Clinico-Microbiological Study of Herpes in Pregnant Females with Bad Obstetric History (BOH)

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ABSTRACT

Background: Herpes simplex virus (HSV) 1 and 2 infection is one of the most common viral infections worldwide. The first-time infection of the mother may lead to severe illness in pregnancy and may be associated with virus transmission from mother to foetus. Neonatal infection is manifested as chorioretinitis, mental retardation, seizures and death.

Objectives: The aim of the study is to assess the seroprevalence of IgG and IgM antibodies for Herpes simplex virus 1 and 2 in women with normal pregnancy, pregnant women with one abortion & pregnant women with history of two or more abortions (BOH). The seropositivity was analysed according to the number of abortions, age, parity and trimester of pregnancy.

Materials & Methods: Serum sample were collected from pregnant women attending antenatal clinic with demographic data. Patients were divided into three groups- normal pregnant women (N), pregnant women with one abortion (A1) & those with two or more abortions (A2) i.e. those with a bad obstetric history. Infection was demonstrated by the presence of IgM and IgG antibodies by ELISA method. A total 78 serum samples were subjected for determination of Herpes 1 and 2 IgM antibodies and 73 for Herpes 1 and 2 IgG antibodies. The study was carried out over a period of two years from November 2012 to October 2014.

Results: Out of 73 cases, 19 cases of normal pregnancy showed 0% IgG prevalence. The distribution showed increase of IgG with increase in number of abortions. In group A1 patients, IgG was equally positive for those with gestational age 2nd trimester (13-28 weeks) and 3rd trimester (29-40 weeks). In this group (A1), IgG was mostly positive for those in the age group of 20-25 years followed by 26-30 years. The distribution for group A2 patients showed an increase of IgG antibodies for those with gestational age 3rd trimester (29-40weeks). The patients of this group also showed increased IgG Antibodies with age group 20-25 years.

Conclusion: High prevalence of HSV 1 and 2 IgG antibodies indicates prior exposure or infection. The incidence of HSV 1 and 2 continue to rise & because the greatest incidence of HSV infection occurs in women of reproductive age, the risk of maternal transmission of the virus to the foetus or neonate has become a major health concern.

Keywords: Microbiological Study, Herpes simplex virus, Seroprevalence, BOH, ELISA.

INTRODUCTION

Herpes Simplex Virus (HSVs) are extremely host-adapted viruses that can cause a wide variety of illness in infected human hosts. There are two types of the HSVs:

1. Herpes Simplex Virus type 1 (HSV-1)
2. Herpes Simplex Virus type 2 (HSV-2) 1

Both the types are closely related in their DNA homology, antigenic determinants, and tissue tropism and disease symptoms but differ in epidemiology. HSV-1 is transmitted primarily by contact with infected saliva whereas HSV-2 is transmitted by sexual contact or by genital tract infection to new-born from an infected mother. 1

HSV 1 and 2 infection is one of the most common viral infection worldwide. The 1st time infection of the mother may lead to severe illness in pregnancy & may be associated with virus transmission from mother to foetus. Since the incidence of this sexually transmitted infection continues to rise and because the greatest incidence of HSV infection occur in women of reproductive age, the risk of maternal transmission of the virus to the foetus or neonate has become a major health concern. 2 The risk of transmission of infection from mother to baby is highest if the mother becomes infected around the time of delivery (30-60%) since sufficient time will have occurred for generation and transfer of protective maternal antibodies before the birth of the child. 3

HSV-1 & HSV-2, herpes virus hominis causes a variety of infections involving mucocutaneous surfaces, the CNS, and on occasion visceral organs. 4

HSV tends to cause more frequent and severe infections in the patients with altered CMI such as HIV and other CMI deficient diseases. Tissue damage occurring in HSV infection is due to a combination of viral pathology and immunopathology. These immunopathology effects of CMI and inflammatory responses are the major causes of symptoms seen during the disease. 1
Aim
The aim of the study was to assess the seroprevalence of IgG and IgM antibodies for HSV for women with normal pregnancy, pregnant women with one abortion and pregnant women with two or more abortions that is those with Bad Obstetric History (BOH).

The sero-positivity was analysed according to number of abortions, age, parity and trimester of pregnancy.

MATERIAL AND METHODS
The study was an experimental and analytical study carried out in the Department of Microbiology, MGM Medical College, Kamothe, Navi Mumbai over a period of two years from November 2012 to October 2014. Ethical clearance was obtained from institutional ethics committee prior to study. Blood samples were collected from pregnant women attending antenatal clinic and patients admitted in antenatal ward of the hospital after taking written informed consent to perform ELISA test for HSV-1 and 2 IgM and IgG on the serum samples.

The IgM and IgG Elisa kit was manufactured by Delta Biological a subsidiary of Erba Diagnostics and supplied by Trans Asia Biomedical Ltd. The HSV IgM Elisa Kit bearing lot no 243-A with expiry date 2014 had a sensitivity of 85.7% and specificity of 95.4%. The HSV-1 and HSV-2 IgG Elisa Kit with lot no-167-A and expiry date 2014-10 had both sensitivity and specificity of 100%. The test was performed as per instructions of the manufacturer.

IgM Elisa -The controls consisted of one negative control and 2 cut off and one positive control. Validity and test results were calculated as per literature of the manufacturer.

Validity Criteria-

a) OD of positive control was 1.5 times that of the cut-off.

b) The ratio between NC & cut off was < 0.6

c) OD of cut off was > 0.16 at 450/620 nm

Ratio between OD value of sample and that of cut off was > 1.2 and was considered as Positive result.

IgG Elisa -The controls consisted of two negative controls (NC), two positive controls (PC) and two cut off calibrators. The mean absorbance of NC, cut off calibrator and PC was calculated.

Validity Criteria-

a) Blank well-Blank absorbance was < 0.050 at 450/630.

b) NEGATIVE CONTROL: The Mean Absorbance after subtracting Blank Absorbance was <0.100 and was considered as valid according to the kit literature.

c) CUT-OFF CALIBRATOR: The Mean Absorbance after subtracting Blank Absorbance was >0.150 and was considered as valid according to the kit literature.

d) POSITIVE CONTROL: The Mean Absorbance after subtracting Blank Absorbance was >1.500 and was considered as valid according to the kit literature.

The index value was calculated to obtain the qualitative specimen results

The cut-off value was obtained by subtracting the blank absorbance from the mean absorbance of the cut off calibrator.

The index value was calculated by dividing the specimen absorbance by the cut off value.

Index value was >1.1 and was considered Reactive.

A predefined proforma was used to collect the data regarding age of the patient, gestational age (trimester) and number of abortions. The result of ELISA test was then subjected to statistical analysis.

RESULTS
A total of 78 samples were taken for detection of IgM antibodies against HSVs out which 19 samples belonged to group N which consisted normal ANC patients, 32 belonged to group A1 which consisted ANC patients with one abortion and 27 belonged to group A2 consisting of ANC patients with two or more abortions (BOH). Whereas a total of 73 samples were taken for detection of IgG antibodies against HSVs out of which 19 samples belong to group N, 22 samples belonged to group A1 & 32 samples belonged to group A2. Prevalence of IgM positivity was 0% and that of IgG was 42.46% (Table 1).

The distribution of abortion has shown that the prevalence of IgG increase from 0% to 62.5% (Table 2). Only 31 patients out of the total of 73 subjected for determination of IgG antibodies against HSV was found to be positive. Maximum distribution of IgG antibodies in A1 & A2 group seen with parity 1 (45.5% & 60%).

The age group distribution has shown high rate of IgG positivity for both A1 and A2 in age group 20-25 years, A1 (36.4%) & A2 (25%). (Table 3). The gestational age distribution shows that IgG was equally positive for group A1 patients with gestational age 2nd and 3rd trimester (36.4%) but for A2 group patients, IgG was most positive for patients with gestational age 3rd trimester (45%). (Table 4). Maximum distribution of IgG antibodies in A1 & A2 group seen with parity 1 (45.5% & 60%). (Table 5).

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Table 1: IgM and IgG positivity in total number of subjects

<table>
<thead>
<tr>
<th>Antibody</th>
<th>No. of Samples</th>
<th>No. of Positive Samples</th>
<th>Positive Percentage (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgM</td>
<td>78</td>
<td>0</td>
<td>0%</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Not Significant</td>
<td></td>
</tr>
<tr>
<td>IgG</td>
<td>73</td>
<td>31</td>
<td>42.46%</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Highly Significant</td>
<td></td>
</tr>
</tbody>
</table>

Graph 1: IgM and IgG positivity

Table 2: HSV IgG Distribution and Abortion

<table>
<thead>
<tr>
<th>Total No of Samples</th>
<th>Positive</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Normal ANC = 19</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>2) One Abortion = 22</td>
<td>11</td>
<td>50%</td>
</tr>
<tr>
<td>3) Two or more Abortions = 32</td>
<td>20</td>
<td>62.5%</td>
</tr>
</tbody>
</table>

Graph 2: IgG and Abortion

Graph 3: Age distribution of subjects (IgG)
Table 3: Age distribution of subjects (IgG)

<table>
<thead>
<tr>
<th>Age Distribution</th>
<th>Normal ANC (0)</th>
<th>One Abortion (11)</th>
<th>Two or more abortion (20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15-19 years</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1 (5%)</td>
</tr>
<tr>
<td>20-25 years</td>
<td>0 (0%)</td>
<td>4 (36.4%)</td>
<td>5 (25%)</td>
</tr>
<tr>
<td>26-30 years</td>
<td>0 (0%)</td>
<td>3 (27.3%)</td>
<td>4 (20%)</td>
</tr>
<tr>
<td>31-35 years</td>
<td>0 (0%)</td>
<td>1 (9.1%)</td>
<td>2 (10%)</td>
</tr>
<tr>
<td>36-40 years</td>
<td>0 (0%)</td>
<td>2 (18.2%)</td>
<td>4 (20%)</td>
</tr>
<tr>
<td>41-45 years</td>
<td>0 (0%)</td>
<td>1 (9.1%)</td>
<td>4 (20%)</td>
</tr>
</tbody>
</table>

Table 4: Gestational Age distribution of IgG positivity

<table>
<thead>
<tr>
<th>Gestational Age</th>
<th>Normal ANC (0)</th>
<th>One Abortion (11)</th>
<th>Two or more abortion (20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st Trimester (1-12 weeks)</td>
<td>0 (0%)</td>
<td>3 (27.3%)</td>
<td>4 (20%)</td>
</tr>
<tr>
<td>2nd Trimester (13-28 weeks)</td>
<td>0 (0%)</td>
<td>4 (36.4%)</td>
<td>7 (35%)</td>
</tr>
<tr>
<td>3rd Trimester (29-40)</td>
<td>0 (0%)</td>
<td>4 (36.4%)</td>
<td>9 (45%)</td>
</tr>
</tbody>
</table>

Graph 4: Gestational Age distribution of IgG positivity

Graph 5: Parity Distribution of IgG
HSV 1 and 2 IgM antibody was detected in the BOH group. In 25 years, 3 BOH group (62.5%) was maximum in the age group of 20-25 years, 3rd Trimester (45%) and with parity -1 (60%). No HSV 1 and 2 IgM antibody was detected in the BOH group.

 DISCUSSION

The HSV 1 and 2 IgG and IgM antibodies were tested by the ELISA method. In this study out of a total of 73 cases subjected to HSV 1 and 2 IgG ELISA, 31 cases (42.46%) were positive for HSV 1 and 2 specific IgG antibodies (p value:0.004 highly significant). Out of 73 cases, 19 cases are of normal pregnancy (NP) with a 0% IgG seroprevalence (0/19), 22 cases are patients with history of one abortion (A1) and the IgG seroprevalence in this group is 50% (11/22), 32 cases are with history of two or more than two abortions (A2) (BOH) with an IgG seroprevalence rate of 62.5% (20/32) (Table 2). The present study shows that seroprevalence for IgG increases with number of abortions (Table 2). Presence of IgG antibodies indicate prior exposure, infection or vaccination. IgG seropositivity also increases with gestational age (Table 4).

A total of 78 cases were subjected to HSV 1 and 2 IgM ELISA and all of them were found to be negative for IgM antibodies (0/78).

The prevalence of HSV 1 and 2 IgG & IgM antibodies in normal pregnancy (NP) was 0% in our study. However, Padmavathy et al. from Bangalore and Denoj Sebastian et al. from Kerala have reported a HSV 1 and 2 IgG antibody prevalence of 5.8% and 38.7% respectively. The prevalence of HSV 1 and 2 IgM antibody was reported as 2.3% by Padmavathy et al.

The prevalence of HSV 1 and 2 IgG antibodies in BOH group in our study was 62.5% which is closer to 67.44% of Kh. Sulochana Devi et al. from Imphal (Manipur). However a much lower prevalence was reported by D.Turbadkar et al. from Mumbai (3.8%) and M.J.Golalipour et al. from Iran (0%). No case of HSV 1 and 2 IgM antibody was detected in BOH group in our study. Similar/closer finding was reported by M.J. Golalipour et al. (0.0%), Padmavathy et al. (2.3%), Kh. Sulochana Devi et al. (2.6%) and D.Turbadkar et al. (3.6%). A much higher finding was reported by Denoj Sebastian et al. (59.2%).

No case of HSV 1 and 2 IgG and IgM antibodies were detected in the NP group. Prevalence of IgG Antibodies in BOH group (62.5%) was maximum in the age group of 20-25 years, 3rd Trimester (45%) and with parity -1 (60%). No HSV 1 and 2 IgM antibody was detected in the BOH group.

 CONCLUSION

HSV IgG antibodies

The overall prevalence of HSV 1 and 2 IgG antibodies was 42.46% (31/73). This antibody was not detected in the NP group. It increased from 50% in A1 to 62.5% in A2 group.

1. The age distribution was 20-45 years in A1 group and 15-45 years in A2 group.

2. Gestational age for A1 and A2 was 1st, 2nd and 3rd trimester.

3. Parity increased up to 3 for A1 and up to 4 for A2 group respectively.

Presence of HSV 1 and 2 IgG antibodies indicate prior exposure or infection. The prevalence of IgG antibodies increased from normal pregnancy group (NP) to patients with one abortion (A1) and two or more abortions (A2). Similarly, age of patient, gestational age and parity increased in NP, A1 and A2 groups.

HSV IgM antibodies

No HSV IgM 1 and 2 antibodies was detected in the entire study group of 78 patients indicating absence of current or recent HSV1/2 infection. The prevalence of IgG antibodies 42.6% indicates a subclinical infection or exposure.

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