Seroprevalence of Herpes Simplex Virus Type-1 IgG Antibody in Healthy Blood Donor from Baghdad, Iraq

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Abstract
Herpes simplex virus type 1 (HSV-1) is a common infectious agent worldwide. It can cause earnest illnesses at each stage of life. Data on seroprevalence of HSV-1 antibody (Ab) in Iraqi population is limited. This study aimed on determination the frequency of HSV-1 IgG Ab in apparently healthy blood donor of Baghdad province, capital of Iraq. In this study, serum samples were collected from 454 apparently healthy blood donor (385 male and 69 female), their ages ranged from 18 to 64 year. This study was conducted through the period from October, 2018 to February, 2019. Demographic data were gathered by a well-designed questionnaire. Five (5) ml of blood sample was collected and centrifuged, all obtained sera samples were tested using Enzyme linked Immunosorbent Assay (ELISA) for determine of HSV-1 IgG Ab. Totally, HSV-1 IgG Ab were positive in 427 out of 454 (94.1%) among studied population. The age groups of (21-30) years and (31-40) years had the highest percentage of seropositivity for anti HSV-1 IgG Ab (39.1% and 32.3%, respectively), while the lowest seropositivity was found in age groups (>50) years and (≤20) years (4.4% and 5.4%, respectively). The frequency of HSV-1 IgG Ab was higher among male (84.8%) compared with females (15.2%), however, it was not statistically significant (P > 0.05). Results confirm a high frequency of anti HSV-1 IgG Ab among Iraqi population. The high frequency of HSV-1 infection underlines the need for focusing on preventive efforts and education among the population about HSV-1 and its complications.

Keywords: HSV-1, ELISA, adult, blood donor, Iraq.

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INTRODUCTION

Herpes simplex virus type 1 (HSV-1), a neurotropic member belong to the alpha herpes subfamily of the Herpesviridae, is an enveloped large dsDNA virus. Herpes is derived from the Greek word “to creep” and this term used for the first time by Hippocrates to describe how this ulcerative lesion appear to be creeping along the skin. However, the virus was not discovered till 1950s. HSV-1 is typically transmitted during childhood through nonsexual routes primarily through oral secretions or by contact with the infected region of the skin during virus reactivation. Exposure to HSV-1 during childhood in western countries was diminished due to hygiene practice and improve the socioeconomic conditions, therefore; young people could reach sexual debut with no defensive antibodies against HSV-1 disease, and subsequently at risk of obtaining the infection genitaly. Herpes simplex virus type-1 may be transmitted vertically from the mother to the fetus, in spite of fact that, the hazard is very low.

HSV-1 IgG Ab detection

All 454 samples were tested by indirect ELISA (Human Gesellschaft für Biochemica und Diagnostica, Germany) for HSV-1 IgG antibody detection. Sera were screened at 1:100 dilution, the procedure was done following manufacturer’s instructions. Blank, positive and negative control was included with each run. The micro titer plate was coated with cell culture derived HSV antigen (HSV1-Ag). The HSV-1 IgG Ab present in patient serum bind to the antigen at the solid phase. At the end of the incubation, the unbound antibodies were washed out. Then a second rabbit anti-human IgG Ab, which are conjugated with peroxidase
are applied. Then after the second incubation step excess components were washed out to remove unbound conjugate. Then the reaction was visualized by the addition of the substrate 3,3,5,5-tetramethylbenzidin (TMB) with hydrogen peroxide and incubate in dark. At the end of the incubation blue color developed at the positive wells that converted to yellow color after addition of the of stopping reagent (sulphuric acid). The intensity of the color is directly proportional to the HSV1-IgG-Ab concentration in the specimen. The optical densities (O.D.) of each well were measured at wave length (450 nm and reference filter 630 nm) within half an hr. after the end of the reaction.

Interpretation of results
For Herpes simplex virus type 1 detection, the cut-off value was calculated by adding 0.1 multiplied by the mean absorbance values of positive control to the measured mean absorbance values of negative control. The net O.D. of each sample was considered positive if their absorbance reading is equal or larger than 15% above the calculated cut-off. While the samples with absorbance reading less than 15% below the calculated cut-off were considered negative. The results lying 15% above or below the cut-off value were considered equivocal. The equivocal samples should repeated in fresh sample taken 7 to 14 days later, therefore, all equivocal sample were excluded from this study.

Statistical Analysis
Data were analyzed using Statistical Package for the Social Sciences program (SPSS program, versions 21). Descriptive statistics were presented as frequencies. Age was categorized into five groups: (≤ 20), (21-30), (31-40), (41-50) and (>50). Cross-tabulation between HSV-1 serostatus and demographic data were performed, the differences were examined by the Chi-square test (X²-test) or Fisher’s exact test wherever appropriate. Correlations between variables were estimated by using Pearson’s correlation (r). Statistical significance was considered at a P value equal or less than 0.05.

RESULTS
In current study, frequency of anti HSV-1 IgG Ab were found in 427 out of 454 (94.1%). The age group of (21-30) years had the highest percentage of seropositivity for anti HSV-1 IgG Ab (39.1%) followed by the age group of (31-40) with seropositivity for anti HSV-1 IgG Ab (32.3%), while age group (> 50) years had the lowest seropositivity (4.4%) followed by the age group of (≤ 20) with seropositivity for anti HSV-1 IgG Ab (5.4%), however, there was no significant association in HSV-1 IgG Ab positivity among different age group (χ²= 8.496, P>0.05). Moreover there was a significant positive correlation between the HSV-1 IgG Ab seropositivity and the extend of age (r=0.127, P<0.05), as shown in table (1).

Table 1. HSV-1 Seroprevalences in Iraqi healthy blood donors, grouped according to age.

<table>
<thead>
<tr>
<th>Age</th>
<th>HSV1-IgG</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>≤ 20</td>
<td>2 (7.4%)</td>
<td>23 (5.4%)</td>
</tr>
<tr>
<td>21-30</td>
<td>17 (63.0%)</td>
<td>167 (39.1%)</td>
</tr>
<tr>
<td>31-40</td>
<td>7 (25.9%)</td>
<td>138 (32.3%)</td>
</tr>
<tr>
<td>41-50</td>
<td>1 (3.7%)</td>
<td>80 (18.7%)</td>
</tr>
<tr>
<td>&gt; 50</td>
<td>0 (0.0%)</td>
<td>19 (4.4%)</td>
</tr>
<tr>
<td>Total</td>
<td>27(100.0%)</td>
<td>427(100.0%)</td>
</tr>
</tbody>
</table>

* Chi-square (χ²) = 8.496, P=0.075
**Pearson Correlation (r) = 0.127, P= 0.007

This study showed that the frequency of HSV-1 IgG Ab seropositivity were (15.2%) in female and (84.8%) in male with no statistically significant differences between male and female in the acquiring of HSV-1 Ab (Fisher’s Exact Test, P>0.05), as shown in table (2).

Table 2. HSV-1 Seroprevalences in Iraqi healthy blood donors, grouped according to gender

<table>
<thead>
<tr>
<th>Gender</th>
<th>HSV1-IgG</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Female</td>
<td>4 (14.8%)</td>
<td>65(15.2%)</td>
</tr>
<tr>
<td>Male</td>
<td>23 (85.2%)</td>
<td>362 (84.8%)</td>
</tr>
<tr>
<td>Total</td>
<td>27 (100.0%)</td>
<td>427 (100.0%)</td>
</tr>
</tbody>
</table>

*Fisher’s Exact Test P value = 0.9999
**Pearson Correlation (r) =0.003, P=0.954
According to occupation, the anti HSV-1 antibody seropositivity were more in those working in private sector (54.6%) while the less prevalent in student (5.4%), however, there was no significant difference between anti HSV-IgG Ab positive and negative groups regarding to the occupation ($X^2= 0.966, P>0.05$), as shown in table (3).

<table>
<thead>
<tr>
<th>Occupation</th>
<th>HSV1-IgG</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Private sector</td>
<td>16 (59.3%)</td>
<td>233 (54.6%)</td>
</tr>
<tr>
<td>Governmental employee</td>
<td>5 (18.5%)</td>
<td>74 (17.3%)</td>
</tr>
<tr>
<td>Military</td>
<td>2 (7.4%)</td>
<td>56 (13.1%)</td>
</tr>
<tr>
<td>Student</td>
<td>1 (3.7%)</td>
<td>23 (5.4%)</td>
</tr>
<tr>
<td>House wife</td>
<td>3 (11.1%)</td>
<td>41 (9.6%)</td>
</tr>
<tr>
<td>Total</td>
<td>27 (100%)</td>
<td>427 (100%)</td>
</tr>
</tbody>
</table>

*Chi-square ($\chi^2$) = 0.966, $P=0.915$

**Pearson Correlation (r) = 0.016, $P=0.726$

This study revealed that (70.7%) of those with positive serology had no history of travel and (29.3%) of them had a history of travel, although there was no significant differences in acquisition of HSV-1 antibodies regarding the travel history (Fisher’s Exact Test, $P>0.05$), as shown in table (4).

<table>
<thead>
<tr>
<th>Travel History</th>
<th>HSV1-IgG</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>No</td>
<td>19 (70.4%)</td>
<td>302 (70.7%)</td>
</tr>
<tr>
<td>Yes</td>
<td>8 (29.6%)</td>
<td>125 (29.3%)</td>
</tr>
<tr>
<td>Total</td>
<td>27 (100%)</td>
<td>427 (100%)</td>
</tr>
</tbody>
</table>

*Fisher’s Exact Test $P$ value = 0.9999

**Pearson Correlation (r) = -0.002, $P=0.969$

DISCUSSION

HSV-1 infection is widespread, and its seropositivity may cover more than 70% of the world population. In the United States, 65% of people have HSV-1 antibodies, which is comparable to the epidemiology in Europe. The variation of HSV-1 prevalence has been related to the diversity of socioeconomic conditions. In the Middle East and North Africa (MENA), seroprevalence was 65.2% in children, and 91.5% in adults. The present study showed that the prevalence rate for HSV-1 IgG antibody was (94.1%) (Table 1) which is nearby to that reported by Hossain (1989) who found that the overall prevalence of HSV-1 IgG antibody in adult were (90%) but it is in contrast to a study that conducted in Baquba-Iraq in two different groups (students and outpatient with different skin disease other than HSV infection) that reported the seroprevalence of HSV-1 IgG Ab and HSV-2 IgG Ab was (56.51%) and (43.45%), respectively, although the same study showed that (79.09%) were positive for HSV IgG Ab in general. This may be due to cross-reactivity for the two Herpes serotypes HSV-1 and HSV-2 or due to using different diagnostic methods that may differ in sensitivity and specificity or due to differences in the populations studied. Other study demonstrated that the seroprevalence of HSV-1 IgG Ab among blood donors living in Qatar was 80.5% among Palestinians, 81.4% among Lebanese, 81.4% among Iranians, 82.3% among type 1 IgG was observed in (71.7%) and (28.3%) among those who did not practice cupping and those who practice cupping with no statistically significant differences between both groups (Fisher’s Exact Test $P>0.05$), as shown in table (5).
Qataris, 86.5% among Jordanians, 88.5% among Syrians, 90.7% among Sudanese, 92.6% among Yemenis and the higher prevalence was 97.5% among Egyptians.

Regarding to age and gender, the current finding of the frequency of HSV-1 IgG Ab was higher in age groups (21-30) and (31-40) years with no significant differences between males and females (Table 1 and Table 2), which close to that demonstrated by Al-Kayalli et al. (2015) who found that HSV infection was predominantly in age group (21-40 years)17. However, in Saudi Arabia, a study reported that the prevalence of HSV-1 IgG showed a progressive increase with age in both males and females with no obvious sex-related variation9. Another study have reported a small increase in the seroprevalence of HSV-1 Ab and HSV-2 Ab above the age of 40 years21. Despite the fact that our specimens were not completely representative of the general population (385 were male while only 69 were female), however, the results about age and gender dispersion aren’t affected by this. Chaabane et al. (2019) mentioned that age and country’s income could explained half of the variation of HSV-1 seroprevalence. While other factors such as gender, population type, year of publication, sample number technique used, were not significantly associated with sero-prevalence7. In addition, the existing evidence suggests that was no major sex-specific differences21,20,22,23,24 and the sample collection bias may be less important for HSV-1, as the virus is primarily orally transmitted within a general-population6.

According to occupation, the current study showed a higher seroprevalence of HSV-1 were found in those working in private sector (54.6%), while the lower seroprevalence were found in students (5.4%), however there was no significant association between occupation and HSV-1 positivity (Table 3). Interestingly, Al-Kayalli et al. (2015) mentioned that the prevalence of HSV Ab were more in student (33.4%) and less prevalent in those working in private sector (19%) although however it was statistically not significant17. The current finding of HSV-1 IgG Ab in Governorate worker were (17.3%) which is comparable to that report by other17. This suggested that HSV-1 existence is high in Iraqi population with no declines in frequency in spite of increasing the income of Iraqi people after the end of the economic siege as the younger person (21-40 years) had higher HSV-1 seroprevalence than older person (>50 years). The route of transmission of HSV-1 is by close contact either sexually or non sexually and infections are acquired during childhood and adolescence. Approximately fifty percent of isolated herpes from hands, fingers and extra-genital regions other than oro-facial area were belong to HSV type-1 while the remaining were belong to HSV type -225.

This study showed that there was no significant association between travel history or cupping practice and HSV-1 IgG positivity (Table 4 and Table 5). Researcher found that different HSV-1 strains in a single individual can be used as a record of the person’s travel history. In addition, viral DNA sequencing may someday become a useful forensic tool and could serve as a surrogate biomarker to study human population structure and migration patterns25,26. Regarding cupping which is a traditional oriental medicine, a study conducted in Korea have reported an uncommon case of cutaneous herpes infection that suspected to be a consequence of cupping due to direct viral inoculation via contamination or HSV reactivation due to mechanical trauma27.

Herpes simplex virus type 1 causes a various diseases such as neonatal herpes, corneal blindness, meningitis, encephalitis and genital herpes7. The fact of the high frequency of HSV-1 IgG Ab in our population suggests that the HSV-1 morbidity should be quantified and addressed. In addition, it is highlight the importance of increasing the effort for vaccine development.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.
AUTHOR’S CONTRIBUTION
AMA, DBH and ZQA designed the study and developed the research methodology. AMA and DBH provided the specimens. AMA conducted laboratory work on the specimens and wrote the initial draft of the article. All authors contributed to the interpretation of the results and drafting and revision of the article.

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DATA AVAILABILITY
All datasets generated or analyzed during this study are included in the manuscript and/or the Supplementary Files.

ETHICS STATEMENT
This study was approved by Institutional Review Board (IRB) at Al-Nahrain College of Medicine number #210.

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