

# Seroprevalence of IgG Antibodies to Herpes Simplex Virus Type-1 in Nigerian Children

<sup>1</sup>Shaibu AM, <sup>\*1</sup>Aminu M, <sup>2</sup>Musa BOP and <sup>3</sup>Bugaje MA

<sup>1</sup>Department of Microbiology, Faculty of Science, Ahmadu Bello University, Zaria

<sup>2</sup>Department of Immunology, Ahmadu Bello University Teaching Hospital Zaria

<sup>3</sup>Department of Paediatrics, Ahmadu Bello University Teaching Hospital Zaria

## ABSTRACT

**Background:** Herpes simplex virus type-1 (HSV-1) can cause chronic ulcerative infection in immunosuppressed children leading to latency with subsequent reactivate in the conjunctiva resulting in scarring, thickening of the cornea and blindness. They are also common cause of fatal sporadic encephalitis in 70% of paediatric patients. This cross sectional study determined the prevalence of HSV-1 in children in Kaduna State, Nigeria.

**Method:** A total of 377 blood samples were collected from children less than five years old attending some selected hospitals in Kaduna State and analyzed for HSV-1 IgG antibodies employing Enzyme immune assay technique by using commercially available ELISA Kits.

**Results:** Sero-prevalence rate of 57.8 % (218 /377) was obtained. The highest prevalence of HSV-1 infection was obtained in children in age group 49-60 months (85.2%) and lowest in children in age group 13-24 months (44.8%). Further analysis of the result of children less than one year old showed that children 9-16 weeks old were more susceptible to HSV-1 infection. HSV-1 infection was significantly associated with age ( $\chi^2=37.92$ ,  $df = 4$ ,  $p = 0.001$ ). Though a higher prevalence was obtained in female children (61.5%) than male children (54.5%), the difference observed in the prevalence was not statistically significant ( $\chi^2=1.84$ ,  $df = 1$ ,  $P = 0.105$ ). HSV-1 infection was significantly associated with children who were in school ( $\chi^2 = 15.28$ ,  $df = 1$ ,  $P = 0.001$ ) with a higher prevalence of 74.3%.

**Conclusion:** Over half of the children sampled were protected from HSV-1 infection while 42.2% were susceptible to the infection and were at risk of developing severe HSV-1 manifestation which includes keratitis, encephalitis and Keratoconjunctivitis.

**Keywords:** Seroprevalence, Herpes Simplex Virus Type-1, Children, ELISA, Kaduna State, Nigeria



## INTRODUCTION

**H**erpes Simplex Virus (HSV) belongs to the family Herpesviridae and is a large enveloped DNA virus of icosahedral symmetry divided into two types, HSV1 and HSV 2 (1-3). The virus is an ever-present pathogen that usually causes either asymptomatic infection or skin and mucosal diseases (4). Herpes Simplex Virus type-1 (HSV-1) is the most common cause of sporadic fetal encephalitis in paediatric children (3,5,6) and accounts for 23% of serious neurological disease in children (7). Neonatal herpes is a potentially devastating consequence of perinatal transmission of HSV, with significant

morbidity and mortality (3, 8).

Prevalence of HSV infection varies throughout the world, poor hygiene, overcrowding, low socio-economic status, birth in developing country have been identified as some of the risk factors associated with the virus (2, 9). Herpes Simplex Virus type-1 is acquired through direct contact with infected lesions and body fluids. The prevalence of HSV-1 infection increases progressively from childhood to adulthood, the seroprevalence being inversely proportional to socioeconomic background (9). The virus may reactivate in the conjunctiva and reoccurrences of HSV-1 results in scarring, thickening of the cornea and blindness (3, 10). In the United States, HSV-1 infections are second only to trauma as cause of corneal blindness (3). There is however no published data on the relationship between corneal

Correspondence: maryamaminu@yahoo.com  
+2348033287031

blindness and HSV-1 in Nigeria where we have many blind individuals.

Herpes Simplex Viruses have become a world health problem because of its devastating clinical manifestations amongst HIV/AIDS children (2, 9). The virus is not a reportable disease in Nigeria and there is a dearth of information on its seroprevalence in neonates and children. The study was therefore conducted to determine the seroprevalence of HSV-1 infection in children 0-5 years attending some selected hospitals in Kaduna state, Nigeria.

## Materials and Methods

---

### Study Area

The study was conducted in Kaduna State, Nigeria. Kaduna state occupies part of the north central region of Nigeria. The Global location of Kaduna state is between 06° and 113° north of the equator and occupies an area of approximately 48,732.2 square kilometers.

### Study Population

A total of 377 blood samples were randomly collected from male and female children 0-5 years old in three selected health care facilities from January 2011 to August 2011. The health care facilities include Barau Dikko Specialist Hospital (BDSH) Kaduna, Ahmadu Bello University Teaching Hospital (ABUTH) Zaria and Institute for Child Health (ICH) Banzazzau, Zaria.

### Ethical Approval

Approval for the study was obtained from the ethical committee of the hospitals and consent for the participation of the children in the study were obtained from parents or care givers. Prior to the sample collection, demographic and clinical information of the children were obtained using structured questionnaires administered to parents or care givers who consented to the study.

### 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 serum collected into clean, sterile dry plain sample bottles using a clean dry Pasteur pipette. The sera were stored at -20°C until needed for analysis (11).

## Analysis of Sera by Enzyme-Linked Immunosorbent Assay

The sera were tested for the presence of HVS-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 obtained were analyzed using SPSS statistical package version 17. Pearson Chi-square test of association was used to determine association between variables and seropositivity to HSV-1 infection in the children at 0.05 significant levels.

## RESULTS

---

Of the 377 serum samples obtained from children aged 0-5 years, 218 were seropositive to HSV-1, giving a prevalence of 57.8% (Table 1). Analysis of the results by gender showed seroprevalence of 54.5% (108/198) and 61.5% (110/179) to HSV-1 for male and female children respectively (Figure1). Although, the prevalence was higher among girls compared to boys, the difference was not statistically significant ( $\chi^2 = 1.84$ ,  $df = 1$ ,  $P = 0.105$ ). The prevalence of HSV-1 according to age group is shown in Figure 2. The highest prevalence (85.2%: 46/54) was recorded among children in age group 49-60 months while the lowest (44.8%: 47/105) was recorded in age group 13-24 months.

There was a statistically significant association between HSV-1 and age ( $\chi^2 = 37.92$ ,  $df = 4$ ,  $P = 0.001$ ). Further analysis of the result of children in the age group 0-12 months showed a significant association between HSV-1 and age ( $\chi^2 = 20.808$ ,  $df = 5$ ,  $P = 0.001$ ). The highest prevalence was recorded in children within 9-16 weeks of age (84.6%), followed

by children within 0-8 weeks (64.3%), 17-24 weeks (62.5%), 25-32 weeks (52.6%), 33-40 weeks (26.3%) and the lowest prevalence was observed in children within 41-48 weeks (23.5%).

The seroprevalence rate was significantly ( $\chi^2 = 15.28$ ,  $df = 1$ ,  $P = 0.001$ ) higher among children in school (74.3%: 75/101) compared to children who were not in school (51.8%: 143/276) as shown in Table 2. Children who were in school were more likely to be infected with HSV-1 infection than those who were not in school (OR = 1.43, 95% CI = 0.5851 – 0.7898).

The results obtained were analyzed according to the mode of delivery of the children enrolled in the study (Table 3). There was a higher preponderance in children delivered by spontaneous vaginal delivery (58.7%: 205/349) as compared to those delivered by caesarian section (46.4%; 13/28) though the difference was not statistically significant ( $\chi^2 = 1.61$ ,  $df = 1$ ,  $p = 0.142$ ).

The prevalence of HSV-1 infection was 54.1% (109/196) in children with 1-3 siblings while children with more than 9 siblings had the highest prevalence (76.9%: 10/13) (Figure 3). The association between number of children per family to the infection was however not statistically significant ( $\chi^2 = 6.45$ ,  $df = 3$ ,  $p = 0.092$ ).

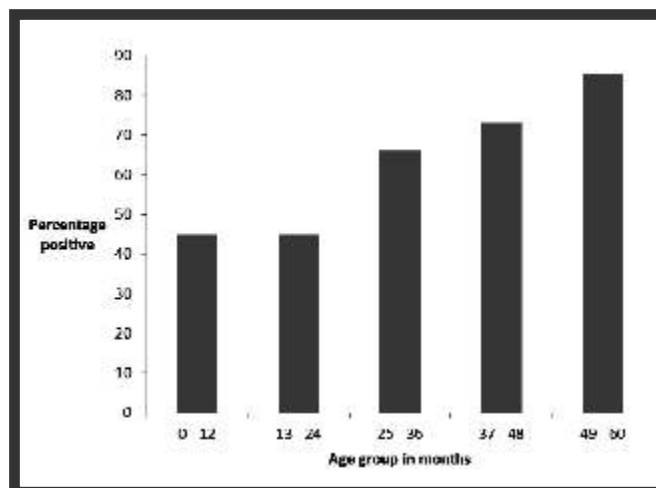


Figure 2: Percentage distribution of IgG antibody to HSV-1 among different age groups in children (0-5 years) in children in Kaduna State, Nigeria.

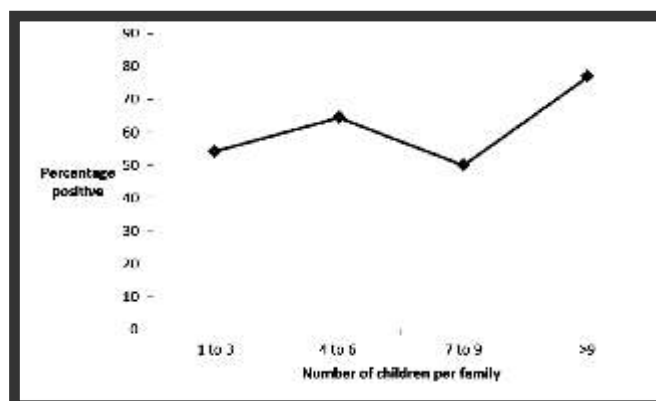


Figure 3: Percentage distribution of IgG antibody to HSV-1 in children 0-5 years old in relation to number of children per family in children in Kaduna State, Nigeria.

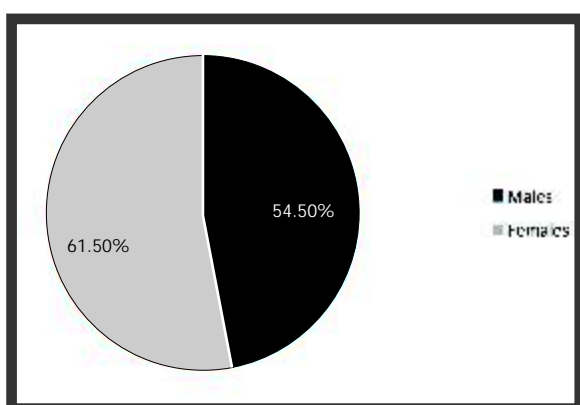


Figure 1: Percentage distribution of IgG antibody to HSV-1 in relation to gender in children 0-5 years in Kaduna state, Nigeria.

Table 1: Seroprevalence of IgG antibody to HSV-1 in children (0-5 years) attending some selected hospitals in Kaduna State

Hospitals	No. of Samples Tested	Number Positive	Percentage Positive	95% CI
BDSH	126	62	49.2	40.63-57.83
ABUTH	125	80	64.0	55.28-71.88
ICH	126	76	60.3	61.59-68.47
<b>TOTAL</b>	<b>377</b>	<b>218</b>	<b>57.8</b>	<b>52.79-62.71</b>

( $\chi^2 = 6.11$ ,  $df = 2$ ,  $P = 0.047$ )

**Key**

BDSH-Barau Dikko Specialist Hospital  
 ABUTH-Ahmadu Bello University Teaching Hospital  
 ICH-Institute for Child Health

Table 2: Prevalence of IgG antibody to HSV-1 in children 0-5 years old in relation to educational status of children in Kaduna State, Nigeria

Child's education	No. of Samples	Number	Percentage	95% CI
	Tested	Positive	Positive	
In school	101	75	74.3	64.95-81.78
Not in school	276	143	51.8	45.93-57.64
<b>Total</b>	<b>377</b>	<b>218</b>	<b>57.8</b>	<b>52.79-62.71</b>

( $\chi^2=15.28$ ,  $df=1$ ,  $P=0.001$ )

Table 3: Prevalence of IgG antibody to HSV-1 in children 0-5 years old in relation to nature of delivery of children in Kaduna State, Nigeria

Mode of delivery	No. of Samples	Number	Percentage	95% CI
	Tested	Positive	Positive	
Normal delivery	349	205	58.7	53.5-63.78
Caesarian section	28	13	46.4	29.53-64.19
<b>Total</b>	<b>377</b>	<b>218</b>	<b>57.8</b>	<b>52.79-62.71</b>

( $\chi^2=161$ ,  $df=1$ ,  $p=0.142$ )

## DISCUSSION

A seroprevalence of 57.8% was obtained in this study which was relatively high and this could in part be attributed to the mode of transmission of HSV-1 which includes direct contact with lesions and through saliva. The result implies that 42.2% of the children in the study were susceptible to HSV-1 infection and were not immunized against the virus. These pockets of susceptible children are at risk of developing severe HSV-1 manifestation which includes gingivitis, Keratoconjunctivitis and fetal encephalitis. If these children are immunocompromised and their cellular immunity is impaired, infection may spread and involve respiratory tract, esophagus and intestinal mucosa as has been shown in children with AIDS and patients under immunosuppressive treatment like bone marrow, renal and cardiac transplant recipients; In addition, if any of these susceptible children is malnourished, he or she will be prone to fatal disseminated HSV infection (3, 5).

The result is similar to the prevalence of 59% obtained in children in Eritrea (12), 59% in New Mexico (13) and 55% in Syria (14) but higher than those reported in Germany, Israel and England where a prevalence of 31%, 38% and 20% were obtained respectively (15-17). The prevalence rate in this study was however; lower than that of 99.7% obtained among older

children in Kaduna state (18) and 69% among adults in Plateau state (19). The lower prevalence obtained in this study may be as a result of age difference in the study population. Earlier studies have found the prevalence of HSV-1 to increase with age progressively (9, 20-22). The published studies in Nigeria sampled older children and adults while the present study sampled younger children 0-5 years old.

Seropositivity of HSV-1 IgG antibody in relation to the selected hospitals showed the lowest and highest prevalence amongst children attending BDSH and ABUTH respectively. The low prevalence seen in BDSH could be due to a number of factors. It could be due to higher number of neonates and younger children who may not have had contact with the virus seen in the unit; or due to increased personal hygiene among the patients as the clinic is situated in Kaduna which is an urban centre; in addition the clinic is a primary health care centre. The higher prevalence recorded for ABUTH may be due to increased number of patients attending the tertiary health care centre where patients with clinical disease are more likely to be referred.

Seroprevalence of HSV-1 in this study was higher in girls than boys. The higher prevalence observed among girls in this study may be due to chance since the difference observed was not significant. The higher prevalence obtained in females from the study agrees with the findings in Europe and USA where female children were more likely to be HSV-1 seropositive than males (20, 23, 24).

There was a highly statistically significant association between age group and infection with HSV-1. The rise in seroprevalence rate with age is similar to the findings in Tanzania, Germany and Israel where HSV-1 seroprevalence was found to increase with increasing age (16, 17, 21). The prevalence obtained for children in age group 0-12 months and 13-24 months were similar. The prevalence of 44.8% in age group 0-12 months in this study is similar to the prevalence (49%) obtained in newborns in England and Wales (20). However, prevalence obtained in this age group could reflect in part maternal antibody (IgG) status, since the antibodies can cross the placenta. The decrease in prevalence (44.8%) in age group 13-24 months could be due to waning of maternal antibody. Studies have also revealed that

early childhood stress can also elevate antibody levels in these children (25). The highest prevalence of the infection in age group 49-60 months could be associated with increased level of interaction among children.

A high prevalence rate of 74.3% was obtained in children who were in school in this study compared to children who were not in school. Earlier studies have not made such comparison. The increased prevalence in children who attended formal education may be associated with the higher degree of interaction amongst children with their peer from variable cultures.

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 secretion from the mucosa lining of female genital tract during labour. Caesarian section on the other hand has been shown to reduce the risk of transmission from mother to child during delivery (26, 27).

The high seroprevalence of HSV-1 among children with high number of siblings seen in this study could be associated to the closer interaction amongst the siblings and overcrowding as also postulated by Brinzli et al (28).

## CONCLUSION

---

Herpes simplex virus type-1 seroprevalence of 57.8% was obtained in children in this study, indicating that over half of the children sampled were protected from HSV-1 infection. However, 42.2% of the children were susceptible to the infection and were at risk of developing keratitis, encephalitis and Keratoconjunctivitis. Younger age and school attendance were risk factors associated with HSV-1 infection among children in the study.

## Acknowledgements

---

We thank Dr HW Idris and the entire staff of Ahmadu Bello University Teaching Hospital, Zaria, Dr .R. Al-Zubair and the entire staff of Barau Dikko Specialist Hospital, Kaduna and Dr E.E. Ella of Department of Microbiology, ABU, Zaria for their support and assistance.

## REFERENCES

---

1. Vittone V, Diefenbach E, Triffett D, Douglas MW, Cunningham AL and Diefenbach, RJ. Determination of interactions between tegument proteins of herpes simplex virus type 1. *J Virol* 2005; 79(15):9566-9571.
2. Willey JM, Sherwood LM and Woolverton CJ. Human diseases caused by viruses and prions: Direct contact diseases: Herpes viruses. In: Willey JM, Sherwood LM and Woolverton CJ. (editors) *Prescott's Microbiology* 8th Edition, New York. The McGraw Hill Companies. International Edition. 2011. pp 914-916.
3. Brooks GF, Carroll KC, Butel JS, Morse SA and Mietzner TA. Virology, Herpes viruses. In: *Jawetz, Melnick and Adelbergs Medical Microbiology*. U.S.A: McGraw Hill Companies Inc. International Edition. 2010; pp 433-455.
4. Fusun AI, Mahir I, Zafer Y, Semra OG, Asuman B, Recep S, Fadil O. Distribution of HSV-1 IgG antibodies by two methods comparing in Turkish atopic children. *New Microbiol* 2007; 30:109-112.
5. Haeheim LR, Pattison JR and Whitley RJ. (editors). *A Practical Guide to Clinical Virology*; Human Herpes simplex virus 1 and 2; Willey John and sons Ltd, 2002. 2<sup>nd</sup> edition; pp 130-131.
6. Ibrahim AI, Obeid MT, Jouma MJ, Roemer K, Lantzsch, MN and Gartner BC. Prevalence of Herpes Simplex Virus (Types 1 and 2), Varicella-Zoster Virus, Cytomegalovirus, and Human Herpesvirus 6 and 7 DNA in Cerebrospinal Fluid of Middle Eastern Patients with Encephalitis. *J Clin Microbiol* 2005; 43(8): 4172-4174.
7. Ward KN, Ohrling A, Bryant NJ, Bowley JS, Ross E M and Verity, C.M. Herpes simplex serious neurological disease in young children: incidence and long-term outcome. *Arch Dis Child* 2011; published online doi: 10.1136/adc.2010.204677.
8. Leung DT and Sacks SL. Current treatment options to prevent perinatal transmission of herpes simplex virus. *Exp Opin Pharmacol* 2003; 4(10):1809-1819.

9. Xu F, Lee FK and Morrow RA, Sternberg MR, Luther KE, Dubin G and Markowitz LE. Seroprevalence of herpes simplex virus type 1 in children in the United States. *J Paed*2007; 151: 374-7.
10. Liesegang TJ. Herpes simplex virus epidemiology and ocular importance. *Cornea*2001; 20 (1):1-13.
11. Cheesbrough M. *District laboratory practice in tropical countries (Part 2)*Cambridge University Press. 2000; pp 248-266.
12. Ghebrekidan H, Ruden U, Cox S, Wahren B and Grandien M. Prevalence of herpes simplex virus types 1 and 2, cytomegalovirus, and varicella-zoster virus infections in Eritrea. *J. Clin Virol*1999; 12:53-64.
13. Becker TM, Magder, L and Harrison HR. The epidemiology of infection with the human herpesviruses in Navajo children. *Amer J Epi*1988; 127:1071-87.
14. Ibrahim AI, Kouwattli KM and Obeid MT. Frequency of herpes simplex virus in Syria based on type-specific serological assay. *Saudi Med. J* 2000; 51: 355-60.
15. Kangro HO, Osman HK, Lau YL, Heath RB, Yeung CY and Ng MH. Seroprevalence of antibodies to human Herpes virus in England and Hong Kong. *J Med Virol* 1994;43 (1):91-96.
16. Wutzler P, Doerr HW and Farber I. Seroprevalence of herpes simplex virus type 1 and 2 in selected German populations-relevance for the incidence of genital herpes. *J Med Virol*2000; 61: 201-7.
17. Isacsohn M, Smetana Z and Rones ZZ. A sero-epidemiological study of herpes virus type 1 and 2 infection in Israel. *J Clin Virol.* 2002; 24: 85-92.
18. Abdulfatai, K. Seroprevalence of Herpes simplex virus type-1 IgG antibodies in some parts of Kaduna State. Unpublished MSc Thesis, Ahmadu Bello University, Zaria- Nigeria. 2011.
19. Rinmecit GS. Seroprevalence of herpes simplex virus in Plateau state of Nigeria, an unpublished MSc research work, 1985 University of Jos, Jos, Plateau State, Nigeria
20. Vyse AJ, Gay NJ and Slomka MJ. The burden of infection with HSV-1 and HSV-2 in England and Wales: implications for the changing epidemiology of genital herpes. *Sex Trans Infect*2000; 76: 183-7.
21. Kasubi JM, Nusen A, Marsden HS, Bergstrom T, Langerland N and Haarr L. Prevalence of antibodies against Herpes Simplex Virus 1 and 2 in children in an urban region of Tanzania. *J Clin Microbiol*2006; 44 (8):2801-2807.
22. Lin H, He NA, Su M, Fangs J, Chen LI and Gao M. Herpes simplex virus infections among rural residents in eastern China. *BMC Infect Dis*2011; 69(11):1-6.
23. Rosenthal SL, Lawrence R, Stanberry FM, Biro M, Slaoui M, Francotte M, Koutsoukos, M, Hayes M and Bernstein DI. Seroprevalence of herpes simplex virus types 1 and 2 and cytomegalovirus in adolescents. *Clin Infect Dis*1997; 24: 135-9.
24. Pebody RG, Andrews N, Brown D, Gopal R, deMeller H, Francois G, Gatcheva N, Hellenbrand W, Jokinen S, Klavs I, Kojoubarova M, Kortbeek T, Kriz B, Prosenk K, Roubalova K, Teocharov W, Thierfelder W, Valle, M, Van Damme P and Vranckx R. The seroepidemiology of herpes simplex virus type 1 and 2 in Europe. *Sex Trans Infect*2004; 80 (3): 185-91.
25. Shirtchiff EA, Coe LC and Pollak SD. Early childhood stress is associated with elevated levels of herpes simplex virus type-1. *NatAcadSci*2009;10 (698):2963-2967. doi:10.1073/pnas.0806660106.
26. Prober CG, Corey L, Brown ZA, Hensieigh PA, Frenkel LM, Bryson YJ, Whitley RJ and Arvin AM. The management of pregnancies complicated by genital infection with herpes simplex virus. *Clin Infect Dis*1992;15(6):1031-8
27. Brown ZA, Wald A, Morrow RA, Selke S, Zeh J and Corey L. Effect of serologic status and cesarean delivery on transmission rates of herpes simplex virus from mother to infant. *JAMA*2003; 289:203-209.
28. Blinzli D, Wiattlisbach V, Barozzoni F, Sahli R and Meylan PRA. Seroepidemiology of Herpes simplex virus 1 and 2 in western and southern Switzerland in adult aged 25-74 in 1992-1993: a population based study. *BMC Infect Dis.*2004; 410. doi:1186/1471-2334-4-10.
29. World Health Organization. Interim WHO clinical staging of HIV/AIDS and HIV/AIDS case definitions for surveillance: African region. Geneva, Switzerland. 2005;2-8