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RESEARCH ARTICLE

Serological Evaluation of Herpes Simplex Virus Type- 1/ Type- 2 Infections in Pregnant Women with Bad Obstetric History in a Tertiary Care Hospital, Kanchipuram

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Herpes Simplex Virus -HSV
Bad Obstetric History- BOH
Enzyme-Linked Immunosorbent Assay- ELISA
Sexually transmitted disease -STD

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Abstract

Intra-uterine Herpes simplex virus infection can cause significant morbidity and mortality in the developing fetus if the pregnant mother gets acute infection during pregnancy. Early diagnosis is essential to start appropriate treatment on time to reduce the transplacental transmission of virus to the fetus. In India the seroprevalence of HSV antibodies varies from 3.6%-61.3%. This study was aimed to record the seroprevalence of HSV infection in women with bad obstetric history (BOH) in our tertiary care hospital, Kanchipuram. A total of 110 pregnant women with bad obstetric history and 50 pregnant women without any bad obstetric history as control were included in the study. All the serum samples were tested for the presence of specific HSV-1/HSV-2 IgM and IgG antibodies using ELISA BASED COMMERCIAL KIT. Total seropositivity in pregnant women with BOH was 36.5% and 4% among control group. More number of seropositivity was observed among women between 26-30 years (50%). Abortion was the commonest form (60%) of BOH, followed by preterm deliveries (25%). Various risk factors such as people living in rural area and lower socioeconomic status had greater association of risk of getting HSV infection than urban population and higher socioeconomic status people. This study revealed that the seroprevalence of HSV infection was significantly high in the study population than in control group. To estimate the real magnitude of the problem, investigative measures should be taken to undertake multicentric study as well as compulsory screening of the antenatal risk group.

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1. Introduction

Bad obstetric history (BOH) implies previous unfavourable fetal outcome in terms of two or more consecutive spontaneous abortion, history of intrauterine fetal death, intrauterine growth retardation, still births, early neonatal death and/or congenital anomalies (Turbadkar et al., 2003, Sadik et al., 2012). Recurrent pregnancy wastage due to maternal infections transmissible *in utero* at various stages of gestation can be caused by a wide array of organisms which include the TORCH complex (*Toxoplasma gondii*, Rubella virus, Cytomegalovirus, Herpes simplex virus) and other microbial agents such as Varicella, *Treponema pallidum* and Parvovirus (Sebastian et al., 2008).

Herpes simplex virus (HSV) infection is one of the most common viral sexually transmitted disease (STD) worldwide. Herpes simplex virus type 2 (HSV-2) is the cause of most genital herpes and is almost always sexually transmitted (Anzivino et al., 2009, Cusini and Ghislanzoni, 2001). Herpes simplex virus type 1 (HSV-1) is usually transmitted during childhood via non-sexual contacts. The acquisition of genital herpes during pregnancy has been associated

with spontaneous abortion, prematurity, and congenital and neonatal herpes (Brown et al., 1997, Biswas et al., 2011).

Herpes simplex viral infection in the neonate is commonly acquired by contact with the mother's infected birth canal (Sadik et al., 2012). Incubation period for herpes virus is between 4 and 21 days (Fleming et al., 2011). Primary infection of HSV enters a latent state in the nerve ganglia and may emerge later to cause recurrent active infection (Corey and Spear, 1988). Neonatal HSV infection is usually acquired at birth, although a few infants have had findings suggestive of intrauterine infection (Sadik et al., 2012).

Seroprevalence estimation of HSV infections allow better understanding of the burden of infection and to know about its evolution with time (Smith and Robinson, 2002). In India 33.3% of individuals are seropositive for HSV-1 and 16.6% are seropositive for HSV-2. Those with both HSV-1 and 2 antibodies are estimated at 13.3% of the population. Seroprevalence of this virus increases with an increasing age (Kaur et al., 2005). In Tamilnadu, there are only limited study reports available that have estimated the seroprevalence of HSV infection during pregnancy. This study was conducted to estimate the seroprevalence of HSV infections among pregnant women attending to our tertiary care hospital with BOH.

2. SCOPE OF THE STUDY

With this background, this study was aimed to record the seroprevalence of of HSV infections among pregnant women attending to our tertiary care hospital with BOH. This study reports the results of screening for IgM and IgG antibodies to HSV among patients attending to antenatal clinic with BOH. Therefore, strategies for early recognition of maternal infection and the institution of appropriate treatment could be started; which in turn necessary to prevent the fetal mortality and morbidity associated with congenital HSV infections.

3. Materials

This cross sectional study was conducted in a tertiary care hospital, Kanchipuram. 110 pregnant women with BOH (previous history of 2-3 pregnancy loss, intra uterine deaths, preterm deliveries and intra uterine growth retardation) and 50 pregnant women without any BOH, as control were included in the study. This study was conducted for duration of 1 year, from April 2012 to March 2013. Detailed history about the age, socio-economic status, area from where they are coming, and past obstetric history including abortions, intrauterine growth retardation, premature deliveries, low birth weight and intrauterine death was taken from all the patients. Oral informed consent was also obtained from all these patients.

4. Methodology

From each patient, 5ml of venous blood was drawn aseptically in a sterile vacutainer and the serum was separated. All the serum samples were tested for the presence of specific IgM and IgG antibodies to HSV-1/HSV-2, using ELISA BASED COMMERCIAL KIT from RATIO DIAGNOSTICS. It is a solid phase immunoassay, intended for *in vitro* use only.

All serum samples were tested according to the manufacturer's literature guidelines provided along with the kits. The test for the assay of HSV-1/HSV-2 IgM and IgG ELISA is based on the principle of the capture of the IgM and IgG immunoglobulins separately in patient's serum and subsequent identification of those, which are specific, making use of their ability to bind an antigen conjugated to peroxidase. The antigen is composed of purified protein of Herpes simplex virus 1 & 2.The capture is performed using monoclonal anti-human IgM and IgG antibodies bound to the solid phase (microtitration strips) separately. Negative control, positive control and the cut-off control were run along with the samples. Optical Density (OD) was read at 450 nm in an ELISA reader. All the samples showing OD reading above the cut-off value were considered to have significant antibody titre for HSV infection. Ratios were calculated between the average OD value and the cut-off.

The sample is considered -

- Positive= if the ratio is > 1.1;
- Negative = if the ratio is < 0.9;
- Doubtful= = if +/- 10% of the cut-off.

5. RESULTS

5.1 Distribution of results among cases and control: In our study, out of 110 women with BOH, 8 (7.27%) were found to be seropositive for IgM, 32 (29.08%) for IgG antibodies for HSV-1/ HSV-2. The total seropositivity (both IgM & IgG) among women with BOH was 40 (36.35%) for HSV-1/ HSV-2 antibodies. None of the control group women were positive for IgM and 2 (4 %) were positive for IgG antibodies to HSV-1/HSV-2 (Table-I).

Table-I: Shows total number of seropositives for HSV-1/HSV-2 antibodies (IgM and IgG) among cases and controls

Type of antibody	No. of seropositives for HSV-1/HSV-2 antibodies in patients with BOH (cases) (n=110)	Percentage (%)	No. of seropositives for HSV-1/HSV-2 antibodies in control group (n=50)	Percentage (%)
IgM	8	7.27%	-	0%
IgG	32	29.08%	2	4%
Total	40	36.35%	2	4 %

Table-II: Shows age distribution of seropositive cases for HSV-1/HSV-2 antibodies

Age in years	No. & % of positive cases for IgM to HSV-1/HSV-2 (n=8)	No. & % of positive cases for IgG to HSV-1/HSV-2 (n=32)	Total positivity (N= 40) (IgM & IgG)
<20	-	4 (12.5%)	4 (10%)
21-25	2 (25%)	4 (12.5%)	6 (15%)
26-30	4 (50%)	16 (50%)	20 (50%)
31-35	2 (25%)	8 (25%)	10 (25%)
36-40	-	-	-

Table-III: Shows distribution of seropositive cases for HSV-1/HSV-2 antibodies in relation to type of BOH

Type of BOH	No. & % of positive cases for IgM to HSV-1/HSV-2 (n=8)	No. & % of positive cases for IgG to HSV-1/HSV-2 (n=32)	Total Seropositivity N=40
Abortion	4 (50%)	20 (62.5%)	24 (60%)
Still birth	2 (25%)	4 (12.5%)	6 (15%)
Pre-term delivery	2 (25%)	8 (25%)	10 (25%)
Congenital anomalies	0	0	0

Table-IV: Shows distribution of seropositive cases for HSV-1/HSV-2 in relation risk factors involved with spread of infection

Variables (risk factors)		Total cases (n=110)	No. & % of positive cases for IgM to HSV-1/HSV-2	No. & % of positive cases for IgG to HSV-1/HSV-2	Total Seropositivity to HSV-1/HSV-2 antibodies N=40
Residence	Rural	68	6 (8.82%)	23 (33.82%)	29 (72.5%)
	Urban	42	2 (4.76 %)	9 (21.42%)	11 (27.5%)
Socio-economic status	High	52	3 (5.76%)	11 (21.15%)	14 (35%)
	low	58	5 (8.62%)	21 (36.20%)	26 (65%)

5.2 Age Distribution:

More number of seropositivity was observed among women between 26-30 years, 20 out of 40 (50%) were seropositives for HSV-1/HSV-2 antibodies (Table-II).

5.3 Distribution of seropositive cases in relation to type of BOH:

The seropositive subjects were analyzed in relation to the type of Bad Obstetric History (BOH) (Table-III).

Study results revealed that abortion (60% for HSV-1/HSV-2) was the commonest form of BOH, followed by preterm deliveries (25%). None of the patient had babies born with congenital anomalies in their past deliveries among seropositives for HSV-1/HSV-2 antibodies.

5.4 Distribution of seropositive cases in relation to risk factors involved with spread of HSV infection:

Risk factors in relation to the spread of HSV infection, like residence (rural or urban) and socio-economic status (high or low) factors were analyzed (Table-IV).

More number of seropositives has been observed in people living with rural area than urban (72.5% vs. 27.5% for HSV-1/HSV-2). Maximum number of patients belongs to lower socioeconomic status than higher socioeconomic status (65% vs. 35% for HSV-1 /HSV-2).

6. DISCUSSION

TORCH screening is now widely requested by obstetricians while investigating the suspected cases of infertility as well as pregnant ladies with recurrent pregnancy loss. There is concern that such requests are inappropriate and should be targeted more specifically as these investigations are costlier (Levin and Myron, 1997). Primary infection with HSV-2 acquired by women in pregnancy accounts for half of the morbidity and mortality from HSV-2 among neonates, another half results from reactivation of old infection (Turbadkar et al., 2003, Vontver et al., 1982). Pregnant woman, who acquires genital herpes as a primary infection in the latter half of the pregnancy, rather than prior to pregnancy, is at greatest risk of transmitting these viruses to her newborn (Anzivino et al., 2009, Baker, 2007). In pregnancy, HSV infection causes fetal damage, which necessitates the need for serological evaluation in all the pregnant women for HSV antibodies for early diagnosis and treatment to prevent the congenital infections in the fetus (2). Detection of IgM and IgG antibodies are the sensitive indicators of acute and remote infections respectively (Knipe and Howley, 2007). HSV-1 and HSV-2 share the majority of their immunogenic sequences. This cross-reactivity results in antibodies that react with almost equal efficiency to both HSV subtypes regardless of whether an HSV-1 or HSV-2 infection has triggered the response (Ashley and Wald, 1999, Field et al., 1993).

According to the various studies conducted in India, the seroprevalence of HSV antibodies varies from 3.6% to 61.3% (Turbadkar et al., 2003, Sebastian et al., 2008, Kaur et al., 2005). In our study, the overall seroprevalence among antenatal women with BOH was 36.5% for HSV-1/HSV-2 antibodies. It is concordant with the studies done by Kaur et al., 2005 (33.3% of individuals are seropositive for HSV-1 and 16.6% are seropositive for HSV-2) and Fujie et al., 2007 (overall HSV seroprevalence was ranging from 22%- 63%). Whereas results are much different in study done by Baker et al., 2011, where 60% were HSV seropositive. In the control group seroprevalence was 4%, it is much lower when compared to study done by Ahmed 2010, where it was 24% among control group. This study showed increased seroprevalence in study group (cases) when compared to control group.

In the present study, overall seroprevalence of IgM antibodies (IgM for HSV-1/ HSV-2) was 7.27%; which is the indicator of acute HSV infection. However; study done by Sadik et al., 2012 it was as low as 1.69%. Seroprevalence of IgG in our study was 29.08%; which is the indicator of latent HSV infection. According to the study done by Sadik et al., 2012, it was slightly lower (16%) and 11% in study done by Ahmed 2010. Low seropositivity for both IgM and IgG was recorded in their study; may be because of random selection of pregnant women and not highly suspected antenatal mothers with BOH, as done in our study.

Maximum number of HSV seropositivity in pregnant women was noted in the age between 26-30 years (50% for HSV-1/HSV-2). The overall prevalence was higher between 26-35 years in our study. The same range has been observed with many studies (sadik et al., 2012, Kaur et al., 2005). In most of the studies, HSV seroprevalence increased consistently with age across the age spectrum or plateaued after age 30 and HSV seropositivity decreased in older women (> 40 years) (Smith and Robinson, 2002).

Among the seropositive antenatal women with BOH, abortion was the commonest reason of pregnancy wastage (60% for HSV-1/HSV-2); which is followed by preterm deliveries (25% for HSV-1/HSV-2). Similar results were observed in studies done by Sadiq et al., 2012 and Ahmed 2010.

The associations of various risk factors were analyzed in relation to HSV seropositive cases.

People from rural area were most commonly infected with HSV (72.5%); when compared to people from urban population (27.5%) for HSV-1/HSV-2 antibodies in our study. It is concordant with the study of Smith et al., 2002. Increased seroprevalence was observed among the rural people probably

because of lack of awareness and health education and also unsafe sexual practices.

Seroprevalence of HSV infection was high in lower socioeconomic group than high socio-economic group in our study. It is matching with the results of study done by Smith et al., 2000 and Sadiq et al., 2012. Lower socio-economic people mainly will be from rural areas and they will be unaware of hygienic health practices; multiple sex partners and unsafe sex practices also will be common among them. These may be the contributing factors for increased HSV seroprevalence among them.

7. LIMITATIONS OF THE STUDY:

Limited research works are available about seroprevalence of HSV infection in pregnant women in southern part of India; as well as in our area. So for comparison and for reference, enough reports were not available. We limited our study group, due to financial constraints; hence to correlate the data obtained by our study to community, large scale studies have to be conducted.

8. CONCLUSION

This study revealed that the seroprevalence of HSV infection was found high in the study population than control. Maximum seropositivity was between 26-35 years and abortion was the commonest form of pregnancy wastage followed by preterm deliveries.

Based on this study, information obtained about the seroprevalence of HSV infection in this area may be just the tip of the ice berg. To find out the real burden of the problem, prompt investigative measures should be followed to undertake multicentric study as well as compulsory screening should be done for all pregnant women with BOH.

Presence of antibodies to HSV-1 and HSV-2 is related to a number of sexual and demographic risk factors. Increased awareness and health education at the patient and public level through government and private agencies are required to minimize the risk of congenital transmission with respect to the various modes of spread. High index of clinical suspicion of congenital HSV infection, identifying the genital herpes in pregnant women, prompt requisition for testing HSV antibodies antenatally, and recognizing the risk factors that contribute to infections should be kept in mind while dealing with a pregnant lady with BOH. Preventive measures like- regular antenatal checkups during pregnancy, promoting healthy life style, avoiding multiple sex partners should be given prime importance in our day to day life.

9. SUMMARY OF RESEARCH:

This sero-prevalence study was helpful; as it provided information about seroprevalence of acute and remote HSV infection in and around Kanchipuram. Effective preventive measures should be undertaken to reduce the disease burden and to prevent fetal mortality and morbidity.

10. DISCLOSURE STATEMENT

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