STUDIES ON THE SEROLOGICAL MARKERS OF HEPATITIS B VIRUS INFECTION AMONG CHILDREN IN A COMMUNITY NORTH CENTRAL NIGERIA.

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 PRESENTED BY: JAMES NDAKO A. (PhD) AT THE AT THE 39TH ANNUAL CONFERENCE AND GENERAL MEETING OF THE NIGERIAN SOCIETY FOR MICROBIOLOGY (NSM) CONFERENCE (UNNNsukka ,2016)

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 (Prescott *et al.*, 2008). The virus primarily interferes with the functions of the liver by replicating in liver cells called the hepatocytes (Kramvis *et al.*, 2005).
- 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 (Lavanchy, 2004).
- It has also been estimated that 25 30% these chronically infected persons are at high risk of death from liver cirrhosis and cancer (WHO, 2004). 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) (Kire, 1996).

INTRODUCTION

- Hepatitis B infection is hyper-endemic in Nigeria (Baba *et al.*, 2000 ; Sirisen *et al.*, 2002). 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 (Custer *et al.*, 2004;Yazigi *et al.*, 2007)[.]
- This chronicity being due to their immature immune system. More than 95% of adults spontaneously recover 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, (Thio *et al.*, 2002 ;Yazigi *et al.*, 2007).

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 (CDC,1994)[.]

Hepatitis B is preventable through vaccination and studies have confirmed protection following vaccination in both industrialized and non-industrialized communities (Alexander *et al.*, 2006)[.] 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.

Materials and Methods

Study area: The study was carried out at selected communities in Riyom Local Government area 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 0-11years. 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 diamine tetraacetic 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 Appropriate statistical analysis.

RESULTS

- 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 Female subjects with a prevalence of 20(10.0%) out of the 90(45.0%) screened, showing a statistical significance<0.005.(Table1).</p>
- Based on Age, Children aged 5-9years 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 2)

- 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>0.05.(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).

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

Table 2: Distributions of Children screened based on Age.

	Total Number Examined (%)	No. Positive (%)	No. Negative p-va (%)	lue OR	95%C.I.
Age (years)					
0-4	41 (20.5)	9 (4.5)	32 (16.0)	0.83	0.34-2.05
5-9	84 (42.0)	30 (15.0)	54 (27.0)	1.64	0.83-3.25
10-14	75 (37.5)	19 (9.5)	56 (28.0) 0.40	50 1	

Total Number	No	No Negative	a n value	OR	959%C T	
Examined (%)	Positive (%)	(%)	e p-value	UK	9370C.I.	
2.505.						-
v						
15 (7.5)	3 (1.5)	12 (6.0)		1		
	185 (92.5)	55 (27.5)	130 (65.0)		0.5816 0.59 0.116-	2.18
			1.0000000000000000000000000000000000000			
8 (4.0)	3 (1.5)	5 (2.5)		1		
192 (96.0)	55 (27.5)	137 (68.5)	0.6138	1.50	0.35-6.47	
	1917-01-020	10.000 (2007)				
4 (2.0)	2 (1.0)	2 (1.0)		1		
196 (98.0)	56 (28.0)	140 (70.0)	0.6280	2.50	0.34-18.19	
15 (7.5)	5 (2.5)	10 (5.0)		1		
185 (92.5)	53 (26.5)	132 (66.0)	0.5873	1.25	0.41-8.82	
	Total Number Examined (%) W 15 (7.5) 8 (4.0) 192 (96.0) 4 (2.0) 196 (98.0) 15 (7.5) 185 (92.5)	Total Number Examined (%) No. Positive (%) W 15 (7.5) 3 (1.5) 185 (92.5) 8 (4.0) 3 (1.5) 192 (96.0) 3 (1.5) 55 (27.5) 4 (2.0) 2 (1.0) 196 (98.0) 2 (1.0) 56 (28.0) 15 (7.5) 5 (2.5) 185 (92.5) 53 (26.5)	Total Number Examined (%) No. Positive (%) No. (%) Negative (%) W 15 (7.5) 3 (1.5) 12 (6.0) 185 (92.5) 55 (27.5) 8 (4.0) 3 (1.5) 5 (2.5) 192 (96.0) 55 (27.5) 137 (68.5) 4 (2.0) 2 (1.0) 2 (1.0) 196 (98.0) 56 (28.0) 140 (70.0) 15 (7.5) 5 (2.5) 10 (5.0) 185 (92.5) 53 (26.5) 132 (66.0)	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$

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

KEY: OR = Odd ratio.CI = Confidence Interval

Table 5 - Overall Prevalence of HBV markers among children screened

		HBsAg	HBeAg	Anti- HBs	Anti- HBe	Anti- HBc	P-Value
CATEGORY	Results	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	
Children	Positive	50 (25.0)	8 (4.0)	35 (17.5)	30(15.0)	27(13.5)	
	Negative	159(75.0)	192(96.0	165(82.0	170(85.0)	173(86.5)	
	Total	200(100)	200(100)	200(100)	200(100)	200(100)	P<0.0001

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.*, 2010 in Kuru Community, North-Central Nigeria with a prevalence rate of 35(9.7%).
- Furthermore, some studies conducted in Nigeria by Sirisena *et al*,2002; Bukbuk *et al*.,2005) corroborated the increasing prevalence of the HBV.

DISCUSSION

- 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.*, 2004) and 6.7% among Saudi Arabian children (Al-Faleh *et al.*, 1992)[.]
- This study also showed a higher prevalence compared to a similar study among children attending a tertiary health institution in Niger Delta, Nigeria (Alikor, *et al.*, 2007) where 12.4% prevalence was recorded.
- However a much more lower prevalence of 4.6% was obtained in another study by Uleanya and Obidike, 2015 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.*, 2010.
- In a similar study by Uleanya and Obidike,2015 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.*, 2002;Bukbuk *et al.*, 2005,corroborated the increasing prevalence with age and showed the overall prevalence to be above 9.7%.

DISCUSSION

- 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, 2002).
- These carriers, though asymptomatic, might serve as reservoir of the virus and medium for spreading infection among other children (Odusanya *et al.*, 2005).
- 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.*, 2005 who found that HBV antigenaemia 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.*, 2015 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.*, 2014 who reported a higher female prevalence of (15.4%) compared to the male subjects with (12.7%).

- 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, and through contact among siblings or children of poorer and larger families(Agbede *et al.*, 2007)

- 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 *et al.*, 2008
 Furthermore, serological evidence of previous HBV infections varies depending on age and socioeconomic class (Ezegbudo *et al.*, 2004)[.]

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 majority of 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

SELECTED REFERENCES

- Baba MM, Ajayi BB, Ekanem IA. (2000). Prevalence of hepatitis B surface antigen among patients suspected of liver diseases in a Nigerian hospital. Niger Postgrad Med Journal,7:91—95.
- Kramvis A, Kew M, François G 2004). March."Hepatitis B virus genotypes".2005; *Vaccine* 23 (19):2409–23. doi:10.1016/j.vaccine.10.045.PMID 15752827.
- Lavanchy D. (2004). Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. Journal of Viral Hepatitis, 11:97—107.
- Kire CF. (1996). The Epidemiology and Prophylaxis of Hepatitis B in sub-Africa: a View from tropical and subtropical Africa. *Gut; 38: S5-S126*.
- Prescott LM, Harley JP and Klein DA Microbiology. 2008; (7th edn), McGraw Hill, New York pp 936.
- Sirisena ND, Njoku MO, Idoko JA. (2002). Carriage rate of hepatitis-B surface antigen (HBsAg) in an urban community in Jos, Plateau State, Nigeria. Niger Postgrad Med Journal,9:7—10.
- World Health Organization (WHO) 2004. Advanced Immunization Management (AIM). Hepatitis B.
 [Cited 2005 August 19]. Available from: <u>www.who.int/mediacentre/</u> factsheets/fs204/en/.
- Yazigi N, Balistrere WF. (2007). Viral Hepatitis. In: Kliegman RM, Behrman RE, Jenson HB, Stanton
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