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A Serological Survey of Herpes Simplex Virus Type 1 and 2 Immunity in Pregnant Women at Labor Stage in Tehran, Iran

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Abstract: This study was carried out to determine the prevalence of neutralizing antibodies to Herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) in pregnant women at labor stage. Blood samples from umbilical cord of four hundred women aging 16 to 40 years at labor stage were collected. After sera separation quantity of anti HSV-1 and HSV-2 antibodies were measured by serum neutralization test. Antibody quantification was assayed by two fold dilution of sera (from 1/2 to 1/256) with 500 Tissue Culture Infective Dose fifty percent (TCID₅₀) of the HSV-1 and HSV-2, separately. Three hundred sixty three (90.75%) of women had neutralizing antibody against HSV-1. Thirty three (8.25%) tested women were seropositive for HSV-2 antibodies. Our results indicate that there is positive correlation between increase of age and seroprevalence of anti-HSV-2 infection in pregnant women. Furthermore, the pattern of HSV-2 infection is similar with other Sexual Transmitted Disease (STD). These results also show that seroprevalence of anti-HSV-1 antibody in our tested population was remarkable. However, the seroprevalence of anti-HSV-1 antibody in different age groups statistically were not significant. These results also showed that most women before fertility age have infections with HSV-1.

Key words: HSV, serology, neutralizing antibodies, sexually transmitted disease, pregnancy

INTRODUCTION

Almost all of the human populations in the world wide are infected with Herpes Simplex Viruses (HSV). There are two different types of HSV which are very close both in genetical and molecular aspects (Ashley and Wald, 1999). However, after a primary infection, these viruses do not eradicate from human body and they remain in regional ganglion as a latent type for life time. Nevertheless, reactivation of viruses can occur anytime due to an external and internal stimulation. Primary and reactivation of infection with HSV-1 normally appear around of mucosal membrane of the mouth, while HSV-2 infection mostly exhibits at a genital area, however oral form of infection also can occur in patients with an abnormal sexual activity (oral sex) (Whitley and Roizman, 2001). Prenatal form of infection in newborn can be observed when, a neonate passing through of the infected birth canal. Contamination of the neonate at this condition may cause meningitis with a serious complication (Brown *et al.*, 1997). Unfortunately transmission of HSV-2 in the human population facilities due to the in-apparent infection and secret behavior of sexual contacts (Russell *et al.*, 2001).

Prevalence of HSV antibodies in our region and in particular qualification of these antibodies in child brings women and her neonates have not been properly investigated. Knowledge of prevalence of HSVs antibodies in this population could help to manage mother and her neonate whenever infecting with these viruses and to predict the possibility of serious infections and disabilities in the newborns (Kimura *et al.*, 2002). Therefore, objective of this study was to survey prevalence of neutralizing antibodies against these viruses in pregnant women at labor stage.

MATERIALS AND METHODS

Patients: Four hundred pregnant women aging 16 to 40 years at labor stage were selected. Blood samples were taken from umbilical cord and their sera were incubated at 56°C for 30 min to inactivate nonspecific inhibitors. The inactivated sera were then stored at -20°C until anti HSV antibodies to be determined.

Cell culture and viruses: Two type of viruses (HSV-1 and HSV-2) which have been identified with florcent monoclonal antibody staining, kindly provided

by Professor M. Shams Shahradi (Rasoul Akram hospital, Tehran, Iran). Each type of virus was culture in Vero cell (National Cell Bank, Pasture Institute, Tehran, Iran) with DMEM medium and 5% fetal bovine serum. When cytopathic effect (CPE) of the infected cells reaches between 70 to 90%, the viruses were harvested by freezing/defreezing of cells. Released viruses were collected by centrifugation of disrupted cells for 20 min at 9000 g at 4°C. The supernatant which contain viruses were then aliquots and quantified with Reed and Muench method to determine TCID₅₀ and stored at -70°C (Reed and Muench, 1932).

To assay cross reactivity of HSV-1 and HSV-2, two groups of rabbits (two rabbits in each group) were inoculated via intramuscular injection and abdominal scarification weekly. To raise maximum cross reacting antibody, inoculation continued for 8 consecutive weeks. Finally the blood samples were taken from the rabbits via heart puncture. The sera were pooled and stored at -20°C.

Micro-sero-neutralization test: Two folds dilution of each serum (from 1/2 to 1/256) in 100 µL volume with DMEM medium was prepared. The diluted sera were mixed with equal volume of 500 TCID₅₀ of HSV and incubated for one hour at 37°C. The treated sera were then added to well of microplate containing Vero cells, DMEM medium with 2% of fetal bovine serum. Three duplicate of controls included 500 TCID₅₀ of HSV and blank wells in 100 µL DMEM media were also added to microplate wells. After 48 h the microplate wells were monitored for CPE, when CPE in viral controls wells approximately reach between 60-70% titer of the antibody in test sample were recorded. These assayed were preformed for each type of the virus in separate microplate to prevent possibility of cross contamination. The potency for the cut off values of cross reacting antibodies against HSV-1 and HSV-2 were measured using rabbit type specific antibody with nonspecific viruses in micro-sero-neutralization test as mention above.

Statistical analysis: To examine statistically significant differences in HSV type-specific seroprevalence rates between different age groups, the Chi-square test was used.

RESULTS

Results for cut off value showed that neutralization power of each rabbit type specific anti serum was at eight folds higher as compared with nonspecific rabbit anti serum. In another words, the anti HSV-1 serum had potency to neutralize 500 TCID₅₀ HSV-1 at 1/128 serum

Table 1: Prevalence of neutralizing antibodies to Herpes Simplex Viruses types 1 and 2 by age in pregnant women

Age group (year)	No. tested	No. positive (%)		HSV negative No. (%)
		HSV1	HSV2	
16-20	104	87 (83.6)	5 (4.8)	17 (16.3)
21-25	125	118 (94.4)	10 (8.0)	7 (5.6)
26-30	113	102 (90.3)	12 (10.6)	11 (9.7)
31-35	44	42 (95.4)	6 (13.6)	2 (4.5)
36-40	14	14 (100.0)	0 (00.0)	0 (0.0)
Total	400	363 (90.75)	33 (8.25)	37 (9.25)

dilution rate, while this anti HSV-1 serum only neutralize 500 TCID₅₀ HSV-2 at dilution rate 1/16. Similar results were also obtained when HSV2 immunized sera were examined with HSV-2 and HSV-1. If test sera at dilution rate 1/2 had potency to neutralize the 500 TCID₅₀ of each two types of the viruses, it was considered as seropositive. In each test serum if difference between neutralizing antibody titer for HSV-1 and HSV-2 was more than 8 folds, positivity of test was accepted for that virus type that patient serum neutralize them at upper titer, while in the case when this difference was lower than eight folds positivity of test was considered for both type of viruses.

Classification of patients: Four hundred of blood samples were classified according to the age of patients to five groups as follows: 16-20 years old 104, 21-25 years old 125, 26-30 years old 113, 31-35 years old 44 and 36-40 years 14 individuals. The seroprevalence of neutralizing antibody at each group was shown in Table 1. Three hundred sixty three (90.75%) of women had neutralizing antibody against HSV-1. Difference between age groups for prevalence of HSV-1 antibody was not statistically significant (p>0.05). Thirty three (8.25%) tested women were seropositive for HSV-2 antibodies. Surprisingly all individuals with HSV-2 antibody were also positive for HSV-1 antibodies. There were positive correlation between seroprevalence of HSV-2 antibody and increase of age in the pregnant women (p≤0.05). Accordingly, these seroprevalence at group aging 16-20 years was 4.8% and rise to 13.6% for group ageing 31 years and more. Nevertheless in 9.25% of our tested population anti HSV antibody was not detected (Table 1).

DISCUSSION

Accumulating evidence indicates HSV-2 infections are gradually increasing worldwide (Ashley and Wald, 1999; Whitley and Roizman, 2001). We conducted this survey to evaluate level of anti HSV-2 and HSV-1 antibody in selected Iranian pregnant women population.

Seroepidemiology studies showed that rate of infection with herpes simplex viruses varied depending to geographical location of county and socio-economical situation of peoples which reflecting to seroprevalence of HSV-2 and HSV-1 Antibodies (Garcia-Corbeira *et al.*, 1999). In the United States of America HSV-2 seroprevalence increased dramatically from 5.6% in 12 to 19 years olds to 27.8% in 30 to 39 year old and remained fairly constant beyond age 39 (Ashley and Wald, 1999). Another study in Canada (Ontario) showed that by the age of 15 to 16 up to 55% of Ontario population had antibodies to HSV-1 and this value increase to 89% in population at early forties. In contrast HSV-2 antibodies were not found among 15 to 16 year old individuals (0 to 3.8%), but positive seroprevalence increased 10-fold to 21% among individuals in their early forties (Howard *et al.*, 2003), consistent with the sexually transmitted nature of HSV-2. In the Germany in people older than 15 years, the seroprevalence of HSV-1 was 76.3% in females and 75.2% in males and the seroprevalence of HSV-2 was 17% in females and 12.5% in males (Rabenau *et al.*, 2002). The results of this study showed that HSV-1 infections were high in our study population. It seems the most women before fertility age, are infecting with HSV-1. However no significant difference observed between different age groups for HSV-1 antibody, while with increase of the age prevalence of HSV-2 antibodies increase significantly. The pattern of increasing of prevalence of the antibodies with age will continues for HSV-2, therefore prevalence of these antibodies are two time more when compared between women aging 21-25 years group with group aging 16-20 years and reach up to 13.6% in women group aging 31 years and over. Although the rate of increase in prevalence of anti HSV-1 antibodies is similar to some western and eastern countries (de Ory *et al.*, 1999; Dong *et al.*, 1998; Ghebrekidan *et al.*, 1999; Morris *et al.*, 2002; Wutzler *et al.*, 2000), but the rate of HSV-2 infection and antibodies in our study are less than some countries (Gottlieb *et al.*, 2002; Morris *et al.*, 2002; Suligoi *et al.*, 2002; Wutzler *et al.*, 2000). One explanation for this difference could be due to cultural and religious behaviors. Nevertheless, prevalence of HSV-1 antibody according to this study in Iranian population is high and similar to other reports whereas age of infection in Iranian population was less than other countries. The reason for this discrepancy can be explained at least partly due to crowded of students in pre and primary school which more contacts between children don't be avoidable.

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