

Sero frequency of Herpes simplex (1&2) virus among pregnant women attending Omdurman maternity hospital

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Abstract:

Background: Primary Herpes simplex virus (HSV) infection may lead to severe illness in pregnancy and may be associated with transplacental virus transmission and fetal infection. The consequences may be abortion, stillbirth and congenital malformations. This study was carried out to detect Herpes simplex (1&2) virus sero- frequency among pregnant women Omdurman Maternity Hospital, Sudan

Methods: this is across –sectional study, serum specimens were collected and analysed by ELISA for HSV (1&2) IgG and IgM antibodies among pregnant women, and the result were correlated with Age , history of abortion trimester of pregnancy and history of chronic illnesses . Generated data were analyzed using SPSS

Results: out of the 90 pregnant ladies tested 82 (91.1%), 9 (10.0%) were positive for IgG and IgM respectively, and 8 (8.9%) were

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negative for both. The result showed higher frequency (50.0 % and 33.3 % for IgM) for IgG among 20 – 30years of age group. HSV (1& 2) serofrequency was significantly associated with body weight but insignificantly associated with age, chronic illness, history of aboraton and gestational age.

Conclusion: *HSV(1&2) can infect pregnant women and their neonates, assessment of HSV infection among them will help in the proper management of HSV infection, besides being useful for epidemiological purposes.*

Key words: Herpes simplex (1&2) IgM, IgG, ELISA, Pregnant Women, Omdurman Maternity Hospital, Sudan

Introduction

The HSV has been considered as one of the most common viral infectious agents in humans. Herpes simplex virus (HSV) is classified in the alpha virinae sub-family within the Family Herpes viridae⁽¹⁾, Herpes simplex virus (HSV) is a DNA virus with 2 subtypes: Herpes simplex virus1 (HSV-1) and Herpes simplex virus⁽²⁾ (HSV-2). Although each is a distinct virus, they share some antigenic components⁽³⁾. HSV-1 is usually transmitted by contact with oral secretions and causes most HSV or official infections, while HSV-2 is usually spread by sexual contact and causes most cases of genital herpes⁽²⁾.

The two Type are closely related viral types, the HSV type 1 (HSV-1) and the HSV type 2 (HSV-2), which are genetically dive rent. In Germany, the prevalence of HSV-1 antibodies reaches high levels of more than 90% in adults whereas nearly 15% of the adult population possesses antibodies to HSV-2⁽³⁾. The number of women, who acquire HSV-1 or HSV-2 infection during pregnancy, has been calculated as 0.5–2%^(4,5)

The most important HSV infection during pregnancy is the primary genital HSV infection, since it can cause the most

severe neonatal diseases. However, a Wrest manifestation of genital herpes during pregnancy is in most cases not a primary infection.

HSV can be vertically transmitted to the infant during the antenatal, intra natal, or postnatal periods ⁽⁶⁾.

HSV establishes latency in sensory ganglia following acquisition, causing an infection that persists for life. HSV-2 infection is the primary cause of genital herpes and is one of the most prevalent sexually transmitted infections STIs worldwide ⁽⁷⁾.

Primary symptomatic genital herpes presents with blistering and ulceration of the external genitalia and cervix leading to valvulas pain, dysuria, vaginal discharge and local lymph adeno pathy. Infection may be complicated by systemic symptoms such as fever and myalgia and occasionally by autonomic neuropathy resulting in urinary retention and meningitis. Women, who acquire genital herpes during the third trimester, are at risk of transmitting HSV to their babies during vaginal delivery. Infection intrapartum can lead to neonatal herpes, also referred to as herpes neonatorum, which is considered a life-threatening illness of the neonate⁽⁸⁾.

Regarding pregnant population, there is a high prevalence of genital herpes. Among Italian pregnant women, the sero prevalence varies from 7.6% to 8.4% sero prevalence[.Nevertheless it is lower than that reported among pregnant women in other countries. For example, in US, approximately 22% of pregnant women are infected with HSV-2, and 2% of women acquire genital herpes during pregnancy, placing their newborn at risk for herpes infection. In Italy, the number of women who acquire HSV infection during pregnancy is about 3%. The acquisition of genital herpes during pregnancy has been associated with spontaneous abortion, intrauterine growth retardation, preterm labour, and congenital and neonatal herpes infections ⁽⁹⁾.

The risk of neonatal infection varies from 30% to 50% for HSV infections that onset in late pregnancy (last trimester), whereas early pregnancy infection carries a risk of about 1%. When primary HSV infection occurs during late pregnancy, there is not adequate time to develop antibodies needed to suppress viral replication before labour. About 85% of perinatal transmission occurs during the intrapartum period while transmission of HSV from mother to foetus during pregnancy is less common. Moreover, studies in HIV infected pregnant women show that co-infection with HSV increases significantly the risk of perinatal HIV transmission above all in women who had a clinical diagnosis of genital herpes during pregnancy ⁽⁹⁾.

This study aimed to detect sero-frequency of HSV (1&2) among pregnant ladies.

Material and Methods:

This was descriptive- cross sectional study which had been conducted in Khartoum state during period from March to April 2015, ninety pregnant ladies were enrolled, Data was collected by using direct interviewing questionnaire; ethical clearance was obtained from research ethical committee of faculty of graduate studies and ministry of health Khartoum state, written consent also was obtained from Pregnant ladies.

Samples collection:

blood samples were collected from 90 pregnant ladies, under direct medical supervision by medial vein puncture using 5 ml syringe into plain tube to obtain serum by centrifugation at 5000 rpm for 10 min. serums was kept in -20°C till serological study was performed. Specimens were processed by Enzyme linked immune sorbent assay (ELISA) (3rd generation ELISA) (Foresight- Germany) for detection IgM and IgG.

Enzyme linked immune sorbent assay for detection anti Herpes simplex IgM and IgG (the same method for both)

All reagents and samples were allowed to reach room temperature for 15 minutes before use. Washing buffer was prepared 1:25 from buffer concentrate with distilled water. 100µl of sample diluents was added into appropriate wells except the blank well and negative well. 5 µl from each sample was added to the appropriate wells and mixed by pipette repeatedly until liquids turn from green to blue. 100µl from negative and positive control was dispensed and added to the negative and positive wells separately without dispensing liquid into the blank control well. Microtiter wells were flicked for 30 seconds and mixed well, then plate was covered and incubated for 30 minutes at 37°C. Plate was taken out and 350µl of wash buffer was added to each well (Washing 1) and aspirated off after 20 seconds. This step was repeated for 5 times until each well became dry, 100µl of Peroxidase-Conjugate Reagent was added in to each well except the blank, the plate was mixed well and covered with the plate cover and incubated for 30 min at 37°C. The plate cover was removed and discarded. The liquid was aspirated and each well was rinsed in wash buffer (Washing 2). This step was repeated for 5 times until each well became dry. 50µl of substrate A and 50µl substrate B solution was added in to each well including the Blank and mixed by tapping the plate gently. The plate was incubated at 37°C for 10 min. 50 µl Stop solutions was added into each well and mixed gently.

Measuring the absorbance:

The plate reader was calibrated with blank well and the absorbance was read at 450 nm. The 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.

$$C.O = *Nc * 2.1$$

*Nc= the mean absorbance value for the two negative controls.

The absorbance was read with micro well reader at 450nm.

Interpretation of Results:

Negative results:

Samples show absorbance less than Cut-off value is nonreactive for this assay.

Positive result:

Sample show 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.

Data analysis:

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

Result:

A total of 90 pregnant women were enrolled in the study. Their age ranged from 15 to 46 years, and their mean age was 28.00 years. Most of them were in third trimester of pregnancy(54.4%), had no chronic illnesses (78.9%), and had no history of abortion (80.0%).

Among the total studied (90 pregnant women) , 9 (10.0%) and 82 (91.1%) showed sero-positivity for Herpes simplex IgM and IgG antibodies, respectively (fig 1,2). While 9 (10.0%) were positive for both. Highest sero-positivity of IgM and IgG was observed among 20 -30 age group range and among whom had no history of abortion, in third trimester, and had no

history of chronic illness, (as demonstrated in tables (2, 3 and 4).

Statistical analysis showed that there was significant association (P. value less than 0.05) between body weight and presence of HSV(1&2) antibodies (table 5), but there is insignificant correlation (P. value more than 0.05) between age, history of abortion, Trimesters, and chronic illness.

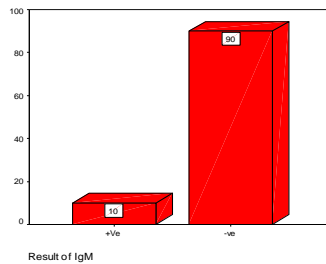


Figure 1: frequency of anti HSV (1&2) IgM among studied population (n= 90)

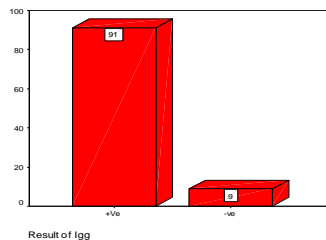


Figure 2: Frequency of anti HSV (1&2) among studied population (n= 90)

Table 1: Serofrequency of HSV (1- 2) among studied population (n=90) according to Age group

Age group in year	Result			
	IgM seropositivity	IgG seropositivity	IgM -IgG seropositivity	Negative
10-20	2(22.2%)	15(18.3%)	2(22.2%)	1(12.5%)
20-30	3(33.3%)	41(50.0%)	3(33.3%)	6(75.0%)
30-40	4(44.5%)	24(29.3%)	4(44.4%)	1(12.5%)
40-50	0(0.0%)	2(2.40%)	0(0.0%)	0(0.0%)
Total	9(100%)	82(100%)	9(100%)	8(100%)
p.vduer	.569**	.584**	.214**	

** Not significant of the 0.05 level

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Table 2: Serofrequency of HSV (1&2) among studied population (n=90) according to past history abortion

History of abortio	Result			
	IgM seropositivity	IgG seropositivity	IgM – IgG Seropositivity	Negative
Yes	2(22.2%)	18(22%)	2(22.2%)	0(0.0%)
No	7(77.8%)	64(78%)	7(77.8%)	8(100%)
Total	9(100%)	82(100%)	9(100%)	8(100%)
p.vduer	.861**	.138**	.156**	

Not significant at the 0.05 leve

Table 3: Seropositivity of HSV (1&2) among studied population (n=90) according to trimester

Trimester of pregnancy	Result			
	IgM seropositivity	IgG seropositivity	IgM – IgG seropositivity	Negative
First trimester	2(22.2%)	31(37.8%)	2(22.2%)	2(25%)
Second trimester	2(22.2%)	7(8.5%)	2(22.2%)	1(12.5%)
Third trimester	5(55.6%)	44(53.7%)	5(55.6%)	5(62.5%)
Total	9(100%)	82(100%)	9(100%)	8(100%)
p.value	.871**	.756**	.871**	

Not significant at the 0.05 level

Table 4: Serofrequency of HSV (1&2) among studied population (n=90) according to chronic illnesses.

History of chronic illnesses	Result			
	IgM seropositivity	IgG seropositivity	IgM – IgG Seropositivity	Negative
Yes	1(11.1%)	16(19.5%)	1(11.1%)	3(37.5%)
No	8(88.9%)	66(80.5%)	8(88.9%)	5(62.5%)
Total	9(100%)	82(100%)	9(100%)	8(100%)
p.vduer	.438**	.234**	.200**	

Not significant at the 0.05 level

Table 5: Serofrequency of HSV (1&2) among studied population (n=90) according to body weight chronic illnesses.

Body weight	Result			
	IgM seropositivity	IgG seropositivity	IgM – IgG Seropositivity	Negative
50-70 k.g	3(33.3%)	55(67%)	3(33.3%)	6(75%)
70-90k.g	5(55.0%)	26(31.7%)	5(55.6%)	2(25%)

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90-110k.g	1(11.1%)	1(1.2%)	1(11.1%)	0.0%
Total	9(100%)	82(100%)	9(100%)	8(100%)
p.value	.0002**	.873**	.198**	

Significant at the 0.05 level for IgG

Discussion

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 ⁽¹⁰⁾. HSV establishes latency in sensory ganglia following acquisition ,causing an infection that persists for life⁽¹¹⁾ .

The result of this study revealed a Herpes simplex virus (1&2) overall sero-frequency rate of 82(91.1%) for IgG and 9 (10.0%) for IgM. We presume this indicates a circulation of wild Herpes simplex virus (1&2) in Sudan, Since Herpes simplex (1&2) vaccination is not practiced in the country. If this compared with related finding, this is considerable higher than in France was 68%. This could be due to the fact that all our samples were from adults, whereas the French study was conducted in general population of all ages.

Also as In Zaire, the seroprevalence of HSV-1 and HSV-2 antibodies in pregnant women was 85% and 32% respectively. The difference in the HSV-2 positivity between our studied population and the Zaire study could well is due to the assay used. The ELISA used in our report is highly specific to HSV-2. The seropositivity for HSV-1 IgG antibodies in our study (90.5%) confirms the findings of previous investigators ⁽⁹⁾.

However different results revealed that sero-positivity of HSV-2 (1&2) IgG antibodies (91.1%) was significantly more than HSV-2 IgG antibodies reported by Ghazi et al ⁽⁸⁾ (27.1%).This could well be due to regional differences within Saudi Arabia.

Conclusion:

This high frequency of HSV (1&2) IgG & IgM antibodies among pregnant ladies suggesting a sustained past and current infection in the population and indicating endemicity also outbreaks and possibly reinfection may occur. Seropositivity of IgG antibody indicate susceptibility of high proportion of the population to the infection and this necessitate the introduction of HSV vaccine.

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