

SEROPREVALENCE OF HERPES SIMPLEX VIRUS TYPE-1 IGG ANTIBODIES IN CHILDREN (0-5 YEARS) ATTENDING AHMADU BELLO UNIVERSITY TEACHING HOSPITAL (ABUTH), ZARIA, NIGERIA.

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Abstract

Herpes simplex encephalitis (HSE) caused by HSV-1 is considered the most common form of fatal sporadic encephalitis in the United States with annual incidence estimated to be 1 in 200,000 patients. Neonatal herpes is a potentially devastating consequence of perinatal transmission of HSV with significant morbidity and mortality. Approximately 1500-2200 neonates with HSV-1 are identified annually in the United State. This study determined the seroprevalence of HSV-1 IgG antibodies in children less than 5 years attending Ahmadu Bello University Teaching Hospital (ABUTH), Zaria. A total of 125 blood samples was collected and serum obtained after centrifugation of blood samples was screened for presence HSV-1 antibodies (IgG) using commercially available Enzyme Linked ImmunoSorbent Assay (ELISA) kit. Out of the 125 blood samples screened 80 representing 64.0% were seropositive. The association of HSV-1 seropositivity in relation to age, sex, and other demographic factors was obtained by the use of structured questionnaires administered to parent or caregivers. From this study, HSV-1 childhood infection in ABUTH was found to be associated with age ( $\chi^2 = 21.180$ ,  $df = 4$ ,  $P = 0.001$ ), children's educational status ( $\chi^2 = 9.37$ ,  $df = 1$ ,  $P = 0.031$ ) and clinical symptoms ( $\chi^2 = 11.94$ ,  $df = 5$ ,  $P = 0.036$ ) presented by the children. The high prevalence of HSV-1 infection observed could be attributable to the mode of transmission of the virus, implying that 36% were still susceptible to this infection. These pockets of unimmunized children are still at risk of contracting the infection

and subsequently coming down with the severe and devastating manifestation of the disease.

Keywords: *Seroprevalence, Herpes Simplex Virus Type-1, Children, ELISA, Zaria, Nigeria.*

#### Background to the Study

Herpes Simplex Virus (HSV) belongs to the family *Herpesviridae*, it is a large enveloped DNA virus of icosahedral symmetry divided into two types, HSV-1 and HSV- 2 (Brooks *et al.*, 2010; Willey *et al.*, 2011). Primary infection is followed by seroconversion and the establishment of latent infection in nerve ganglia while recurrent infections occur periodically due to virus reactivation (Vyse *et al.*, 2000). The major clinical manifestations associated with HSV-1 infections are gingivostomatitis, keratitis and conjunctivitis, vesicular eruptions of the skin, encephalitis, eczema and lethal infections of newborns (Fusun *et al.*, 2007). Neonates have poor cell mediated immunity and therefore are at increased risk of disseminated infection, if exposed to the HSV in the perinatal period (Leung and Sacks, 2003). Neonatal herpes is a potentially devastating consequence of perinatal transmission of HSV, with significant morbidity and mortality (Brooks *et al.*, 2010; Leung and Sacks, 2003). Herpes simplex encephalitis (HSE) caused by HSV-1 infection is the most common cause of fatal sporadic encephalitis and without treatment 70% of paediatric patients die (Ibrahim *et al.*, 2005); with serious neurological sequelae among those that survive (Ward *et al.*, 2011).

Herpes simplex virus type-1 causes oral, labial, and occasionally facial lesions, initial infection is the most severe with ulcerative, painful stomatitis that usually occurs in children and is often associated with fever, anorexia and local oedema of oral mucosa interfering with swallowing (Amir *et al.*, 1999). The virus may reactivate in the conjunctiva and reoccurrences of HSV-1 results in scarring, thickening of the cornea and blindness (Liesegang, 2001; Davidson, 2002). Herpes simplex virus type 1 infections are second only to trauma as cause of corneal blindness in the United States (Brooks *et al.*, 2010). Babies with neonatal herpes infection may develop disseminated generalized herpes with many affected organs, including the central nervous system (CNS), intravascular coagulopathy, hepatic and adrenal necrosis, pneumonitis and/or encephalitis followed by permanent neurological sequelae if the patient survives (Waggoner-Fountain and Grossman, 2004; Ward *et al.*, 2011). Congenital infection may induce malformations such as microcephaly or microphthalmia, or other symptoms such as jaundice, hepatosplenomegaly, bleeding diathesis, seizures, irritability, chorioretinitis and herpetic vesicles of the skin (Tjotta and Hoddevik, 2002).

Herpes Simplex Viruses Type 1 is not a reportable disease in Nigeria; therefore there is dearth of data on its seroprevalence in neonates and children. The study was conducted to determine the seroprevalence of HSV-1 infection in children 0-5 years attending Ahmadu Bello University Teaching Hospital (ABUTH) Zaria and to analyze factors associated.

## Materials and Methods

### Study Area

This study was conducted in Ahmadu Bello University (ABUTH), Zaria. ABUTH is located in Mili goma village in Giwa local government area of Kaduna State. Kaduna State occupies part of the north western region of Nigeria It shares boundaries with Kano state, Niger state, Nasarawa state, Federal Capital Territory, Abuja and Katsina state. The Global location of Kaduna state is between 06<sup>o</sup> and 113<sup>o</sup> north of the equator and occupies an area of approximately 48,732.2 square kilometers. ABUTH is the major referral center of the state.

### Study Design

This study is a cross-sectional study to determine the prevalence of serum IgG against HSV-1 infection among children between ages of 0-5 years. Information on the children was obtained through questionnaire.

### Ethical Approval

Approval for the study was obtained from the ethical committee of the hospital and consent for the participation of the children in the study was obtained from parents or care givers.

### Study Population

A total of 125 blood samples were randomly collected from male and female children 0-5 years attending Ahmadu Bello University Teaching Hospital (ABUTH) Zaria from April 2011 to July 2011.

### Sample Collection and Processing

Using a sterile disposable syringe, 3ml of venous blood was collected aseptically by a clinician and dispensed into a plain sterile sample bottles and transported safely to the laboratory. The blood samples were centrifuged at 2,500 rpm and sera collected into clean, sterile dry plain sample bottles using a clean dry Pasteur pipette. The sera were stored at -20<sup>o</sup>C until needed for analysis (Cheesbrough, 2000). Prior to the sample collection, socio-demographic and clinical information of the children were obtained using self designed questionnaires administered to parents or care givers who consented to the study.

### Analysis of Sera by Enzyme-Linked Immunosorbent Assay

The sera were tested for the presence of HSV-1 IgG antibodies using a commercially available IgG enzyme-linked Immunosorbent assay (ELISA) Kit manufactured by DIAGNOSTIC AUTOMATION, INC. USA. The ELISA uses HSV-1 antigens for the detection of anti-HSV-1 IgG antibodies in serum. The absorbance was read at 450 nm using an ELISA micro titer plate reader (Sigma Diagnostic). The presence or absence of anti-HSV-1-specific IgG antibodies in the test samples was calculated according to the manufacturer's instructions. Results were obtained by comparing the antibody titers with the cut off values of the positive and negative controls.

### Statistical Analysis

Data generated from questionnaires and results obtained from the laboratory analysis were entered into Microsoft Excel and reduced to percentages by Descriptive Statistics. Special package for social sciences (SPSS) Version 17.0 was used to analyse the data. Chi square was used to determine association between HSV-1 infection and the various parameters studied at 95% Confidence Interval (CI).

### Results

Out of the 125 blood samples screened for presence of HSV-1 IgG antibodies, 80 representing 64.0% were seropositive. The results obtained were further analysed based on different age groups. Age group 0-12 months recorded a prevalence of 52.8%, while age groups 13-24 months, 25-36 months, 37-48 months and 49-60 months recorded prevalences of 41.7%, 91.7%, 87.6% and 78.9% respectively (Table 1). Chi Square showed a highly significant statistical association ( $\chi^2 = 21.180$ ,  $p=0.001$ ) between the prevalence of the infection and age. There was no statistically significant association between the prevalence of HSV-1 IgG antibodies and gender but a higher prevalence was observed in male (69.1%) than female (59.4%). Children who were in school recorded a higher prevalence (80.6%) than those who were not in school (58.5%). Children born via normal delivery (spontaneous vaginal delivery) recorded seroprevalence of 67.0% while children born via caesarian section recorded a seroprevalence of 43.8% (Table 3.0). Eight (8) out of nine (9) of the children who had orofacial lesions at the time of visit to the hospital were seropositive representing a seroprevalence of 88.9%. All the children who presented to the hospital with skin infection and conjunctivitis were seropositive to HSV-1 IgG antibodies. Children who had infections like sickle cell anaemia and respiratory tract infection recorded the lowest prevalence of 55.0% (Table 3.0).

Table 1.0: Distribution of HSV-1 IgG antibody in relation to age in ABUTH, Zaria

Age in Months	Number. of Sample tested	Number of samples Positive	% Positive
0 -12	34	18	52.9
13 -24	36	15	41.7
25 -36	12	11	91.7
37 -48	24	21	87.6
49 – 60	19	15	78.9
Total	125	80	64.0

(  $\chi^2 = 21.180$ ,  $df=4$ ,  $p=0.001$ )

Table 2.0: Distribution of HSV-1 IgG antibody in relation to sex in ABUTH, Zaria

Sex	Number of Sample tested	Number of samples positive	% Positive
Male	55	38	69.1
Female	70	42	59.4
Total	125	80	64.0

(  $\chi^2 = 1.105$ ,  $df=1$ ,  $p=0.293$ )

Table 3.0: Risk factor associated with the distribution of HSV-1 IgG antibody in ABUTH,

Variables	Number of Sample tested	Number of samples positive	Percentage Positive	$\chi^2$	p-value
Educational status of children					
In school	31	25	80.6	4.957	0.026
Not in school	94	55	58.5		
Nature of Delivery					
Normal delivery	109	73	67.0	3.266	0.094
Caesarean section	16	7	43.8		
Clinical symptoms					
Orofacial lesions	9	8	88.9		
Skin infection	10	10	100.0		
Conjunctivitis	2	2	100.0	11.941	0.036
Febrile illness	41	26	63.4		
Jaundice	43	23	53.5		
Others	20	11	55.0		
Total	125	80	64.0		

## Discussion

Our study determined seroprevalence of HSV-1 IgG antibody collected from 125 children within the ages of 0 and 5 years in ABUTH, Zaria. Results showed prevalence of 64.0%. The high prevalence obtained may be due to the fact that the hospital is a tertiary health care centre and people including children with clinical courses are more likely referred. The prevalence obtained from this study is similar to prevalence of 62.6% obtained in atopic children in Turkey (Fusun *et al.*, 2007). The results obtained is higher than those reported in children from Germany (31%), Sweden (31%), Israel (38%), England (20%), Eritrea (59%) and Syria (55%) (Kangro *et al.*, 1994; Ghebrekidan *et al.*, 1999; Ibrahim *et al.*, 2000; Wutzler *et al.*, 2000; Isacsohn *et al.*, 2002; Tunbck *et al.*, 2003) The result is however lower than earlier studies in Nigeria, where prevalences of 99.7% and 69.0% were reported among primary school children and adults in Kaduna and Plateau States respectively (Rinmecit, 1985; Abdulfatai, 2011). The lower prevalence obtained from this study may be due to the age difference in the study populations. The earlier studies sampled older children and adults while this study sampled neonates and younger children. This also agrees with other findings in different parts of the world where the prevalence of the infection increases progressively from childhood to adulthood (Becker *et al.*, 1988; Ghebrekidan *et al.*, 1999; Vyse *et al.*, 2000; Kasubi *et al.*, 2006; Fusun *et al.*, 2007; Lin *et al.*, 2011).

From our study, school attendance is a risk factor for HSV-1 infection and this could be attributed to increased peer interaction among children from different backgrounds and cultures. Children who are in schools find themselves in communities more crowded than their homes, this increases susceptibility to the infection and subsequent development of antibodies. Herpes simplex virus type 1 is acquired through direct contact with lesions and through saliva, therefore the rate of contracting the infection is increased by factors that support crowding and close interactions. This close interaction may however be attributed to the very high prevalence of the infection seen in children in age group 25-36 months. This is age (2-3 years) in which most children start attending schools especially in the northern parts of Nigeria, where the hospital is located. Previous studies have revealed that early childhood stress can elevate HSV-1 antibody levels in children (Shirtchiff *et al.*, 2009).

In our studies, there was no association between seroprevalence with gender showing that sex differences do not play any significant role in the acquisition of the infection. This agrees with reports from England and Tanzania (Vyse *et al.*, 2000; Kasubi *et al.*, 2006). There was no statistically significant association between the mode of delivery of children and seropositivity to HSV-1 infection, although a higher prevalence was observed in children delivered via normal delivery

(spontaneous vaginal delivery) than in children delivered via caesarian section. The higher prevalence seen in children born via normal delivery may be as a result of contact with infected secretions from the mucosa lining of female genital tract during labour and caesarian section on the other hand reduces the risk of transmission from mother to child during delivery (Prober *et al.*, 1992; Brown *et al.*, 2003).

The prevalence of HSV-1 infection in relation to clinical presentations of the children indicated highest seropositivity in children with orofacial lesions, skin infection and conjunctivitis. This was followed by children with jaundice and other infections. Association between the clinical symptoms and seropositivity to HSV-1 infection was statistically significant. Similar studies carried out among HIV infected children reported prevalence of oral lesions to be 87.2% ((Arotiba *et al.*, 2005; Agbelusi and Wright, 2005).

Children have low resistance to infection so presence of HSV-1 infection, other underlining diseases and malnutrition which is peculiar to developing countries like Nigeria may lead to severe manifestations of the disease and consequently death. Clinical manifestations of chronic HSV infection among HIV/AIDS patients have been regarded by the World Health Organization (WHO) as an important presentation defining the disease progression of HIV/AIDS (WHO, 2005).

### Conclusion

Our study revealed a seroprevalence of 64.0% to HSV-1 IgG antibodies obtained in children in ABUTH, Zaria, which is high and could be attributed to the modes of transmission of the virus. Implying however that 36.0% of these children are still at risk of acquiring the infection and consequently coming down with the devastating manifestation of the disease. There is need for early therapy since the severity and duration of the disease is directly related to the degree of immunosuppression and children have low immunity. From this study, HSV-1 childhood infection in ABUTH was found to be associated with age, children's educational status and clinical symptoms presented by the children.

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