Positive (%)</th>
<th>No. Negative (%)</th>
<th>p-value</th>
<th>OR</th>
<th>95% C.I.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>15 (7.5)</td>
<td>3 (1.5)</td>
<td>12 (6.0)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>185 (92.5)</td>
<td>55 (27.5)</td>
<td>130 (65.0)</td>
<td>0.5816</td>
<td>0.59</td>
<td>0.116-2.18</td>
</tr>
</tbody>
</table>

### Table 5: Overall Prevalence of HBV markers among children screened

<table>
<thead>
<tr>
<th>CATEGORY</th>
<th>HBsAg</th>
<th>HBeAg</th>
<th>Anti- HBs</th>
<th>Anti- HBe</th>
<th>Anti- HBc</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children</td>
<td>Positive</td>
<td>50 (25.0)</td>
<td>8 (4.0)</td>
<td>35 (17.5)</td>
<td>30 (15.0)</td>
<td>27 (13.5)</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>159 (79.5)</td>
<td>192 (96.0)</td>
<td>165 (82.0)</td>
<td>170 (85.0)</td>
<td>173 (86.5)</td>
</tr>
</tbody>
</table>
DISCUSSION

In this study, two hundred (200) children were screened, overall prevalence of 58(29.0%) was recorded, which is much higher than the outcome of a similar study conducted by Ndako et al.,[24] in Kuru Community, North-Central Nigeria with a prevalence rate of 35(9.7%). Furthermore, some studies conducted in Nigeria by Sirisena et al.,[25] Bukbuk et al.,[26] corroborated the increasing prevalence of the HBV.

The value obtained in this study was however higher than 7.6% prevalence reported in a study among primary school children in Nnewi, Nigeria, Chukwuka et al. [27] and 6.7% among Saudi Arabian children Al-Faleh et al.[28] This study also showed a higher prevalence compared to a similar study among children attending a tertiary health institution in Niger Delta, Nigeria by Alikor and Erhabor [29] where 12.4% prevalence was recorded. However a much more lower prevalence of 4.6% was obtained in another study by Uleanya and Obidike, [30] among children attending outpatient clinic (CHOP) of the University of Nigeria Teaching Hospital, Ituku.

Age distribution of HBV infection in this study showed that, among different age groups screened, subjects aged 5-9 had the highest prevalence rate of 15.0%, compared to 3.9% highest prevalence recorded among children aged 7-9 years as obtained in the work of Ndako et al.,[24] In a similar study by Uleanya and Obidike, [30] the least prevalence was observed among children aged 1-5 years (2%) while the highest prevalence of HBsAg was observed among those aged 6-10 years with (6.5%) prevalence. Studies by Sirisena et al.[25] Bukbuk et al.,[26] corroborated the increasing prevalence with age and showed the overall prevalence to be above 9.7 %.

The age of acquiring infection remains a major determinant of incidence and prevalence rates. It is believed that 25.0% of children are infected at 1 - 5 years of age; while about 1.0 - 5.0% of those infected are older children that end up as carriers Goldstein [31] These carriers, though asymptomatic, might serve as reservoir of the virus and medium for spreading infection among other children Odusanya.[32] However, there was no statistical significant relationship between age and the viral infections in this study.

Considering the prevalence of HBsAg based on gender, this study showed that gender is critical to the acquisition of HBV infection. 38(19.0%) of the males tested were positive for the infection compared to 20(10.0%) females. This is similar to the work of Bukbuk et al.,[26] who found that HBV antigenemia was higher among male subjects studied with 47.2 % positivity compared to female subjects with 38.1%. Another similarity was observed in the work of Isa et al.,[33] who showed that, males were more prevalent with hepatitis B virus infection with (10%) than their female counterparts with (6.7%). However, the result obtained from this study disagrees with the work of Donbraye et al.,[34] who reported a higher female prevalence of (15.4%) compared to the male subjects with (12.7%).

Similarly, the result obtained by Uleanya and Obidike [30] recorded a slightly higher prevalence of HBsAg among the females subjects studied. Gogos et al. [35] concluded that males are known to increase the risk of Serum hepatitis infection. This is was corroborated by the results obtained from this study where risk factor in males were considered with regards to the rate of HBV infection among male subjects that had the traditional method of circumcision recorded a higher rate of infection rate compared to male that had their circumcision in clinics. Though the prevalence rates in this study was similar with the findings of Emechebe[36] in Alikor [37] and Bukbuk [26] who observed a higher prevalence among males. Considering risk factor, children with history of HBV infection in the family recorded 3(1.5%) positivity, compared to those without any history of HBV infection. Similarly, Findings from this work showed that
children positive for HBsAg with history of HBV infection in the family recorded 15(7.5%) positivity. This suggests that they may have contacted the virus from their mother, family members or peer groups. It has been shown that children can acquire HBV infection during delivery or post-partum through breast feeding or from chronic carrier mothers, Agbede et al. [38] and through contact among siblings or children of poorer and larger families, Toukan et al. [39].

It has been shown that children can acquire HBV during delivery or post-partum, through breast feeding or from chronic carrier mothers (Agbede et al., [38]) and Wolf [39] added that HBV could be transmitted through infected family members while children without HBV history in the family could have contacted the virus from other predisposing factors. The social class of the parents in this study was not significantly associated with HBsAg positivity. This may be because of equal exposure to the risk factors of HBV, among children of different social classes.

However, it has been suggested that the higher the social class, the lower the number of children positive to HBsAg. This could be because people in the lower socioeconomic class are more likely to indulge in activities that may promote infection with HBV such as alternative medicine, share sharp objects and toothbrushes. This is similar to the findings of Emechebe [36] Furthermore, serological evidence of previous HBV infections varies depending on age and socioeconomic class Ezegbudo et al. [41].

Other risk factors such as sharing of toothbrush among siblings or household members16 (8.0%) prevalence rate was recorded, this was found to be statistically insignificant, however the alkaline nature of the saliva may be a contributing factor at reducing transmission rate of the virus through bite. Meanwhile, an intraoral trauma during brushing could be the source of transmission; making the chance of transmitting the virus through this route is likely substantial as such sharing is most likely to occur over prolonged time period. This finding is similar to the work of Nwokediuko. [42]

Liver enzyme analysis on HBsAg seropositive subjects 59 (100%), showed that 55(93.2%) had normal ALT levels while 10(16.9%) had elevated ALT levels. This indicates the risk of HBV-related liver disease such as hepatocellular carcinoma or eventual liver cancer in the nearest future if no prompt attention is sought by these positive subjects. World health organization WHO. [43] stated that about 25% of adults who become chronically infected during childhood die from HBV-related liver cancer or cirrhosis. Uneke and Ogbu [44] reported that at the stage of non-replication the ALT levels are within the reference range while HBsAg may still be actively replicating.

In the study conducted by Tsai et al, [45] Liver function tests are used to determine if the liver has been damaged or its function impaired among the children that tested positive for the HBV, ALT result showed 6.9% abnormality. ALT levels have been correlated positively with liver inflammation, while patients with persistently normal ALT levels had significantly lower liver damage compared with patients with either intermittent or persistently elevated ALT Levels. [46] Therefore measurement of aminotransferase levels by serial observations and analysis remain the most common and convenient way to identify liver inflammation in patients particularly with chronic HBV infection.

From this study the highest rate of positivity recorded involved the HBsAg with 25% among children. According to Lee [47] The presence of HBsAg for longer than 6 months after acute infection indicates chronic infection. The detection of HBsAg and absence of IgM anti-HBc in a single serum specimen also generally indicates chronic HBV infection. Shortly after the appearance of the HBsAg, the hepatitis B e antigen (HBeAg) generally becomes evident.
(Ganem and Prince, [48] although serum HBV DNA assays will show the presence of HBV DNA prior to the appearance of HBsAg or HBeAg, with HBV DNA levels. Rehermann et al. [49] The HBeAg recorded in this study showed 4.0% among the children screened. Previous study reported (Yang et al [50]) the presence of HBeAg in serum indicate active viral replication.

The continued presence of HBeAg generally reflects higher HBV DNA levels and greater infectiousness. Some patients with chronic HBV infection may have resolution of their HBeAg along with appearance of anti-HBe, and this usually correlates with low (or absent) HBV levels and relatively normal levels of hepatic aminotransferase levels. Lee. [21] Anti-HBe positivity among the Children screened showed a record of 15.0% positivity. Anti-HBs which indicates antibody to the HBsAg showed 35(17.5%) positivity for those patients who resolve their infection, HBsAg disappears at about 3 to 6 months, often just prior to the detection of antibodies to hepatitis B surface antigen (anti-HBs).

The presence of anti-HBs following acute infection generally indicates recovery and protective immunity against re-infection. In addition, patients with resolution of infection have disappearance of HBeAg and development of antibodies to hepatitis B e antigen (anti-HBe). Patients with resolved infection have persistence of anti-HBc for life, but about 4 to 6 months after the appearance of anti-HBc, the total anti-HBc predominantly consists of IgG. Rehermann, [49] Earlier studies Zhang et al, [46] reported that the history of vaccination could be attributed to HBsAb positivity, which should increase the confidence of the population on immunization process. Anti-HBc Positivity showed that children screened recorded 13.5%, while Anti-HBc recorded a positivity of 9.3% among subjects aged 5-9 years, About the time that clinical symptoms develop, antibody to hepatitis B core antigen (anti-HBc) appears, primarily detectable as the IgM class (IgM anti-HBc) Mast et al, [51]

CONCLUSION
The prevalence rate of Hepatitis B virus recorded from this study is alarming going by the predisposing risk factors outlined which calls for a prompt enlightenment on the various risk factors that can predispose our study subjects to HBV infections. It is most disturbing that most parents and guardian of the subjects screened had no knowledge of hepatitis B virus (HBV), which emphasizes the need for public enlightenment campaign coupled with routine screening, prompt vaccination regimen and management of infected individuals, these measures would help reduce the cycle of transmission in the population.

REFERENCES
7. Baba MM, Ajayi BB, Ekanem IA. Prevalence of hepatitis B surface antigen among patients suspected of


27. Chukwuka, J.O., Ezechukwu, C.C., Egbononu, I. and Okoli, C.C. Prevalence of Hepatitis B Surface Antigen in Primary School Children in...


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