Seroprevalence of Herpes Simplex Virus type-Iantibodies (IgM,IgG) in smokers in Kirkuk city-Iraq
Lezan Medhat Mohammed Zanana
Medical Laboratory technology, Technical College / Kirkuk, Kirkuk, Iraq

Abstract:
Herpes Simplex Virus type-1 (HSV-1) is classified in the α-herpes virus group of the Herpesviridae and HSV-1 infections are very common in the human population. The objective of this study is to determine the prevalence of anti-herpes simplex virus type-1 IgM and anti-herpes simplex virus type-1 IgG among smokers and nonsmokers. In the current study, A total of one hundred two serum samples from males were collected (70 smokers and 32 nonsmokers), there ages were ranged between 20- 50 year; this study which was done during the period between September 2014 to the January 2015. All obtained sera samples from all persons were tested using ELISA method for determine of Herpes simplex virus type-1 antibodies. Results in this study showed that the herpes simplex virus type-1 IgM seropositivity among smoker persons was 24 (34.3%). In addition, the result revealed that the percentage rate of the herpes simplex virus type-1 IgG among smokers was 61(87.1%). There was a significant effect of smoking on the seroprevalence of Herpes simplex virus type-1 antibodies (IgM , IgG).

Key words: Herpes simplex virus type 1 (HSV-1), Seroprevalence, Smoking and Kirkuk.

Introduction
Herpes simplex virus type-1 and Herpes simplex type-2 (HSV-1 and HSV-2), also known as Human herpes virus type-1 and Human herpes virus type-2 (HHV-1 and HHV-2), are two members of the herpes virus family, Herpesviridae, that infects human [1]. Hippocrates first used the word “ herpes “ derived from the Greek word “ to creep “ to describe how the lesions of this contagious ulcerative disease seemed to creep or crawl along the skin [2]. The virus itself was not identified until the 1950 s [3]. Herpes simplex virus type-1 is an enveloped virus with a large 150-250 nm in size, linear, double-stranded DNA and contains at least 74 genes within their genome [4, 5, 6, 7]. The worldwide rates of Herpes simplex virus type-1 are between 60%-90% in adults [8]. Herpes simplex virus type-1 are transmitted by contact with an infected area of the skin during reactivation of the virus [9]. Herpes simplex virus type-1 may also be transmitted vertically during childbirth, although, the real risk is very low [10]. About 1/3 of HSV-1 patients do not show their symptoms at all, for this reasons, it can be mistaken or many particles can be unaware of it [11]. During the primary infection (usually 2-12 days) after contacting virus, the initial pimple-like clear vesicle form, which contain infectious virus fluid and the bottom part of the vesicles have a reddish color [11,12,13] , then painful shocking ulcerating blisters are dried out and healed within (7-10 days) without leaving scars [11, 14]. The virus remains and spreads in to proximal nerve travelling from one cell to another without ever leaving the internal environment of the cell going into a latency period (not contagious during this period) [11,15] . Smoking, poor nutrition, and reactivational drug used, all of which cause stress that is detrimental to our immune system [16]. When certain stimulus like physical emotional stress, fever, and UV-light impact a patient, a secondary lesions breaks out, which is less severe than the primary [15,17]. All these signs and symptoms may show along with fever, muscle ache, chills, headache, swollen lymph nodes, and flu-like symptoms [11]. The incubation period of Herpes simplex virus type-1 infection is 2-12 days (mean 4 days)[18]. Herpes simplex virus type-1 is usually associated with infections of the lips, mouth, and face. HSV-1 often causes sores ( lesions ) inside the mouth such as cold sores (fever blisters ,oral infection) [19,20]. The diagnosis of Herpes simplex virus type-1 infection is usually made by the appearance of the lesions and the patient’s history. However, if the pattern of the lesions is not specific to Herpes simplex virus type-1, its diagnosis can made by viral culture, PCR, serology, direct fluorescent antibody testing, or Tzanck test [21,22,23,24]. Current treatment is effective only on replicating virus and does not work against latent virus; this means that treatment will never terminate the virus [12]. The most common antiviral agent is nucleoside analog Acyclovir (Zovirax), There are medicines that are precursor of Acyclovir: Val acyclovir, Penciclovir and Famciclovir[17]. One of the initial ways to prevent HSV-1 is avoid contact with the virus (for example, having good hygiene will prevent possible initial contact with the virus ), using water and soap usually kills the virus [12,25]. Now, most of the vaccines being developed are to lessen the severity of the symptoms, and to minimize the possible secondary effects and further recoccurrence of infection [14]. The aim of this study was to detect the seroprevalence of Herpes simplex virus type-1 antibodies in smokers in Kirkuk city because screening for HSV-1 in blood is not mandatory in the city.

Materials and Methods
Blood samples collected from total 102 male persons (70 smokers) and ( 32 nonsmokers, as a control group), All samples were centrifuged at (2000 rpm) for 15 minutes and serum separated, then screened for presence of herpes simplex virus type-1 antibodies (IgM and IgG) by classical ELISA technique. Criteria for selection were smokers, male without history of smoking, age.
ELISA test for the detection of IgM antibodies to Herpes Simplex Virus in human serum principle:
The human HSV IgM ELISA is based on the classical ELISA technique. The microtiter strip wells as a solid phase are coated with cell culture derived Herpes Simplex virus antigens (HSV 1-Ag). In the first incubation step corresponding specific antibodies (HSV-IgM-Ab) present in patient specimens or controls bind to the antigens at the solid phase. The sample dilution buffer contains anti-human IgG to prevent rheumatoid factor interference and competition from specific IgG present in the specimen. At the end of the incubation unbound components are washed out. For the second incubation step anti-IgG conjugate (anti-human IgG antibodies, peroxidase conjugated) is added which binds specifically to IgG class antibodies resulting in the formation of typical immunocomplexes. After a second washing step to remove excess conjugate, TMB/Substrate is added (step3). A blue colour develops changing to yellow after stopping the reaction. The intensity of the colour is directly proportional to the HSV-IgG-Ab concentration in the specimen.

The absorbance of controls and specimen is determined by using ELISA microplate readers or automated ELISA systems. Results for patient samples are obtained by comparison with a cut-off value[Classical ELISA, Germany].

Interpretation of results
A patient \( \leq \text{COV} + 15\% \) : anti-HSV-1 IgM ( IgG)-Ab-positive
A patient \( \geq \text{COV} + 15\% \) : anti-HSV-1 IgM ( IgG)-Ab-negative

Statistical analysis: \( \chi^2 \) (chi-square) test was used. The significant level used was \( P < 0.05 \).

Result and Discussion
Table 1 demonstrates the seropositivity of Herpes Simplex virus type-1 IgM antibodies among smokers according to different age groups. 11(36.7%) occurred in age between 20-30 years, 9(32.1%) occurred in age between 30-39 years, and 4( 33.3%) occurred in age more than 40 years.

Table 1 Distribution of Herpes Simplex virus type-1 IgM antibodies in smokers according to age group

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>20-29</th>
<th>30-39</th>
<th>40+</th>
<th>Total</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgM Positive</td>
<td>Count</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% within Age</td>
<td>36.7%</td>
<td>32.1%</td>
<td>33.3%</td>
<td>34.3%</td>
<td>0.9</td>
</tr>
<tr>
<td>Negative</td>
<td>Count</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% within Age</td>
<td>63.3%</td>
<td>67.9%</td>
<td>66.7%</td>
<td>65.7%</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>Count</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% within Age</td>
<td>100.0%</td>
<td>100.0%</td>
<td>100.0%</td>
<td>100.0%</td>
<td></td>
</tr>
</tbody>
</table>

Table 2 shows the prevalence of Herpes Simplex virus type-1 titer IgG antibodies among smokers according to different age groups. 61(87.1%) out of 70 smokers have positive anti-herpes simplex virus type-1 titer IgG.

Table 2 Distribution of Herpes Simplex virus type-1 IgG antibodies in smokers according to age group

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>20-29</th>
<th>30-39</th>
<th>40+</th>
<th>Total</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG Positive</td>
<td>Count</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% within Age</td>
<td>80.0%</td>
<td>92.9%</td>
<td>91.7%</td>
<td>87.1%</td>
<td>0.3</td>
</tr>
<tr>
<td>Negative</td>
<td>Count</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% within Age</td>
<td>20.0%</td>
<td>7.1%</td>
<td>8.3%</td>
<td>12.9%</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>Count</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% within Age</td>
<td>100.0%</td>
<td>100.0%</td>
<td>100.0%</td>
<td>100.0%</td>
<td></td>
</tr>
</tbody>
</table>
Figure (1). Reveals that the correlation between Herpes Simplex virus type-1 IgM antibodies and smoking are statistically significant (P-value: 0.003)

![Figure 1](image1)

**Figure (1): Correlation between Herpes Simplex virus type-1 IgM antibodies and smoking**

Figure (2). Illustrates that the correlation between anti-herpes simplex virus type-1 titer IgG and smoking are statistically significant (P-value: 0.005)

![Figure 2](image2)

**Figure (2): Correlation between Herpes Simplex virus type-1 IgG antibodies and smoking**

Because the primary infection is often asymptomatic, reactivation can be the first clinical manifestation of infection. Classically, HSV-1 infects squamous epithelium. Factors promoting reactivation includes trauma, smoking, burns, radiation therapy or chemotherapy[26]. Herpes simplex viruses are very sensitive to their environment. In a young persons with a healthy immune system, they will attack a very low level to avoid large-scale activation of the immune system, which could deliver damaging blow to the herpes viruses sense that stress, aging, cancer, smoking, or physical injury has weakened the body, they accelerate their attack, just as they do if they sense that the immune system is preoccupied fighting off another infection such as a cold or flu.[16]. The Chi-square analysis revealed that the prevalence of IgG seropositivity were 61(87.1%) in smoker men, this results was nearly compatible with another study that revealed the prevalence of anti-herpes simplex virus type-1 was (55.4%) [27]. Another study also nearly compatible with the current study that show that the antibody titers (IgG&IgM) were significantly higher in the smoking group than in controls [28]. Another study shows that the individuals in the lowest age group had less reactivation; these results demonstrate that herpesvirus reactivation is associated with variables such as age, and may play a role in poorer health outcomes in both younger and...
older adults [29]. A studied done in Romania showed that among the men, seroprevalence increased from 4.0% in 20–24-year-olds to 27.1% in 40–44-year-olds [30]. Another studied done in china also nearly compatible with the current study, showed that the age specific seropositive rate reaching 80% among those over 30 years [31]. The current study show the

References
correlation between seropositivity of IgM & IgG herpes siplex virus type1and smoking are significant, this results are agreed with another studied which show that there was a significant correlation between herpes simplex virus type-1 antibodies and smoking are significant (p-Value < 0:05)[32].

انتشار اضداد فايروس الهيربس البسيط النوع الأول في المدخنين في محافظة كركوك - العراق

لبنى محدث محمد زنكنة
قسم التحليلات المرضية ، الكلية التقنية / كركوك ، كركوك ، العراق

الملخص

فايروس الهيربس البسيط النوع الأول مصنف من مجموعة الفا هيربس فايروس وعائلة هيربس فيريداي. إن اصابات فايروس الهيربس البسيط النوع الأول شائعة جدا في الإنسان. هدف هذه الدراسة هو تقدير اضداد فايروس الهيربس البسيط النوع الأول (IgG, IgM) في المدخنين وغير المدخنين. في الدراسة الحالية جمعت مائة واثنان عينة مصل من المدخنين (70 مدخنين و 32 غير مدخنين). ود تراوحت أعمارهم بين (20-50) سنة. أجريت هذه الدراسة في محافظة كركوك للفترة من شهر ايلول/2014 ولغاية شهر كانون الثاني/2015. جميع المصلات التي تم الحصول عليها من جميع الأشخاص المعنيين بالدراسة تم اختبارها باستخدام طريقة ELISA لتحديد الأجسام المضادة لفايروس الهيربس البسيط النوع الأول (IgM). بينت النتائج في الدراسة الحالية أن نسبة انتشار اضداد فايروس الهيربس البسيط النوع الأول (IgM) في المدخنين كان موجبا بنسبة (34.3%) (24)، وإن نسبة انتشار اضداد فايروس الهيربس البسيط النوع الأول (IgG) كان موجبا بنسبة (87.1%) (61). حيث بينت الدراسة أيضا أنه توجد دلالة معنوية في تأثير التدخين على انتشار اضداد فايروس الهيربس البسيط النوع الأول (IgM). (gGl).

الكلمات الدالة : فايروس الهيربس البسيط النوع الأول، الاختبار المصل، التدخين، كركوك.