Original article:

Study to determine the prevalence of Herpes Simplex Virus type II antibody in female genital tract lesion patients in a district of Andhra Pradesh, India

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Abstract:

Introduction: Approximately 60% of world’s sexually transmitted diseases (STDs) among women are found to be due to Herpes Simplex virus type 2 (HSV-2). The virus is also widely associated with uterine cervical cancer and may also cause aseptic viral meningitis in neonates. In this study, type specific serological tests were used to assess the prevalence of HSV-2 infection among patients of STD and carcinoma cervix.

Materials & Methods: This cross-sectional study was conducted in Eluru district in Andhra Pradesh to find out the incidence of HSV-2 antibody positive cases among patients attending the gynaecological OPD with diagnosed STD and to assess association of HSV-2 in carcinoma cervix patients. 91 samples were collected and screened for HSV-2 type-specific IgG as per the protocol given in the diagnostic kit used i.e. Euroimmun ELISA kit. Of them, 35 patients were suffering from postmenopausal or postcoital bleeding, whose Pap smear and/or cervical biopsy tests were done as the clinical situation demanded. 40 samples from STD cases and 16 samples from apparently healthy, married females (control group) were tested.

Observations: 53.33% of the 75 cases and 25% of control group were seropositive. 65% of the 40 STD patients and 40% of cancer patients had tested positive for HSV-2 IgG.

Results & Conclusion: HSV-2 seropositivity among cases was statistically significant. The difference in proportion of seropositivity between both the groups, i.e. cancer cervix versus STD cases, was found to be statistically significant.

Keywords: Herpes Simplex Virus Type 2, sexually transmitted diseases, Enzyme linked immunosorbent assay

INTRODUCTION:
Herpes Simplex Virus Type 2 (HSV-2) is one of the commonest primary cause of Genital Ulcer Diseases (GUDs) worldwide, also much reported to be associated with cancers of the uterine cervix. The herpes viruses are double stranded DNA viruses with icosahedral capsid. The two antigenic types HSV-1 and HSV-2 share some antigenic cross reactivity but have different glycoproteins and hence have different neutralization patterns and tend to produce different clinical symptoms. HSV-1 is mainly associated with “Fever blisters” and affects the trigeminal ganglia. The HSV-2 virus primarily infects the genital mucosa of both sexes, may also produce neurological diseases. Viral transmission is through genital routes, replication occurs at the site of infection; virus then invades local nerve endings and is transported by retrograde axonal flow to the dorsal root ganglion, where after replication, latency is established in sacral ganglia. Viraemia is common. Provocative stimuli like immune-suppression, ultraviolet rays can cause reactivation. HSV-2 may cause aseptic meningitis, especially in neonates, which may occur when in utero, and during or after birth, mother being the most common source. 75% of infections occur during vaginal delivery; hence
mothers diagnosed with HSV-2 should be delivered abdominally. The newborn seems unable to limit the replication and spread of the virus and thus, develops severe disease. HSV-2 has long been associated with uterine cervical carcinoma. An antigen designated ICP 10/AG4 was found to be a valid candidate for the role of virus-encoded protein involved in the maintenance of a transformed phenotype. Conversion to AG4 occurs during primary infection by HSV-2, it is transient and related to tumor growth.

HSV2 is known to contain regions in its genome capable of transforming cells in vitro, suggesting a possible oncogenic potential in vivo. Some studies showed that HSV-2 changes some genetic material in a cell and then moves on, leaving it susceptible to further infections, the 'hit and run theory'. Tremendous inflammatory response to HSV-2 may exacerbate the HPV induced damage to the cells and lead to cancer.

The diagnosis of HSV-2 from lesions us made by:
(a) cytopathology with Giemsa stain;
(b) isolation and identification of the virus;
(c) Polymerase Chain Reaction (PCR);
(d) serology.

Serology is useful in diagnosis of primary infection. Antibodies develop within a few days of infection and rise in titre may be demonstrated by Enzyme Linked Immunosorbent Assay (ELISA), Neutralization, or Compliment Fixation Test (CFT).

When type specific antigen preparations based on glycoprotein G are used, type specific antibody may be detected. Type specific serology is becoming more readily available and will enhance the ability to make the diagnosis and guide the clinical management in select patients, when other virological methods are unavailable or yield negative results; or in identifying carrier of infection. Majority of transmission occurs when the person is asymptomatic.

Most of the data that are available are based on studies in other countries. Also, there are very few studies available on seroprevalance of HSV 2 antibodies in sexually transmitted diseases and carcinoma cervix patients. Hence, this study was conducted to find out the seroprevalance of HSV-2 IgG antibodies by ELISA method, in STD clinic attendees and also to look for association of HSV 2 in carcinoma cervix patients at ASRAMS, Eluru, West Godavari District, Andhra Pradesh. The aim of this study was to determine the incidence of HSV-2 infection in women with genital tract lesions and also to assess the association of HSV-2 with different histopathological types of carcinomas of cervix.

MATERIALS & METHODS:
The present cross-sectional study was conducted in the Department of Microbiology, ASRAMS, Eluru to know the incidence of HSV2 among the female patients attending the Department of Gynaecology, ASRAMS, Eluru from April 2010 to December 2010 (9 months).

A total of ninety one (91) whole blood samples were collected. Among them seventy five (75) were the cases that constituted the test group. Among the 75 test samples, 40 were from women suffering from sexually transmitted diseases (STDs) and 35 were from carcinoma cervix patients. Women in carcinoma group were subjected to PAP smear examination or cervical biopsy as the clinical situation demanded.
Remaining 16 samples were collected from apparently healthy individuals.

Selection criteria:
Study group: Among the Gynaecology Outpatient Attendees (between April 2010 and December 2010) samples were collected from the women who satisfied the following criteria:
- All sexually active women more than 20 years of age;
- The STD group constituted those women with disease confirmed both clinically and by laboratory diagnoses.
- The samples screened under Carcinoma cervix group were from those cases with history of postmenopausal or postcoital bleeding and with histopathologically confirmed carcinoma cervix.

Control group: 16 healthy, married, sexually active women were included in this study as control group. Informed consent was taken from all women included in the two groups. Institutional Ethics Committee clearance was taken as per protocol.

I. Collection and processing of blood samples:
Blood samples were collected from all the women included in the study by venipuncture method using standard aseptic precautions.
Results were evaluated semi quantitatively by calculating a ratio of the extinction value of the control or patients samples over the extinction value of calibrator 2.
Extinction of the control or patient sample and extinction of calibrator 2 was taken as ratio.
EUROIMMUN recommends interpreting results as follows:
- Ratio < 0.8  Negative
- Ratio ≥ 0.8 to <1.1  Borderline
- Ratio ≥ 1.1  Positive

II. Collection and processing of cytopathological and histopathological specimens: The samples for cytology and histopathological confirmation were collected in the Department of Gynaecology and processed in the Department of Pathology, ASRAMS, Eluru as follows:

1. Collection of PAP (Papanicolaou) smear was done with the help of Ayre’s spatula, properly stained and studied under the microscope.
2. Collection of Biopsy specimen was done with the help of cervical biopsy forceps under colposcopic examination after highlighting the abnormal areas (if any) by applying acetic acid solution. Histopathological examination was done.  

STATISTICAL METHODS:
The data collected were analysed by means of EXCEL-2007 using Proportion, Chi square and t-test.

RESULTS:
A total of 75 serum samples constituted ‘Study group’ with two major subgroups. 40 samples were collected from sexually transmitted disease patients and 35 samples were collected from carcinoma cervix patients. 16 samples were collected from healthy married sexually active women constituting the ‘Control group’. A total of 91 samples were tested by EUROIMMUN, HSV2 Specific IgG antibody. (Table I)

Out of 75 cases tested for HSV2 IgG Antibody, 53.33% (40/75 cases) were seropositive and 46.67% (35/75 cases) were seronegative. Among 16 controls, 25% (4/16 samples) were seropositive and 75% (12/16 samples) were seronegative for HSV2 IgG antibodies. The HSV2 IgG seropositivity among cases was statistically significant. (P < 0.05). (Table II)
• Out of total 40 cases in STD subgroup 26 (65%) cases were seropositive and 14 (35%) cases were seronegative for HSV2 IgG antibodies.

• Out of 35 cases of carcinoma, 14 (40%) patients showed seropositivity and 26 (60%) patients were seronegative for HSV2 IgG antibodies.

The difference in proportion of seropositivity between both the groups i.e. carcinoma cervix versus STDs group was found to be statistically significant (P< 0.005). (Table III)

Correlation of HSV-2 seropositivity with type of carcinoma cervix based on histopathological examination is shown in Table IV.

• Among 35 carcinoma cervix cases, 25 had squamous cell carcinoma (SCC) and 10 had adenocarcinoma (AC), based on Histopathology reports.

• 14 of total 35 cases were seropositive and 21 cases were seronegative for HSV2 IgG specific antibodies.

• Among the 14 seropositive cases 8 cases (57.14%) were seropositive in squamous cell carcinoma and 6 cases (42.85%). (Table IV)

DISCUSSION:

Herpes Simplex virus type 2 is among one of the several factors that boost the risk of cervical cancer. HSV-2 has been reported to trigger repeated STDs generating DNA-damaging free radicals resulting in Cervical cancers. (16) The aims of the present study were to assess the seroprevalence of HSV2 specific IgG antibody in women with sexually transmitted diseases attending STD clinic and in carcinoma cervix patients attending gynaecology OPD at ASRAMS, Eluru, West Godavari district. 40 samples were collected from STD clinic attendees and 35 samples from confirmed cases of carcinoma cervix patients. 16 samples were collected from women who were apparently healthy as control group. A total of 91 samples were tested by EUROIMMUN HSV-2 IgG ELISA in this study.

(Table 1). Table II shows that, an overall seropositivity of 53.33% (40 / 75 samples) was observed among the cases and among controls 25% (4/ 16 samples) were found seropositive for HSV2 IgG antibody. The seropositivity among controls represents presence of HSV-2 antibodies in normal women. (17)

In TABLE III the seropositivity observed among Carcinoma cervix cases was 40% and 65% patients in the sexually transmitted diseases (STDs) group (26/ 40 cases) were seropositive. Studies conducted by Barkley (16) and by Smith (18) et al showed similar results. This observation might support the fact that HSV2 may be responsible for cancer cervix. In TABLE IV, correlation of seropositivity with histopathological type of cervical cancer matched with results obtained by Smith et al. (17) Here, 8 cases (57.14%) of SCC and 6 cases (42.85%) of Adenocarcinomas were seropositive.

CONCLUSION:
The overall seropositivity observed in this study for HSV2 antibody, IgG antibody detection among study group was high.

1. Seropositivity for HSV2 IgG antibodies was higher among sexually transmitted disease cases when compared to carcinoma cervix cases.

2. Among carcinoma cervix cases, the incidence of HSV2 antibodies was more in squamous cell carcinoma patients when compared to adenocarcinoma cases.

3. As Herpes is a latent disease, the seropositive people among control
group indicate asymptomatic infection. They might have suffered with subclinical illness. These people might not have any acquisition of STD but the risk of developing carcinoma cervix in them cannot be overruled. Based on the observations in this study, it should be made mandatory to test HSV-2 infections in high risk women with genital tract infections attending Gynaecology & Obstetrics department in any hospital to reduce the morbidity and mortality of women as well as newborns.

Acknowledgements: We are extremely thankful to the patients who cooperated throughout the study, the technicians and the teaching & non-teaching staffs of ASRAMS, Eluru.

**GRAPH 1:** TOTAL NUMBER OF CLINICAL SAMPLES COLLECTED FROM CASES AND CONTROLS

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<table>
<thead>
<tr>
<th>Case Group</th>
<th>Control (n=16)</th>
<th>Carcinoma cervix (n=35)</th>
<th>Total No</th>
</tr>
</thead>
<tbody>
<tr>
<td>STIs (n=40)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcinoma cervix (n=35)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total No</td>
<td>91</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A total of 75 serum samples constituted Case group with two major subgroups i.e., sexually transmitted diseases and carcinoma cervix. 40 samples were collected from sexually transmitted disease cases and 35 samples were collected from carcinoma cervix patients. 16 samples were collected from healthy married sexually active women constituting Control group. A total of 91 samples were tested by EUROIMMUN, HSV2 Specific IgG antibody.
Out of 75 cases tested for HSV2 IgG Antibody, 53.33% (40 / 75 cases) were seropositive and 46.67% (35 / 75 cases) were seronegative. Among 16 controls, 25% (4 / 16 samples) were seropositive and 75% (12 / 16 samples) were seronegative for HSV2 IgG antibodies. The HSV2 IgG seropositivity among cases was statistically significant. (P < 0.05)
**TABLE 3: COMPARISON OF HSV2 IgG SEROPOSITIVITY AMONG CARCINOMA CERVIX AND STDs CASES**

<table>
<thead>
<tr>
<th>HSV2 SEROLOGY</th>
<th>SEXUALLY TRANSMITTED DISEASES (STDs)</th>
<th>CARCINOMA CERVIX</th>
<th>TOTAL NO OF CASES</th>
</tr>
</thead>
<tbody>
<tr>
<td>SERO-POSITIVE</td>
<td>26 (65%)</td>
<td>14 (40%)</td>
<td>40</td>
</tr>
<tr>
<td>SERO-NEGATIVE</td>
<td>14 (35%)</td>
<td>21 (60%)</td>
<td>35</td>
</tr>
<tr>
<td>TOTAL</td>
<td>40 (100%)</td>
<td>35 (100%)</td>
<td>75</td>
</tr>
</tbody>
</table>

Chi square = 4.67; df=1; P<0.05 (SIGNIFICANT)

**HSV2 IgG antibodies in Sexually transmitted diseases:** Out of total 40 cases in STD subgroup 26 (65%) cases were seropositive and 14 (35%) cases were seronegative for HSV2 IgG antibodies.

**HSV2 IgG antibodies in Carcinoma cervix patients:** Out of 35 cases 14 (40%) patients showed seropositivity and 26 (60%) patients were seronegative for HSV2 IgG antibodies.

The difference in proportion of seropositivity between both the groups i.e. carcinoma cervix versus STDs group was found to be statistically significant (P< 0.005)
**TABLE IV: CO-RELATION OF HSV2 SEROPositIVITY WITH TYPES OF CARCINOMA CERVIX BASED ON HISTOPATHOLOGY REPORT:**

<table>
<thead>
<tr>
<th>TYPE OF CARCINOMA</th>
<th>CASES POSITIVE FOR HSV2 IgG Ab</th>
<th>CASES NEGATIVE FOR HSV2 IgG Ab</th>
<th>NUMBER OF CASES</th>
</tr>
</thead>
<tbody>
<tr>
<td>SQUAMOUS CELL CARCINOMA</td>
<td>8 (57.14%)</td>
<td>17</td>
<td>25</td>
</tr>
<tr>
<td>ADENO CARCINOMA</td>
<td>6 (42.85%)</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>14 (100%)</td>
<td>21</td>
<td>35</td>
</tr>
</tbody>
</table>

Chi$^2$=2.33 ; df=1; P>0.05 (not significant)

**GRAPH 4: CO-RELATION OF HSV2 SEROPositivity WITH TYPES OF CARCINOMA CERVIX BASED ON HISTOPATHOLOGY REPORT:**

References: