Seroprevalence and risk factors of herpes simplex virus type 2 among pregnant women in Dhamar city, Yemen

Abstract

Herpes simplex virus type 2 is the common cause of genital ulcer disease worldwide. Genital herpes infection is a major concern in pregnancy due to the risk of neonatal transmission.

Materials and methods: A descriptive cross-sectional survey was conducted from August 2016 to March 2017 in some hospitals and health centers to assess the seroprevalence and risk factors of herpes simplex virus type 2 infection among pregnant women attending antenatal care in Dhamar city, Yemen. After taking written consent, socio-demographic, behavioral and obstetric history along with blood samples were collected from 200 pregnant women using a pre-structured questionnaire. Sera were analyzed for HSV-2 specific IgG using Electro-Chemiluminescence Immunoassay (ECLIA) method.

Results: The overall seroprevalence of HSV-2 infection was 6% (12/200) among pregnant women in Dhamar city. Levels of education and some obstetrical history such as numbers previous abortion (P=0.03), stillbirth (P=0.001). Although the study showed, there are significant differences in some symptoms, such as itching (P=0.02) and inflammation during urination (P=0.03).

Conclusion: Overall, the seroprevalence of HSV-2 infection among pregnant women in Dhamar city is low. There is a critical need to adopt screening of HSV-2 into the antenatal profile tests. There is also need for more health education of this virus infection, methods of transmission, associated risk factors, and effective prevention and control strategies.

Keywords: seroprevalence, risk-factors, hsv-2, pregnant women, eclia, Dhamar, Yemen

Introduction

Herpes simplex virus (HSV) is an enveloped, double stranded DNA virus, belonging to Herpesviridae family. Genital herpes may be caused by either HSV-1 or HSV-2 but the majority of cases worldwide are caused by HSV-2. HSV-2 is a pathogen sexually transmitted and causes a severe chronic infection at both the symptomatic and asymptomatic periods. It is the worldwide natural cause of genital ulcers.

In women, transmitted through mucus membranes and intact skin, who migrate to the nerve tissues, where they remain in a state of latency, the group of pregnant women is important since they are ready to infect their partner. HSV-1 dominates in orofacial lesions and causes a severe chronic infection at both the symptomatic and asymptomatic periods. HSV-2 is almost sexually transmitted and is the common cause of genital ulcer disease worldwide. Genital ulcer infection causes during pregnancy which has been a problem of neonatal herpes, a severe condition because of the high mortality and long-term neurological sequelae noted in about 20% of survivors.

HSV disease of the newborn can be acquired during one of the three-time periods: in utero, prenatally, or postnatally. The most common mode of transmission is via direct contact of the baby with infected vaginal secretions during delivery. Pregnant women then become a target for surveillance of the infection to avoid the risk of neonatal transmission. Seroprevalence studies showed wide variations in infection rates by geographic location. This study aimed to assess the seroprevalence of HSV-2, and associated risk factors among pregnant women in Dhamar city, Yemen.

Materials and methods

Study area and population

This is a cross-sectional descriptive study conducted in Dhamar city. It is situated in northern Yemen, about 100 km2 away from Yemen’s capital. In terms of population, Dhamar is Yemen’s six largest cities. The population of the Republic of Yemen was 1,330,108, according to the last population census in 2004, and 6.8%. The percentage is estimated to be from 660,553 to 669,555 of men to women. Nevertheless, the population may change as a result of the civil war, due to emigration to or from Dhamar.

Data collection

In order to collect information about sociodemographic properties, reproductive and obstetric features, clinical symptoms and risk-related data, the questionnaire was developed. It was planned to coincide with this study, following several previous studies, with slight modifications. The questionnaire was first created in English and translated into Arabic to help researchers complete the survey in some cases. Many were “yes or no” questions and it took about 5 minutes to complete the questionnaire.

Keywords: seroprevalence, risk-factors, hsv-2, pregnant women, eclia, Dhamar, Yemen

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Sample collection, handling and storage

After having received written and verbal permission from the participants, each consenting subject has been taken by venipuncture from a blood sample (five ml) which was then transferred to a non-anticoagulating bottle and may coagulate. Then it centrifuged and the serum was moved to cryovials (3000 rpm, 5 min). The serum obtained from each study subject has been put in two different tubes of Eppendorf. The initial sample was one and the second was the backup sample. It was done to prevent prolonged freeze / thaw (reduced) or incomplete serum cycles.

The second sample was used as a backup sample if something was incorrect or the quantity was insufficient. At -10 to -20 °C, serum samples were stored. These samples were saved in two separate locations at various freezers and checked daily in case of power failure, with emergency power supply. Once in the morning and once in the afternoon, samples were taken once in the freezer for the first serologic test and placed overnight in a refrigerator at 4 °C. The samples were removed early in the morning from the refrigerator for serologic tests after thawing in the refrigerator overnight.

Serologic testing

Serological assays were performed by using ECLIA on the Cobas e411 system (Roach Diagnostic GmbH, Mannheim, Germany). Two hundred microliter of serum was pipetted from each Eppendorf tubes and placed in the reaction cell (sample well) of ECLIA. A new sterile disposable pipette tip was used for each serum sample. The study number (SNO) on the Eppendorf tubes was cross-checked with the SNO entered into the Cobas e411 system to ensure that there were no numbering errors of samples. All samples equal to or above the index of ≥1.0 COI were considered positive. The entire samples were interpreted as negative with < 0.51 COI index and the samples were equivocal with a 0.51-< 1.0 COI index.

Ethical consideration

This research was ethically carried out following the corresponding procedure by the Biology Department of the Faculty of Sciences of the Ethics Committee of the University of Sana’a. Before taking the sample, all women were explained in the questionnaire for this study. The use of numbers instead of names ensured confidentiality. The study was also obtained oral and written consent.

Results

The present study conducted on a total of 200 pregnant women at antenatal clinics in some hospitals and health centers in Dhamar city during the period from August 2016 to March 2017. This section describes the results of the overall prevalence of HSV-2 IgG antibody among pregnant women, distribution of HSV-2 IgG antibody among respondents with a socio-demographic view, obstetrical history, risk factor and clinical characteristic for HSV-2 IgG antibody.

Socio-demographic characteristics of pregnant women:

A total of 200 pregnant women were enrolled in the study. The age of pregnant women ranged from 16 to 40 years. The mean (SD) age of the pregnant women was 25.5 (5.7) years. One Hundred fifty-nine (78.0%) of these women were born in Dhamar Governorate and 41 (22.0%) were born in other regions. From the studied pregnant women, 78 (39%), were illiterate, while only 44 (22%) were in the basic educational level. Other women distributed in primary, secondary, and university 26 (13%), 45 (22.5), and 7 (3.5%), respectively. According to the socio-economic status, most of the studied subjects 156 (78%) were at the medium economic level, while only 16 (8%) were at a relatively high economic level. Most of these women, 196 (98.0%) were housewives (Table 1).

Table 1 Overview of socio-demographic variables of studied pregnant women in Dhamar city (n= 200)

| Variable                  | Participants |
|---------------------------|--------------|
| Age                       |              |
| < 20                      | 51 25.50     |
| 21-30                     | 111 55.50    |
| 31-40                     | 38 19.0      |
| Marriage                  |              |
| First                     | 184 92.0     |
| Second or more            | 16 8.0       |
| Marriage type             |              |
| Monogamous                | 176 88.0     |
| Polygamous                | 24 12.0      |
| Residence                 |              |
| Rural                     | 98 49.0      |
| Urban                     | 102 51.0     |
| Place of birth            |              |
| Dhamar                    | 159 80.30    |
| Other                     | 41 19.70     |
| Educational level         |              |
| Illiterate                | 78 39.0      |
| Basic                     | 44 22.0      |
| Primary                   | 26 13.0      |
| Secondary                 | 45 22.50     |
| University                | 7 3.50       |
| Occupation                |              |
| Housewife                 | 196 98.0     |
| Employee                  | 4 2.0        |
| Socio-economic level      |              |
| High                      | 16 8.0       |
| Medium                    | 156 78.0     |
| Low                       | 28 14.0      |

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Overall prevalence of HSV-2 IgG antibodies:

Analysis of the results showed 6% (12 of 200) pregnant women were positive for the HSV-2 IgG antibodies, while 94% (188 of 200) cases were negative (Figure 1).

Distribution of HSV-2 IgG antibodies according to the socio-demographic characteristics:

Socio-demographic characteristics such as age, marriage and marriage type, place of birth, residence, education level, occupation, and socio-economic level. From all the socio-demographic characteristic there was only a statistically significant association between education level and seropositivity of HSV-2 IgG antibody (P = 0.02).

Distribution of HSV-2 specific IgG antibodies according to the reproductive characteristics:

Reproductive characteristics, such as the trimester, number of term deliveries, preterm delivery, live births, stillbirths, spontaneous abortions, surviving children, cesarean-section, and malformed children were studied. The means (SD; Minimum, Maximum) of trimester, term deliveries, stillbirths, abortions, preterm birth, live births, surviving children, and malformed children were 4.75(2.60; 1.0, 9.0), 2.62(2.33; 0.0, 11.0), 0.15 (0.49; 0.0, 4.0), 0.66 (1.03; 0.0, 5.0), 0.16 (0.52; 0.0, 4.0), 1.51(1.69; 0.0, 8.0), 1.47(1.65; 0.0, 7.0), respectively; 74 (37.0%), 23 (11.50%), and 4(2.0%) of the pregnant women who had a history of spontaneous abortion, stillbirth, preterm birth, and malformed children, respectively. Table (3) showed that more than a third of the pregnant women (39.50%) were in their second trimester. There was a statically significant association between seropositivity of HSV-2 IgG antibody and abortion (χ² = 4.50; P = 0.03) and stillbirth (χ² = 11.41; P=0.001). While other reproductive characteristics show no statistically significant association between seropositivity of HSV-2 IgG antibodies (P > 0.05).

Table 2 Seroprevalence of HSV-2 specific IgG antibodies according to the socio-demographic characteristics

| Variable                  | Participants | IgG Positive | p. value |
|---------------------------|--------------|--------------|----------|
|                           | No (%)       | No (%)       |          |
| Age                       |              |              |          |
| < 20                      | 51 (25.50)   | 2 (3.92)     | 0.39     |
| 21-30                     | 111 (55.50)  | 6 (5.41)     |          |
| 31-40                     | 38 (19.0)    | 4 (10.53)    |          |
| Residence                 |              |              |          |
| Rural                     | 98 (49.0)    | 9 (9.18)     | 0.06     |
| Urban                     | 102 (51.0)   | 3 (2.94)     |          |
| Educational level         |              |              |          |
| Illiterate                | 78 (39.0)    | 1 (1.28)     |          |
| Basic                     | 44 (22.0)    | 7 (15.91)    |          |
| Primary                   | 26 (13.0)    | 1 (3.85)     | 0.02     |
| Secondary                 | 45 (22.50)   | 3 (6.67)     |          |
| University                | 7 (3.50)     | 0 (0.0)      |          |
| Occupation                |              |              |          |
| Housewife                 | 196 (98.0)   | 12 (6.12)    | 0.60     |
| Employee                  | 4 (2.0)      | 0 (0.0)      |          |
| level of household income |              |              |          |
| High                      | 16 (8.0)     | 0 (0.0)      | 0.34     |
| Middle income             | 156 (78.0)   | 9 (5.77)     |          |
| Low                       | 28 (14.0)    | 3 (10.71)    |          |
| Place of birth            |              |              |          |
| Dhamar                    | 159 (80.30)  | 11 (6.92)    | 0.40     |
| Other                     | 41 (19.70)   | 1 (2.4)      |          |
Table 3: Seroprevalence of HSV-2 specific IgG antibodies according to the reproductive characteristics

| Variable                  | Participants | IgG Positive | IgG Negative | χ²   | P- value |
|---------------------------|--------------|--------------|--------------|------|----------|
|                           | No (%)       | No (%)       | No (%)       |      |          |
| Trimester                 |              |              |              |      |          |
| First                     | 67 (33.50)   | 5 (7.46)     | 62 (92.54)   | 0.77 | 0.67     |
| Second                    | 79 (39.50)   | 5 (6.33)     | 74 (93.67)   |      |          |
| Third                     | 54 (27)      | 2 (3.70)     | 52 (96.30)   |      |          |
| Term of pregnancy         |              |              |              |      |          |
| First                     | 47 (23.50)   | 3 (6.38)     | 44 (93.62)   | 0.01 | 0.89     |
| Second or more            | 153 (76.50)  | 9 (5.88)     | 144 (94.12)  |      |          |
| Preterm birth             |              |              |              |      |          |
| Yes                       | 23 (41.0)    | 3 (1.35)     | 20 (86.96)   | 2.28 | 0.13     |
| No                        | 177 (54.0)   | 9 (8.73)     | 168 (91.27)  |      |          |
| Cesarean-section          |              |              |              |      |          |
| Yes                       | 16 (8.0)     | 1 (6.25)     | 15 (93.75)   | 0.001| 0.96     |
| No                        | 184 (92.0)   | 11 (5.98)    | 173 (94.02)  |      |          |
| Abortion                  |              |              |              |      |          |
| Yes                       | 74 (37.0)    | 1 (1.35)     | 73 (98.65)   | 4.5  | 0.03*    |
| No                        | 126 (63.0)   | 11 (8.73)    | 115 (91.27)  |      |          |
| Stillbirth child          |              |              |              |      |          |
| Yes                       | 23 (11.50)   | 5 (21.74)    | 18 (78.26)   | 11.41| 0.001*   |
| No                        | 177 (88.50)  | 7 (3.95)     | 170 (96.05)  |      |          |
| Live births               |              |              |              |      |          |
| None                      | 82 (41.0)    | 4 (4.88)     | 78 (95.12)   | 1.2006| 0.54 |
| 01-Apr                    | 108 (54.0)   | 8 (7.41)     | 100 (92.59)  |      |          |
| > 4                       | 10 (5.0)     | 0 (0.0)      | 10 (100)     |      |          |
| Surviving children        |              |              |              |      |          |
| None                      | 85 (42.5)    | 4 (4.71)     | 81 (95.29)   | 1.2768| 0.52 |
| 01-Apr                    | 106 (53.0)   | 8 (7.55)     | 98 (92.45)   |      |          |
| > 4                       | 9 (4.5)      | 0 (0.0)      | 9 (100)      |      |          |
| Malformed children        |              |              |              |      |          |
| Yes                       | 4 (2.0)      | 0 (0.0)      | 4 (100)      | 0.26 | 0.6      |
| No                        | 196 (98.0)   | 12 (6.12)    | 184 (93.88)  |      |          |

Distribution of HSV-2 antibodies according to the symptoms characteristics:

Symptoms, such as fever, headaches, itching, tingling, inflammation during urination, swelling and blisters, were studied (Table 4). 96 (48.0%), 104 (52.0%), 86 (43.0%), 92 (46.0%), 125 (62.50%), 19 (9.50%) and 39 (19.50%), of the pregnant women had a symptoms of fever, headaches, itching, tingling, inflammation during urination, swelling and blisters respectively. There was a statistically significant association between the itching (χ² = 5.33; P = 0.02) and inflammation during urination (χ² = 4.63; P = 0.03) seropositivity of HSV-2 IgG antibody. While other symptom characteristics did not show any statistically significant association between seropositivity of HSV-2 IgG antibodies (P > 0.05).

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### Discussion

From our investigation, there is no previous community-based seroepidemiological study investigating HSV-2 IgG antibodies among pregnant women in Dhamar city, Yemen. Hence, we focus on concerning the prevalence of HSV-2 IgG antibodies among pregnant women in Dhamar city. The study also assessed some associated risk factors that increase the infection rate among pregnant women. It would provide not only current information on the numbers of pregnant women at risk of these viral infections but also provide some useful information for health planners, care providers and health promotion activities.

The percentage of HSV-2 IgG observed in this study was (6.0%). similar to the previous study in other different countries such as Saudi Arabia 5.5%, 6.5%, 6.8%,15–17 Iraq 9%,18 Sudan 7.6%,19 Turkey 4.4% ,5%,19–26 India 8.7%, 6.7%,21,22 Kashmir 7.5%,21 Bangladesh 9.91%,24 Their previous studies showed similar findings in terms of the rate of HSV-2 IgG infection. This similarity in their studies may be related to several reasons, such as the size of the sample and type of target category. Another effect factor as a place of study is in the rural or the urban, perhaps due to lack of knowledge and awareness about this virus. However, the main reasons for increasing the prevalence rate of virus infection. It could be due to the poverty absence of a vaccine in those regions that plays hygiene and religion. Poverty has a significant role in increasing the incidence of infection.

The analytical data have, there was no significant association observed between increasing age with seropositivity of HSV-2 IgG antibodies but increasing the seropositivity with advanced age (p=0.39). It must be reiterated that as the women advanced in age, the rate of exposure to risk factors increases, leading to the high prevalence observed. Aging is known to be associated with a gradual decrease in immunity. It could contribute to the high prevalence observed which is in agreement with the previous studies reported by14,20 While in India inconsistent published by29.

Regarding the high level of HSV-2 IgG, seropositivity was reported also by other countries researchers such as in Saudi Arabia 27.1% (9), Sudan 34.6%, 63.3%, 91.1%,20 Iraq 22.2%,27 Syria 52%,28 India 64.9%, 34.86%,29 Ethiopia 32.1%,19 Nigeria 82.2%,33,38 Ghana 78.3 %,32 Zimbabwe 49.1%, 51.10%,13,34 Eastern Nepal 35.8 %,35 Cote D’ivoire 96.5%,36 Germany 82%,13 Switzerland 21.2%,38 Brazil 15.6%39 and USA 22%. These shades as mentioned above showed higher findings in terms of the rate of HSV-2 IgG infection. These higher rates may be related to several reasons, such as the size of the sample and type of target category. Another effect factor as a place of study is in the rural or the urban, perhaps due to lack of knowledge and awareness about this virus. However, the main reasons for increasing the prevalence rate of virus infection. It could be due to the poverty absence of a vaccine in those regions that plays hygiene and religion. Poverty has a significant role in increasing the incidence of infection.

In our study, there was a significant association between education level and HSV-2 seropositivity. This finding similar to some
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While other studies found increases in the infection with the level of education by (17), studies show high sero-prevalence increased with education women. However, found no significant between education level and HSV-2 sero-prevalence. All of other socio-demographic data were no signs with the sero-prevalence of HSV-2 IgG antibodies; this result agrees with the study by. The sero-prevalence of HSV–2 IgG was found statistically significantly associated with the occupation of the mother. However, found no significant between occupation and HSV-2 sero-prevalence.

Statistical analysis showed, there was a significant association observed between stillbirth with sero-prevality of HSV–2 IgG antibodies (P<0.00). This finding similar to the study of. They found 73 mothers with various obstetric problems like abortion, stillbirth, premature birth and intrauterine developmental retardation, reported HSV–1 positivity in 71 (97.3%) HSV–2 IgG positivity in 65 (89%) and HSV–2 IgM positivity in 6 (8%). None of the characteristics considered to be risk factors were a statistically significant predisposing to HSV–2 infection. We were reported that 5% of the women had multiple sexual partners during pregnancy. Having multiple sexual partners and being in polygamous unions increased the risk of HSV–2 acquisition threefold and fourfold, respectively. Other studies also found multiple sexual partners and polygamy to be risk factors for HSV–2 seroconversion. Having multiple sexual partners increases the risk of one of the partners having HSV–2 and transmitting to the others. Some of the common clinical symptoms associated with HSV–2 infection were observed in the pregnant women in this study, included fever, headaches, itching, tingling, inflammation during urination, swelling and blisters with studies of.

In this study, there were significantly associated with the risk of infection (positive for HSV–2 IgG antibody) and itching and inflammation during urination. The pregnant women made complaints mostly of delirium. Very few of these women were positively for HSV–2 infection (IgG antibody). The overall clinical picture of HSV–2 infection is very similar to that reported by other workers in some geographical areas. Related work has reported that fever was significantly associated with HSV–2 infection; this could arise as a result of the immune response during infection. However, who reported a sero-prevalence of 54.3% amongst fever patients. On the other hand, blisters and ulcers in the genital area were found to be statistically associated with HSV–2 infection (P<0.05). This finding agrees with earlier reports by who reported that a 40.3% prevalence infection of HSV–2 also observed a significant association between genital ulcerations with the virus infection, also reported a high HSV–2 sero-prevalence among subjects associated with genital ulcers. Painful urination was observed to be significantly and associated with HSV–2 infection (P<0.05). This finding disagrees with an earlier report by, who found that 21% of his study population had painful urination, but there was no significant association with the infection. One study disagrees with our study reported by who observed sero-prevalence itching (pruritus) among subjects and significantly associated with high HSV–2 infection (P<0.85). This finding disagrees with our study.

Conclusion

Overall, the sero-prevalence of HSV–2 infection among pregnant women in Dhamar city is low. There is a critical need to adopt screening of HSV–2 into the antenatal profile tests. There is also need for more health education of this virus infection, methods of transmission, associated risk factors, and effective prevention and control strategies.

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Conflicts of interest
None.

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