Seroprevalence of Herpes Simplex Virus Type 1 among Pregnant Women Attending Abo Gota Antenatal Care Clinic, AlGezira State, Sudan

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ABSTRACT

Background: Infection with HSV are unaware they have contacted the virus, and most new infections in pregnant. Herpes infection can be passed from mother to child during pregnancy, childbirth, or in the newborn period, resulting in a potentially fatal neonatal herpes infection. This study aimed to determine the presence of HSV-1 IgM and IgG antibody and to detect relation between seropositivity of HSV-1 and other factors (age, trimesters, history of abortion, gravidity).

Methods: This was descriptive cross sectional study in which 90 pregnant women attending AboGota antenatal care clinic during January to March 2015 were included their age ranged from 15 to 45 years with mean 30. Serum specimens were collected and tested by semi-quantitative enzyme linked immune sorbent assay (ELISA) for presence or absence of anti herpes simplex virus type-1 (IgM) and (IgG).

Results: Most of the enrolled pregnant ladies were multigravidae (52.2%), and in third trimester of pregnancy (66.7%), the results showed that 8(8.9%), 32 (35.6%) were positive for IgM antibody and IgG respectively, while 2 (2.2%) were positive for both. High frequencies of positive IgM and results were observed among 15-20 years age groups. The study shown statistically significant relationship (P value more than 0.05) between trimesters and HSV-1 IgM infection. However there were insignificant correlation (P value more than 0.05) between age, Gravidity, history of abortion and presence of HSV-1.

Conclusion: In the present study about high seropositivity was observed among pregnant ladies further studies were recommended to validate this result.

Keywords: sero frequency, anti-herpes simplex virus type-1, IgM, IgG, pregnancy, ELISA, Abo Gota, Sudan.
INTRODUCTION

Herpes simplex virus (HSV) is an ubiquitous, enveloped, and double stranded DNA virus, belonging to the family of Herpesviridae transmitted across mucosal membranes and non intact skin, that migrate to nerve tissues, where they persist in a latent state. HSV-1 predominates in orofacial lesions, and it is typically found in the trigeminal ganglia, whereas HSV-2 is most commonly found in the lumbosacral ganglia [1].

HSV-1 infection typically occurs during childhood and adolescence through direct oral exposure and, if symptomatic, it is characterized by orolabial or facial lesions. However, recent studies have showed that HSV-1 has become a major causative agent of genital herpes in some developed countries [2, 3].

Infected with HSV are unaware they have contacted the virus, and most new infections in pregnant [4].

Intrauterine HSV infection accounts for 5% of HSV infections in neonates. The highest risk of intrauterine infection has been observed in pregnant (about 50%) who develop disseminated HSV infections and 90% of those are related to HSV-2. Both primary and recurrent maternal infection can result in congenital disease, even if the risk after recurrent infection is small [5].

Intrauterine viral transmission is highest during the first 20 weeks of gestation leading to abortion, stillbirth, and congenital anomalies. The perinatal mortality is 50% [5].

Neonatal herpes is one of the most serious complications of genital herpes. Healthcare providers should ask all pregnant women if they have a history of genital herpes. Herpes infection can be passed from mother to child during pregnancy, childbirth, or in the newborn period, resulting in a potentially fatal neonatal herpes infection. During pregnancy there is a higher risk of perinatal transmission during the first outbreak than with a recurrent outbreak, thus it is important that women avoid contracting herpes during pregnancy. [6]

Turkey asymptomatic pregnant women who were admitted to Gynecology and Obstetrics clinics of Izmir Ataturk Research and Training Hospital for routine control, were investigated for IgG and IgM antibodies specific for HSV-1 and HSV-2 were screened by commercial ELISA Total IgG seropositivity rates for HSV-1 were found as 94.7% (108/114), while IgM seropositivities were 0 (0/114) [10].

The aim of this study was to determine the presence of HSV-1 IgG and IgM antibody and to detect relation between seropositivity of HSV-1 and other factors (age, trimesters, abortion, and gravidity).

MATERIALS AND METHODS

Design:
The present study was descriptive cross sectional study carried out in ALNeelain University in which 90 pregnant women at reproductive aged (12-45) years attending AboGota antenatal care clinic during January to March 2015 were enrolled.

Ethical consideration:-
Written consent was taken from patients under study, and approval from research ethical comitee ALNeelin university.

Experimental work:
Collection of specimens:
Before collecting the specimen Each subject completed a structured questionnaire. Relevant sociodemographic variables were analyzed. A total of 90 blood specimens were collected, serum was separated from a pregnant women, the samples were then stored at -20°C refrigerator.

Processing of specimens:
Serum samples were analyzed using Enzyme-linked immune sorbent assay (indirect ELISA) kits (VirionSerion, Germany) for anti-Herpes Simplex virus type-1 (HSV-1) (IgM) and (IgG) the same method for both. Negative and positive controls were used, and the ELISA kits tested within analyzing the serum samples.

All sera and reagents were brought to room temperature for at least 15-30 minutes before the test was carried out. The washer buffer diluted 3% with distilled water. The strips needed were set in strip-holder and numbered sufficient number of wells including one negative control two stander and one calibrator. The specimens were diluted 1:100 with sample buffer for IgG and were diluted 1:100 with and rheumatoid factor absorbent and sample buffer (2:8) to avoid interfere of IgM with rheumatoid factor, samples incubated for 10 minutes at room temperature. The controls are ready to use as supplied. 100μl of samples were added in to each well and 100μl stander and negative controls in to their respective wells. A separated disposal pipette tip was used for each specimen. The plate was covered with the plate cover and incubated for 1 hour at 37°C in moisture. At the ends of the incubation the plate covers was removed and discard, then washed each well automatically 3 times with 400μl working strength wash buffer. Each time the micro wells were allowed to soaked for 30-60 seconds. After the final washing cycle, the plate was blotted on to a clean towel, to remove any remaining buffer. 100μl of peroxidase labeled anti- IgM human (goat) were
added in to each well except the blank. The plate was covered with the plate cover and incubated for 30 minutes at 37°C. Then the plate cover was removed and washed. 100μl of chromogen/substrate solution were added in to each well including the Blank, then was Incubated at 37°C for 15 minutes. 100μl of Stop solution was added in to each well in the same order and the same speed as the chromogen/substrate solution was introduced. The plate reader was calibrated with the calibrator well and read the absorbance at 450nm.

Measurement:
Photometric measurement of colour intensity was red at 450 wavelength within 15 minutes of added stop solution.

Calculation of results and interpretation:
Results were semi quantitatively.
Formula to calculation:
The mean between two standers value*factor
Recommend interpreted results as follows:

IgM:
Ratio <0.5: negative
Ratio ≥ 0.5 to 0.8 border line
Ratio ≥ 0.8 positive

IgG:
Ratio <0.18: negative
Ratio ≥ 0.18 to 0.26 border line
Ratio ≥ 0.26 positive

Data Analysis:
Data retrieved from the questionnaires were analyzed using the Statistical Package for Social Sciences (SPSS) version 16 and the Microsoft Excel (MS) software program. The seroprevalence of anti-HSV-1 (IgM and IgG), age, trimesters, and gravidity distribution were analyzed among pregnant women. The degree of association of seroprevalence (IgM and IgG) with age, trimesters, gravidity and were determined using Chi square test. Statistical significance was set at p-value of less than or equal to (0.0

RESULT
A total of 90 pregnant ladies who enrolled in this study. Their age ranges from 15-45 years with mean 30 Figure (1), most of them were multigravidae (52.2%), in third trimester of pregnancy (66.7%). The results showed that 8(8.9%), 32 (35.6%) were positive for IgM antibody and IgG respectively (fig 2,3), while 2 (2.2%) were positive for both. High frequencies of positive IgM and IgG results were observed among 15-20 age groups (table 1) and who were in third trimester 6 (6.7%) for HSV IgM, while it was 22 (24.4%) for IgG (tables 2). Regarding abortion 1(1.1%) for HSV IgM, while it was 4 (4.4%) for IgG were had past history of abortion (table 3), and were multigravidae (tables 4). The statistical analysis showed significant relationship (p=0.05) between trimester and HSV-1 IgM infection and there were insignificant correlation (P value more than (0.05) between age, Gravidity, past history of abortion and presence of HSV-1.

DISCUSSION
Herpes Simplex virus infection occurs worldwide but its epidemiology varies between different countries and between groups of individuals. The seroprevalence of HSV-1 and HSV-2 antibodies is an accurate method of determining the epidemiology of this infection[7,8].
Even though genital herpes infection in pregnant women is common and rarely serious, the risk of vertical transmission to the infant when the mother develops a primary infection during the third trimester is high and this risk increases the closer to the time of delivery[8, 9].
This w study was carried in AboGota antenatal care clinic aimed to detect the presence of HSV-1 IgM and IgG antibody.
Our study included 90 pregnant women were investigated for HSV IgM and IgG. seropositivity of HSV IgM were 8(8.9%), IgG were 32(35.6%) among pregnant women, when compared with other study carried out in Gynecology and Obstetrics clinics of Izmir Ataturk Research and Training (2009) it found to be higher than their results was 0( 0%) HSV-1 IgM positive while IgG seropositivity rates for HSV-1 were found as (108/114) 94.7% [10], other study carried out in Nigeria (2013) it found to be highest than other results was 0(0%) HSV-1 IgM positive while IgG seropositivity rates for HSV-1 were found as (255/264) 96.6% [11].
In the present study seropositive result was high among 15-20 years age range HSV IgM were 1(1.9%), IgG were 16(30.8%).
In the present study the relationship between seropositivity and trimester demonstrated that seropositivity of HSV IgG was the highest for third trimester 22 (24.4%); while it was the highest for third 6 (6.7%) trimester for HSV IgM.
In Allahabad (India) Seropositivity of HSV IgG was the highest for third trimester and it was followed by second and first trimester; while it was the highest for first trimester for HSV IgM and it was followed by third and second trimester. Seroprevalence of HSV IgG IgM was 32 (53.3%), 61 (66.3%), and 27 (69.2%) for first, second and third trimester, respectively. Seroprevalence of HSV IgG IgM group
was 02 (3.3%), 01 (1.1%) and 01 (2.6%) for first, second and third trimester, respectively. Seroprevalence of HSV IgG IgM group was 26 (43.3%), 30 (32.6%), and 11 (28.2%) for first, second and third trimester, respectively.\(^{(12)}\).

In the present study the relationship between seropositivity and abortion demonstrated that seropositive of HSV-1 IgM were 1 (1.1%) , IgG were 4 (4.4%) among pregnant women were had history of abortion while HSV-1 IgM were 7 (7.8%) , IgG were 28 (31.1%) had not , when compared with study carried out in Tehran, Iran\(^{(14)}\) demonstrated that There were no significant relationship between abortion and serologic results of HSV-1 7 among pregnant women had history of abortion while 146 had not\(^{(13)}\).

CONCLUSION
This study reported high result for serofrequency of HSV-1 among pregnant women that indicate the important of screening for HSV-1 infection for all pregnant women. We recommended vaccinating all pregnant women attending antenatal care clinic. Further confirmation and mentoring with large scale population is recommended.

ACKNOWLEDGMENT
Our thanks to all pregnant women attending AboGota Antenatal Care Clinic who contributes in this study, department of Medical Microbiology in Faculty of Medical Laboratory Sciences AL Neelain University, and all staff and Research lab for their technical support.
Figure 3
Frequency of anti HSV-1 IgG among study population (n= 90)

Table 1
Serofrequency of HSV-1 according to age group of study group (n=90)

<table>
<thead>
<tr>
<th>Age groups</th>
<th>Results</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgM seropositive</td>
<td>IgG seropositive</td>
</tr>
<tr>
<td>15---&gt;20</td>
<td>2 (2.2%)</td>
<td>9 (10%)</td>
</tr>
<tr>
<td>20---&gt;25</td>
<td>2 (2.2%)</td>
<td>9 (10%)</td>
</tr>
<tr>
<td>25---&gt;30</td>
<td>1 (12.5%)</td>
<td>6 (6.7%)</td>
</tr>
<tr>
<td>30---&gt;35</td>
<td>1 (1.1%)</td>
<td>4 (4.4%)</td>
</tr>
<tr>
<td>35---&gt;40</td>
<td>2 (2.2%)</td>
<td>3 (3.3%)</td>
</tr>
<tr>
<td>more than 40</td>
<td>0 (0%)</td>
<td>1 (1.1%)</td>
</tr>
<tr>
<td>Total</td>
<td>8 (8.9%)</td>
<td>32 (35.6%)</td>
</tr>
</tbody>
</table>
Table 2
Serofrequency of HSV-1 among pregnant ladies (n=90) according to trimesters of pregnancy

<table>
<thead>
<tr>
<th>Trimesters</th>
<th>Result</th>
<th>Total</th>
</tr>
</thead>
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<tr>
<td></td>
<td>IgM seropositive</td>
<td>IgG seropositive</td>
</tr>
<tr>
<td>First</td>
<td>2 (2.2%)</td>
<td>4 (4.4%)</td>
</tr>
<tr>
<td>Second</td>
<td>0 (0%)</td>
<td>6 (6.7%)</td>
</tr>
<tr>
<td>Third</td>
<td>6 (6.7%)</td>
<td>22 (24.4%)</td>
</tr>
<tr>
<td>Total</td>
<td>8 (8.9%)</td>
<td>32 (35.6%)</td>
</tr>
</tbody>
</table>

Table 3
Serofrequency of HSV-1 among pregnant ladies (n=90) according to history of abortion

<table>
<thead>
<tr>
<th>History of abortion</th>
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<th>Total</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>IgM seropositive</td>
<td>IgG seropositive</td>
</tr>
<tr>
<td>Yes</td>
<td>1 (1.1%)</td>
<td>4 (4.4%)</td>
</tr>
<tr>
<td>No</td>
<td>7 (7.8%)</td>
<td>28 (31.1%)</td>
</tr>
<tr>
<td>Total</td>
<td>8 (8.9%)</td>
<td>32 (35.6%)</td>
</tr>
</tbody>
</table>

Table 4
Serofrequency of HSV-1 among pregnant ladies (n=90) according to gravidity

<table>
<thead>
<tr>
<th>Gravidity</th>
<th>Result</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgM seropositive</td>
<td>IgG seropositive</td>
</tr>
<tr>
<td>Primagravidae</td>
<td>6 (6.7%)</td>
<td>13 (14.4%)</td>
</tr>
<tr>
<td>Multigravidae</td>
<td>2 (2.2%)</td>
<td>19 (21.1%)</td>
</tr>
<tr>
<td>Total</td>
<td>8 (8.9%)</td>
<td>32 (35.6%)</td>
</tr>
</tbody>
</table>

REFERENCES
8. Gungor S, Kocabeyoglu O, Gun H et al. Herpes simplex virus type 2 antibody levels in sera


