

Seroprevalence of Herpes Simplex Virus Type -2 among Pregnant Women Attending Ibrahim Malik Teaching Hospital ,Khartoum state,sudan.

Hadeel Elsir Ahmed Idress^{*1}, Wafa Ibrahim Elhag^{**2}

*1 M.Sc Student Microbiology Department of Faculty of Medical Laboratory Sciences, Al Neelain University, Sudan

**2 Associate Professor- Microbiology Department, Faculty of Medical Laboratory Sciences, Al Neelain University

Corresponding author: Hadeel Elsir Ahmed Idress
E-mail: hadeel.elsir103@gmail.com , hadeeelsir380@gmail.com
Tel: +249-910303806

Abstract

Background: Infection with herpes simplex is one of the most common sexually transmitted infections. Because the infection is common in women of reproductive age it can be contracted and transmitted to the fetus during pregnancy and the newborn. The greatest risk for miscarriage, premature labor, inhibited fetal growth, or transmission of the herpes infection to the infant cause of neonatal infection, which can lead to death or long-term disabilities. Rarely in the uterus, it occurs frequently during the transmission delivery. To the fetus and the newborn occurs in case of an initial maternal infection contracted in the second half of pregnancy.

This study aimed to detect seroprevalence of HSV-2 IgM, IgG antibodies among pregnant women.

Methods: This was descriptive cross sectional study in which a total of 90 serum specimens were collected from pregnant women and analyzed for IgM and IgG antibodies to Herpes simplex viruse _2 by using Enzyme-linked Immunosorbent Assay (ELISA) .

Generated data were analyzed by using SPSS program (version11)

Results: Out of the 90 samples , HSV-2 IgM Abs were detected in 2 (2.2%) of pregnant women, while HSV-2 IgG Ab were detected in 57 (63.3%) of pregnant women.

Conclusion: A high seroprevalence of HSV_2 was detected in the examined samples. It was much higher for IgG compared to IgM. Therefore, it is necessary to diagnose the pregnant women for the possibility of HSV infection so that it could be minimized by proper medication.

Key words: Seroprevalence, IgM, IgG, HSV, ELISA, Pregnant women, Ibrahim Malik Teaching Hospital

{ **Citation:** Hadeel Elsir Ahmed Idress, Wafa Ibrahim Elhag. Seroprevalence of herpes simplex virus type -2 among pregnant women attending Ibrahim Malik Teaching Hospital, Khartoum state, Sudan. American Journal of Research Communication, 2015, 3(11): 149-160} www.usa-journals.com, ISSN: 2325-4076.

Introduction

Genital herpes infection is the primary cause of genital ulcer disease (GUD)⁽¹⁾ worldwide mainly caused by herpes simplex virus type 2 (HSV-2). Herpes simplex virus (HSV) is neurotropic virus that has a large linear, double-stranded DNA genome protected by a capsid with icosahedral symmetry surrounded by an envelope consisting of a lipid bilayer with embedded glycoproteins, having yet a proteinaceous region between the capsid and envelope called tegument⁽²⁾. The HSV belongs to the family of Herpesviridae, subfamily Alphaherpesvirinae, Alphaherpesvirinae, and genus Simplex virus^(3,4). It is a virus that has a very complex life cycle and stands out as one of the most common pathogens in the etiology of sexually transmitted diseases worldwide^[5].

HSV-2 can cause genital herpes with greatest incidence among women of reproductive age, with risk of maternal transmission of the virus to the fetus and neonate⁽⁶⁾. The acquisition of genital herpes during pregnancy has been associated with spontaneous abortion, intrauterine growth restriction, prematurity, and congenital and neonatal herpes. Vertical transmission from an infected mother to her baby can cause severe disease resulting in sequelae or death of the infant. Most neonatal infections result from exposure to HSV in the genital tract during passage through

the birth canal, although they can also be transmitted to the fetus during the intrauterine phase⁽⁷⁾ The risk of disease in the newborn is significantly higher when the mother acquires genital infection for the first time with HSV-2 during pregnancy. Recurrent infections are rarely associated with disseminated neonatal disease in the newborn of immune-competent mothers. In fact, the pregnant women who acquire genital herpes as a primary infection in the latter half of pregnancy, rather than prior to pregnancy, are at the greatest risk of transmitting the virus to their newborn^(8,9). The baby is at greatest risk during a vaginal delivery, especially if the mother has an asymptomatic infection that was first introduced late in the pregnancy

In recent years, genital herpes has become an increasing common sexually transmitted infection^(10,11) From the late 1970s, HSV-2 seroprevalence in the US has increased by 30%, resulting that one out of five adults is infected^(10,12) .Comparing the developing countries, substantially higher rates of HSV2 have been observed in sub-Saharan Africa, where prevalence in adults ranges from 30% to 80% in women, finally more than 80% of female commercial sex workers are infected⁽¹¹⁾ . In South America, available data are mainly for women, in whom HSV2 prevalence ranges from 20% to 40%. Prevalence in the general population of Asian countries shows lower values, from 10% to 30%^(11,15) HSV seroprevalence in patients attending STD clinics varies from 17% in Italy (6% in the general population) to 40% in Australia (14% in pregnant women).^(13,14)

Materials and Methods

Design

This is across sectional study included HSV infected pregnant women aged between (18-41) years old with mean 30.4 years old conducted Ibrahim Malik Teaching Hospital , khartoum State, Sudan, during October 2014 to Junaury2015. The data was collected by structured questionnaire. Ethical approval was taken from Al Neelain University research ethical board and from patients verbally.

Experimental work

Serum specimens were collected from pregnant women, and screened for Herpes simplex virus type -2 IgG and IgM antibodies using (ELISA) kits (SERION Elisa Classic -Germany) technique at Research Laboratory (AL Neelain University).

Collection of specimens and processing

Three milliliters of blood were collected under aseptic technique into plain container, the sera obtained after centrifugation was kept at -20°C . The serum samples were tested for the presence of HSV-2 IgG and IgM antibodies, using ELISA kit SERION Elisa Classic Germany), (4th generations), All reagents and Samples were allowed to reach the room temperature for at least 15-30 minutes. The washer buffer concentrate was checked for the presence of salt crystals. The washer buffer diluted 1 to 30 with distilled water.

The strips were set in strip-holder and the wells were labeled including 1 for negative controls, 2 for Standard serum and 1 for blank.

Before running the test patient sample must be diluted in dilution buffer as following 10 μl from patient sample each to 1000 μl dilution buffer.

100ul of diluents was added to each well except the blank. Then 100 μl of negative controls and Standard serum and specimens were added into their respective wells, plate was covered with the plate cover and incubated for 60 minutes at 37°C .

At the end of incubation the plate cover was removed and each well was washed 4 times with diluted wash buffer. After final washing cycle the plate was blotted onto clean towel to remove any remaining liquid.

100ul of Alkaline Phosphates-conjugate (AP) was added to each well except blank then covered with plate cover and incubated for 30 minutes at 37°C . Plate cover was removed and each well washed 4 times with diluted wash buffer, then blotted. 100 μl of chromogen were added into each well including blank, plate was covered with plate cover and incubated for 30 minutes at 37°C . The enzymatic reaction between the chromogen solution and Alkaline phosphates–

conjugate (AP) produced yellow to green color in positive standard serum and positive sample wells.

The plate cover removed and 100µl of stop solution were added into each well, intensive yellow to green color was developed in positive control and positive sample wells.

Measuring the Absorbance

The plate reader was calibrated with blank well and the absorbance was read at 405nm.

Results were calculated by relating each sample optical density (OD) value to the Cut off value of plate.

Calculation of Cut off (C.O) value.

$$\text{C.O} = * \text{mean of St} * 0.352$$

*St=the mean absorbance value for the Tow Standard serum.

Interpretation of the Results

Negative results

Samples giving absorbance less than Cut-off value are negative for this assay.

Positive result

Sample giving absorbance equal to or greater than Cut-off considered initially reactive.

Borderline

Sample with absorbance to Cut-off value are considered borderline and retesting of these samples in duplicate is recommended to confirm the results.

Data Analysis

Data was analyzed by SPSS (Statistical Package of Social Science) software version 16.

Results

A total of 90 pregnant women, attending Ibrahim Malik Teaching Hospital, Khartoum State, Sudan, during October 2014 to January 2015, were enrolled in this study, their mean age range (30.4) years old the seroprevalence of Herpes Simplex Virus (HSV) was 2 (2.2%) and 57 (63.3%) for HSV IgM and HSV IgG, respectively (fig_1), (fig_2). The positivity for HSV IgM indicates current or recent infection of HSV, while the positivity for HSV IgG indicates past infection of HSV, highest seropositivity of HSV IgG, IgM was observed among first trimester (Table_2). Statistical analysis showed insignificant correlation between HSV IgG, IgM and age groups of pregnant women ($P > 0.05$) (table_1).

According to gestational age seroprevalence of HSV IgG group was 36 (62.0%), 20 (47%), and 1 (20.0%) for first, second and third trimester, respectively, while seroprevalence of HSV IgM group was 01 (1.7%), and 01 (3.7%) for first and second trimester respectively (table_2).

Among pregnant women 35 were primigravida and 55 multigravida. 2 (5.7%) IgM positive, 33 (94.3%) IgG positive were primigravida, while 24 (43.6%) were IgG positive, 31 (56.3%) IgG, IgM negative result in multigravida women.

Out of the total, 22 (54.4%) pregnant women have symptoms like discharge which are continuous more than two weeks out of them 13 (59.1%) are IgG positive, 8 (36.4%) are negative, only one (4.5%) pregnant woman was IgM positive, no lesion was observed in any pregnant women in this study.

Regarding to history of genital herpes infection 12 (13.3%) patients had past history, while 78 (86.7%) had no history. From them 5 (41.7%) have IgG positive and 7 (58.3%) IgG, IgM negative.

In our study 7 (7.8%) pregnant women had past history of abortion, while 83 (92.2%) had no abortion, however the seropositive HSV was observed among the second group only.

Discussion

Genital herpes is a large global problem, and for the person infected, it is a very painful

disease. It can cause significant distress, and can have a devastating impact on the social and psychological wellness of an individual, In addition, mothers with genital herpes caused by infection by HSV-2 can transmit the virus to the neonate at birth, causing neonatal herpes, which is a potentially preventable cause of neonatal mortality and morbidity⁽¹⁷⁾.

In the present study 90 pregnant women were enrolled, the result showed that 2(2.2%) were seropositive of HSV-2 IgM among pregnant women, while 57(63.3%) seropositive IgG, the result slightly similar to study conducted by Abul-Razak , *et al* (2013), who found 2(2.2%) IgM positive, 2(2.2%) IgG positive out of 91 pregnant⁽¹⁸⁾

Our study higher than other study done by El-Amin *et al* (2013), in Sudan who found 45 (34.6%) tested positive for the IgG of Herpes virus but none of them was positive for the IgM test out of 130 pregnant women⁽¹⁹⁾. but lower than studies of Omer *et al* (2015),in india who reported that 124(64.9%)and 4(2.1%) samples were positive HSV IgG and IgM respectively out of 191 pregnant women⁽²⁰⁾.

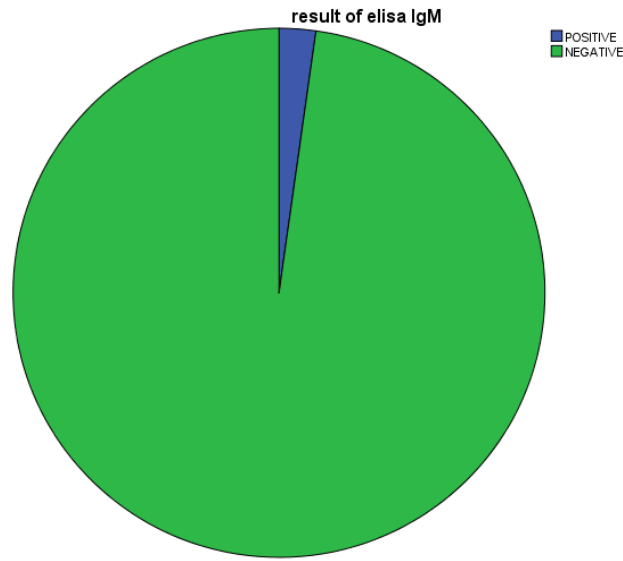
The variation may be due to sample size technique use for analysis. Compared to study conducted in Saudi by Obeid O, *et al* (2007) Of the 459 pregnant Saudi women who were included in the study, 31 (6.8%) were found to be seropositive for HSV-2 IgG antibodies, 27 (5.9%) were found to be seropositive for HSV-1 IgM antibodies⁽²²⁾.

Conclusion

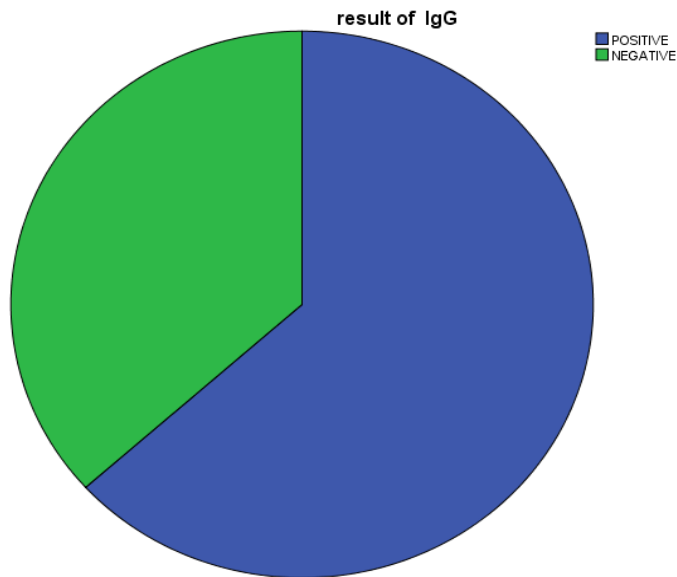
This study revealed high sero frequency of HSV IgG among pregnant ladies , further studies using advanced techniques is important to validate this result .

Acknowledgements

Our thanks to Hamza Abdallh, Ministry of Health, Virology lab, Khartoum, State, Sudan and staff of Medical Microbiology Department AL Neelain University ,Sudan



Fig_1: Seroprevalance of herpes simplex viruse_2 IgM among pregnant women (n=90).



Fig_2: Seroprevalance of herpes simplex viruse_2 IgG among pregnant women (n=90).

Table_1 Seroprevalence of HSV-2 among pregnant women according to their age (n=90)

Age group (years)	Total number (%)	IgM Positive (%)	IgG Positive (%)
18-25	42 (46.7)	1 (2.8)	28 (66.6)
26-33	33 (36.7)	1 (3.3)	26 (78.7)
34-41	15 (16.7)	0	3 (20.0)
Total	90 (100)	2 (2.2%)	57 (63.3%)

Table_2 Seroprevalence of HSV-2 among pregnant women according to gestational age: (n=90)

Gestation age	Total Number (%)	IgM Positive (%)	IgG Positive (%)
First trimester	58 (55.6)	1 (1.7)	36 (62.0)
Second trimester	27 (60.1)	1 (3.7)	20 (74.0)
Third trimester	5 (5.6)	0 (0)	1 (20.0)
Total	90	2 (2.2%)	57 (63.3%)

References

1. World Health Organization. Herpes simplex virus type 2 programmatic and research priorities in developing countries, WHO/HIV_AIDS/2001.05. Report of a WHO/UNAIDS/LSHTM Workshop, London. 14–16 February 2001, 2.
2. Grünewald K, Desai P, Winkler DC, et al. Three-dimensional structure of herpes simplex virus from cryo-electron tomography. *Science*. 2003;302(5649):1396
3. Anzivino E, Fioriti D, Mischitelli M, et al. Herpes simplex virus infection in pregnancy and in neonate: status of art of epidemiology, diagnosis, therapy and prevention. *Virology Journal*. 2009;6, article 40.
4. Straface G, Selmin A, Zanardo V, de Santis M, Ercoli A, Scambia G. Herpes simplex virus infection in pregnancy. *Infectious Disease in Obstetrics and Gynecology*. 2012;2012:6 pages.3856972
5. Peña KC, Adelson ME, Mordechai E, Blaho JA. Genital herpes simplex virus type 1 in women:detection in cervicovaginal specimens from gynecological practices in the United States. *Journal of Clinical Microbiology*. 2010;48(1):150–153.
- 6- Anzivino E, Fioriti D, Mischitelli M, et al. Herpes simplex virus infection in pregnancy and in neonate: status of art of epidemiology, diagnosis, therapy and prevention. *Virology Journal*. 2009;6, article 40
7. Corey L, Wald A. Maternal and neonatal herpes simplex virus infections. *The New England Journal of Medicine*. 2009;361(14):1328–1385.
8. Anzivino E, Fioriti D, Mischitelli M, et al. Herpes simplex virus infection in pregnancy and in neonate: status of art of epidemiology, diagnosis, therapy and prevention. *Virology Journal*. 2009;6, article 40.
9. Straface G, Selmin A, Zanardo V, de Santis M, Ercoli A, Scambia G. Herpes simplex virus infection in pregnancy. *Infectious Disease in Obstetrics and Gynecology*. 2012;2012:6 pages.385697
- 10-Cusini M, Ghislanzoni M: The importance of diagnosing genital herpes. *JAntimicrob Chemother* 2001, 47:9-16

11-Weiss H: Epidemiology of herpes simplex virus type 2 infection in the developing world. *Herpes* 2004, 11:24A-35A.

12-Fleming DT, McQuillan GM, Johnson RE, Nahmias AJ, Aral SO, Lee FK, St Louis ME: Herpes simplex virus type 2 in the United States, 1976 to 1994. *N Engl J Med* 1997, 337:1105-1111.

13-Cusini M, Cusan M, Parolin C, Scioccati L, Decleva I, Mengoli C, Suligo B, Palú G: Seroprevalence of herpes simplex virus type 2 infection among attendees of a sexually transmitted disease clinic in Italy. *Italian Herpes Forum. Sex Transm Dis* 2000, 27:292-295.

14-Cunningham AL, Lee FK, Ho DW, Field PR, Law CL, Packham DR, McCrossin ID, Sjögren-Jansson E, Jeansson S, Nahmias AJ: Herpes simplex virus type 2 antibody in patients attending antenatal or STD clinics. *Med J Aust* 1993, 158:525-528.

15-Paz-Bailey G, Ramaswamy M, Hawkes SJ, Geretti AM: Herpes simplex virus type 2: epidemiology and management options in developing countries. *Sex Transm Infect* 2007, 83:16-22.

16-Fatahzadeh M, Schwartz RA. Human herpes simplex virus infections: epidemiology, pathogenesis, symptomatology, diagnosis, and management. *J Am Acad Dermatol.* 2007 Nov;57(5):737-63. 18

17-Kim ID, Chang HS, Hwang KJ (2012). Herpes Simplex Virus 2 Infection Rate and Necessity of Screening during Pregnancy: A Clinical and Seroepidemiologic Study. *Yonsei Med. J.* 53(2):401-40

18-Abul-Razak .H , Abbas A. A AsmaaH, WasanA, ZeinaGany F. et al Seroprevalence of Anti-Herpes Simplex Virus Type 2 IgG, IgM Antibodies , *Diyala Journal of Medicine* October 2013

19- El-Amin EO, Elamin OE, Ahmed RAM, Abdulla AK, Elamin SE. Sero-Prevalence of Herpes Virus Infection in Sudanese Pregnant Women et al *Tropical Medicine & Surgery.* (2013)

20- Omar A, Harish B, Neena G, Ankit S, Harison M Prevalence of Herpes Simplex Virus in Pregnant Women from Gangetic Plain Region of Allahabad, India et al *June* (2015)

21- Obeid E Obeid , et al Prevalence of herpes simplex virus types 1 and 2 and associated Sociodemographic variables in pregnant women attending King Fahd Hospital of the university. J Family Community Med. 2007 Jan-Apr; 14(1): 3-7.