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Research Article

Sero Frequency of *Herpes Simplex Virus* Types 1&2 IgG Among Pregnant Women Attending Saada Bouelella Hospital

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Abstract

Background*: Herpes simplex virus (HSV)* infection is one of the most common sexually transmitted infections. Due to its prevalence among women of reproductive age, the infection can be contracted during pregnancy and subsequently transmitted to the fetus or newborn. *HSV* is a significant cause of neonatal infections, which can result in death or long-term disabilities. The greatest risk of transmission to the fetus and newborn occurs when the mother contracts the initial infection in the second half of pregnancy. The risk of maternal-fetal-neonatal *HSV* transmission can be reduced through antiviral treatment or, in certain cases, by opting for a caesarean section. This research aims to determine the rate of *HSV* infection among pregnant women attending Saad Abou Ella Hospital, shedding light on the extent of the problem.

Methodology: Blood samples were collected from 101 pregnant women under direct medical supervision via venipuncture using a 5 ml syringe. The blood was drawn into plain tubes, and serum was separated after centrifugation at 5000 rpm for 15 minutes. The sera were stored at -20°C until serological tests were conducted. Specimens were processed using a third-generation Enzyme-Linked Immunosorbent Assay (ELISA) (Foresight, Germany) to detect IgG antibodies against *HSV-1* and *HSV-2.*

Results: A total of 101 pregnant women participated in the study, with 45 from Khartoum, 25 from Omdurman, 9 from Bahri, 19 from Al Jazirah, and 3 from White Nile State. The participants' ages ranged from 16 to 41 years, with a mean age of 27.10 years. Most of the women were in the third trimester of pregnancy (67.7%) and had no history of abortion (80%). Among the 101 pregnant women studied, 32% were seropositive for *HSV-1* IgG antibodies, and 17% were seropositive for *HSV-2* IgG antibodies, with 5 women testing positive for both. The highest seropositivity for IgG was observed in the 32-38 age groups, among those with no history of abortion, and in women in the third trimester of pregnancy. Statistical analysis revealed no significant correlation (P-value > 0.05) between age, history of abortion, trimesters, and the frequency of *HSV* antibodies.

Keywords: *HSV, Seroprevlance, Sudan.*

Introduction

Human herpes virus (*HHV*) infections have been known since ancient times, with *Herpes simplex virus* (*HSV*) infections first recorded in humans in ancient Greece. Hippocrates used the term "herpes (Sakaoka et al., 1986)," meaning to creep or crawl, to describe the spreading nature of skin lesions. Although the vesicular nature of *HSV*-associated lesions was well understood by the late eighteenth century, it wasn't until 1893 that Vidal first recognized the person-to-person transmission of *HSV* (Sakaoka et al., 1986). The virus was isolated for the first time in 1919 (Sakaoka et al., 1986), and it took several decades before the identification of two distinct serotypes, *HSV*-1 and *HSV*-2 (Umenc and Yoshida, 1994). *HSV* is one of the most prevalent viral infections in humans, classified within the alphaherpesvirinae sub-family of the Herpesviridae family (Alzahrani et al., 2005). This DNA virus has two subtypes: *HSV*-1 and *HSV*-2 (Peter et al., 2009). Although these subtypes are distinct, they share some antigenic components (Wutzler et al., 2000). *HSV*-1 is mainly transmitted through contact with oral secretions and is the leading cause of orofacial infections, while *HSV*-2 is primarily spread through sexual contact, responsible for most cases of genital herpes (Peter et al., 2009). In Germany, more than 90% of adults have antibodies to *HSV*-1, while approximately 15% have antibodies to *HSV*-2 (Wutzler et al., 2000). The rate of *HSV* infection during pregnancy is estimated to be between 0.5% and 2% (Brown, 2002). Primary genital *HSV* infections during pregnancy are particularly concerning, as they can lead to severe neonatal diseases. However, in most instances, genital herpes manifestations during pregnancy are not primary infections. *HSV* can be transmitted to the infant vertically during the antenatal, intranatal, or postnatal periods (Nancy et al., 2007). Once acquired, *HSV* establishes latency in sensory ganglia, resulting in a lifelong infection. *HSV*-2 is the primary cause of genital herpes and is one of the most common sexually transmitted infections (STIs) worldwide (Jennifer et al., 2002). Primary symptomatic genital herpes presents with blistering and ulceration of the genitalia and cervix, causing vulvar pain, dysuria, vaginal discharge, and local lymphadenopathy. Systemic symptoms such as fever and myalgia, and complications like autonomic neuropathy and meningitis, may also occur. Women who acquire genital herpes during the third trimester are at risk of transmitting *HSV* to their babies during vaginal delivery, which can lead to neonatal herpes, a potentially life-threatening condition for the newborn (Sauerbrei and Wutzler, 2004). The seroprevalence of genital herpes among pregnant women varies internationally, with rates between 7.6% and 8.4% in Italy and approximately 22% in the US, where 2% of women contract genital herpes during pregnancy (Gianluca et al., 2012). In Italy, around 3% of women contract *HSV* during pregnancy, which is associated with risks such as spontaneous abortion, intrauterine growth retardation, preterm labor, and congenital and neonatal herpes infections. The risk of neonatal infection is highest, ranging from 30% to 50% for *HSV* infections that occur in late pregnancy, while early pregnancy infections carry a risk of about 1%. Intrapartum transmission accounts for 85% of prenatal *HSV* transmission (Gianluca et al., 2012). *HSV* is a significant cause of neonatal infections, leading to serious conditions like generalized encephalitis, which can be fatal. This study was conducted to determine the seroprevalence of *HSV*-1 and *HSV*-2 among pregnant Sudanese women attending Saad Abuo Elella Hospital, with the aim of contributing to the reduction of the virus's impact on public health.

Materials and Methods

Study design: This is a Health facility based cross-sectional study involving patients with *HVS* in Khartoum city.

Study area: Saad Abuo Elella Hospital was the major center for sampling and date collection.

Study population: All pregnant women attending the study area during the study period were considered eligible to be included.

Ethical consideration: All individual included in this study were informed about the objective of these study and its importance, each their acceptance to participate in the study was obtained, Data obtained will never be used for any other purpose. Acceptance of the ethical committee of Sudan Academy of Science was obtained and consent of the subject under study was taken.

Data collection: Through a well structured questionnaire (Appendixes), information on name, age and regional residency trimester of pregnancy. The patent was also asked for the history of abortion. **Patients and samples**: Venous bloods were collected from each subject under aseptic technique 5 ml from each.

Specimen Collection: Blood samples were obtained from 101 pregnant women under direct medical supervision through venipuncture, using a 5 ml syringe. The blood was collected into plain tubes, and serum was separated by centrifugation at 5000 rpm for 10 minutes.

The serum samples were stored at -20°C until the serological tests were conducted. Specimens were analyzed using a third-generation Enzyme-Linked Immunosorbent Assay (ELISA) (Euro – Imune , Germany) to detect IgG antibodies against Herpes simplex virus (*HSV*). The same ELISA method was used to detect anti-*HSV* IgG for both *HSV-1* and *HSV-2*.

ELISA Procedure: All reagents and samples were allowed to reach room temperature for 15 minutes before use. Washing buffer was prepared at a 1:25 dilution from a buffer concentrate with distilled water. 100µl of sample diluent was added to the appropriate wells, excluding the blank and negative control wells. Then, 5 µl of each sample was added to the respective wells and mixed thoroughly with a pipette until the solution turned from green to blue. Additionally, 100µl of negative and positive controls were dispensed into their respective wells, with no liquid added to the blank control well. The micro titer wells were gently flicked for 30 seconds to ensure thorough mixing, and the plate was then covered and incubated at 37°C for 30 minutes. After incubation, 350µl of wash buffer was added to each well, and the liquid was aspirated after 20 seconds (Washing 1). This washing step was repeated five times to ensure each well was dry. Subsequently, 100µl of Peroxidase-Conjugate Reagent was added to each well except the blank, followed by mixing. The plate was covered again and incubated at 37°C for another 30 minutes. After incubation, the plate cover was removed and discarded, and the liquid was aspirated. Each well was rinsed with wash buffer (Washing 2), and this step was repeated five times until the wells were dry. Next, 50µl of substrate A and 50µl of substrate B solution were added to each well, including the blank, and the plate was gently tapped to mix. The plate was incubated at 37°C for 10 minutes. Finally, 50 µl of Stop Solution was added to each well, and the plate was gently mixed.

Measuring: The plate reader was calibrated using the blank well, the absorbance was read at 450 nm. Results were calculated by comparing each sample's optical density (OD) value to the plate's cut-off value.

Calculation of Cut-Off (C.O) Value: The cut-off value was determined using the formula C.O = Nc * 2.1, where *Nc* represents the mean absorbance value of the two negative controls. Absorbance was measured using a micro plate reader at 450 nm.

Interpretation of Results

Negative Result: Samples with absorbance less than the cut-off value were considered non-reactive for this assay.

Positive Result: Samples with absorbance equal to or greater than the cut-off value were considered initially reactive.

Borderline: Samples with absorbance close to the cut-off value were considered borderline, and retesting of these samples in duplicate was recommended.

Results

A total of 101 pregnant women were enrolled in the study, they was distributed as 45 from Khartoum, 25 from Omdurman,9 from Bahri 19 from Aljazera and 3 from Nile White, Their age ranged from 16 to 41 years, and their mean age was 27.10 years. Most of them were in third trimester of pregnancy (67.7%) and had no previous abortion (80%). Among the total studied (101 pregnant women), 32 showed sero-positivity for *Herpes simplex virus HSV*-1 IgG antibodies, and 17 showed sero-positivity for *Herpes simplex virus HSV*-2 IgG antibodies respectively (table 1,2 and 3). While 5 were positive for both. Highest sero-positivity IgG was observed among 32 -38 age group range and among who had no history of abortion, in third trimester. As demonstrated in tables (1, 2 and 3). Statistical analysis showed that there was insignificant (P. value more than 0.05) between age, history of abortion and Trimesters.

Table 1. Serofrequency of *HSV*-1&2 among study population according to age group.

(Years)	Total Tested	IgG Type 1 +ve		IgG Type 2 +ve	
		Frequency	Percent	Frequency	Percent
16-24	36	30.5	11%	3	8.3%
25-31	44	40.9	18%	9	20.4%
32-38	13	23	3%	4	28.5%
>39	8	0	0%	1	12.5%
Total	101		32%	17	84%

Table 2. Serofrequency of *HSV*-1&2 among study population according to history of previous abortion.

Previous abortion	Total examined	IgG Type 1 +ve		P.Valeb /t group	IgG Type 2 +ve		P. Vale b/t group
		Frequency	Percentage		Frequency	Percentage	
Abortion (yes)	20	9	45	0.153	6	30	0.79
Abortion (No)	81	23	28		11	14	
Total	101	32	69		17	84	

Table 3. Sero-frequency of *HSV*-1&2 among study population According to trimester.

Trimester of pregnancy	Total	IgG Type 1 +ve		IgG Type 2 +ve	
		Frequency	Percentage	Frequency	Percentage
First trimester	10	5	50%	1	10%
Second trimester	24	7	29%	4	17%
Third trimester	67	20	30%	12	18%
Total	101	32	69	17	84

The analysis was done by chi-square test and the p. Value at <0.05 considered significant.

Discussion

Based on our result of a total of 101 pregnant women enrolled in the study, their mean age was 27.10 years which is considered an excellent target for this study considering literature review. As the most of these women were in third trimester of pregnancy (67.7%) and had no previous abortion (80%) it was important to study this group for monitoring risk of infection and its relation to pregnancy complications. Our study has also shown result of (101 pregnant women), of them 32 showed sero-positivity for *Herpes simplex HSV*-1 IgG antibodies, and 17 showed sero-positivity for *Herpes simplex HSV-2* IgG antibodies respectively compared to other studies in France where Seroprevalence's of *HSV*-1 antibody in pregnant women was 68%. This is considerably higher than the values obtained in study (32.3% for *HSV*-1). This could well be due to the fact that all sample collected in this study were from adults, whereas the French study was conducted in general population of all ages. *HSV*-1 seropositivity is known to increase with age. The seropevalence of *HSV*-2 in the French study in the same population was 17.3% (Whitley 2001) which is in agreement with our studies 17.2%. Also, in our study where 5 cases were positive for types of viruses and the highest sero-positivity IgG was observed among 32 -38 age group range and among who had no history of abortion, in third trimester with (P.value more than 0.05) between age, history of abortion, Trimesters. We used to target this group of women in our studies because the maternal genital herpes infection at the time of delivery may result in infection of the newborn infant during passage through the infected birth canal, or by ascending infection after rupture of the membranes (Ades et al, 1989) *HSV* establishes latency in sensory ganglia following acquisition, causing an infection that persists for life (Jennifer et al, 2002). However, compared to our study and in Saudi Arabia, the seroprevalence of *HSV*-1 and *HSV*-2 antibodies in pregnant women was 90.5% and 6.5% respectively (Alzahrani et al, 2005). The difference in the *HSV*-1 positivity between this studied population and the Saudi Arabia study could well be due to the assay used. The ELISA used in these studies is highly specific to *HSV*-1. The seropositivity for *HSV*-1 IgG antibodies in this study (32.3%). The estimated seropositivity of *HSV*-2 IgG antibodies in this study (17.3 %) is significantly less than the *HSV*-2 IgG antibodies reported by (Ghazi et al, 2002) (27.1%). This could well be due to regional differences within Saudi Arabia. Using cell culture, (Ades et al 1989) estimated the positivity of *HSV* isolation in pregnant women to be 50%. The difference in positivity between this data and data generated using the cell culture may well be due to the difference in sensitivity serological diagnosis of *HSV* and detection of *HSV* by cell culture. Serological assays are far more sensitive than cell culture techniques.

Conclusion

In this study the seroprevalence of *HSV*-1 and *HSV*-2 IgG antibodies were found to be 32.3% and 17.2% respectively. Patients with high prevalence of abortion were found to have high rates of infection *HSV*-1 and *HSV*-2 IgG antibodies compared to those without past history of abortion rate for both types of infection.

Recommendations

Further in-depth studies including large sample size and other areas are recommended. Pregnant women are recommended to be screened for *HSV* infection.

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