ORAL ULCERATION IN SYSTEMIC LUPUS ERYTHEMATOSUS IN IRAQI PATIENTS AND THE LEVELS OF SERUM VASCULAR ENDOTHELIAL GROWTH FACTOR-A

Maytham R. Ali1*, Taghreed F. Zaidan2

1Faculty of Dentistry, Babylon University, Iraq
2Faculty of Dentistry, University of Baghdad, Iraq

*Corresponding author E-mail: dr.maythem_d@yahoo.com (Ali)

ABSTRACT

The aim of this study was to estimate the levels of serum vascular endothelial growth factor (VEGF-A) among systemic lupus erythematosus (SLE) patients with and without oral ulceration in Iraqi sample.

The study took place in Mirjan teaching hospital in Hilla city, Babylon (Rheumatology unit) during the period from May 2018 till January 2019. All the subjects were from the same ethnic group (Arabic) and were from the same geographical region. The American College of Rheumatology (ACR) 1997 criteria of SLE was used for diagnosis of SLE. A total of 52 SLE patients and 31 control subjects were recruited for this study.

The mean age of SLE patients was (30±9.57) years, while for control subjects was (29±9.44) years, and female to male ratio was (12:1), oral ulcerations was found in 24(46.15%) SLE patients, most of patients were with multiple oral ulcers (54.17%) and with non-painful oral ulcer (62.5%). The mean of serum level of VEGF was found significantly higher (p value 0.001) in SLE patients (142.89±24.37 pg/ml) than control (79.61±12.71pg/ml), and significantly higher in non-painful oral ulcerations than with painful ulcerations (p value 0.04). No significant differences in the level of serum VEGF-A found between patients with oral ulceration (P+OU) and patients without oral ulceration (P-OU). Oral ulceration associated with SLE usually painless, multiple, nonspecific, and most common sites were tongue and labial mucosa. Serum VEGF-A in SLE patients was significantly higher than in than control.

Keywords: Oral ulcerations, Systemic lupus erythematosus, vascular endothelial growth factors


INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease with a highly variable course and prognosis, may involve several tissues and organs and genetically is a complex rheumatic disease with heterogeneous clinical manifestations [1,2]. The etiology of SLE is unknown, but the highly variable clinical expression of SLE is the result of its complex immunopathology involving the production of autoantibodies and immune complex vasculitis with endothelial cell damage. There are certain risk factors have been identified e.g. genetic, environmental and hormonal factors may related to the disease [3].

The SLE occurs more common in women at childbearing age and tends to be more severe in black and Asian persons, with incidence rate around the world approximately 1 to 10 per 100,000 person-years, while the
prevalence rates range from 20–70 per 100,000 person-years with a wide range of age from children to the elderly and a narrower gender distribution, peak female-to-male ratio of 12:1 [4].

Oral ulcers are the most common mucosal sign in (SLE). The oral ulcers (OU) are one of the key clinical features; however, the terminology of oral ulcers, especially in SLE patients, is often vague and ill-defined [5].

Oral ulcerations or ulcers in the setting of SLE have long been considered to be predictors of systemic vasculitis and worse prognosis [6]. It is the most common mucosal sign in (SLE), and are one of the key clinical features; however, the terminology of oral ulcers, especially in SLE patients, is often vague and ill-defined [5].

Oral manifestations of SLE are frequently encountered, and may include oral ulceration, honeycomb plaque, raised keratotic plaque, nonspecific erythema, and cheilitis [7].

The oral ulcers occur at buccal mucosa, palate or tongue without other causes (e.g., Infection) [8]. Moreover, some ulcers (e.g., palatal erythematous ulcers and aphthous ulcers) occur particularly when disease is active. However, other types of ulcers may appear without being related to disease activity and severity [9].

It is of great importance to emphasize early detection of these lesions as a mean of diagnosis of SLE and early initiation of treatment [10].

Vascular endothelial growth factor (VEGF) is a cytokine-stimulating angiogenesis. It has been suggested to play a role in inflammation and pathogenesis of vasculitis processes and may play a role during the course of oral aphthous lesions in Behçet's disease (BD) [11].

The VEGF family plays an integral role in angiogenesis, lymphangiogenesis, and vasculogenesis. The human VEGF family consists of five members: VEGF (or VEGF-A), VEGF-B, VEGF-C, VEGF-D, and placental growth factor (PIGF). Each of these proteins contains a signal sequence that is cleaved during biosynthesis [12].

The VEGF-A, also known as vascular permeability factor (VPF), is a signal protein produced by cells that stimulates the formation of blood vessels. The VEGF-A is a sub-family of growth factors, the platelet-derived growth factor family of cystineknot growth factors. They are important signalling proteins involved in both vasculogenesis and angiogenesis, so it is part of the system that restores the oxygen supply to tissues when blood circulation is inadequate [13]. Angiogenesis plays a significant role in the pathogenesis of systemic lupus erythematosus [14].

One of the key players in the process of new vessel formation is VEGF-A [15]. Several studies have reported elevated levels of VEGF-A in the plasma and serum of SLE patients, and the high VEGF-A levels may be associated with the disease activity in SLE [16,17].

In addition to association of VEGF-A levels with disease activity in SLE, it was also found to be correlated with other disease manifestations as lupus nephritis, and with higher mean carotid intima media thickness [18].

MATERIALS AND METHODS

A case-control study was included "52" Iraqi patients from Babylon Province with (SLE) and "31" of apparently healthy subjects.
All patients with (SLE) and control subjects were examined by using dental mirror and probe with artificial light. Oral examination was done for each subject, detection of oral mucosal ulceration at time of investigation and their features such as “duration, number, location of lesion”, was done for each subject involved in this study.

Five milliliters of venous blood were taken from anti-cubital vein from each subject in this study, and then permitted to clot at room temperature for 2 hours before centrifugation for “15 minutes at 3000×g” and the remaining sera stored frozen at -20 ºC in polyethylene tubes until analyzed. Blood collection tubes should be disposable, non-pyogenic, and non-endotoxic.

The estimation of human VEGF-A in the samples was done by using Sandwich-ELISA principle. In which the micro ELISA plate has been pre-coated with an antibody specific to Human VEGF-A.

RESULTS

Eighty three subjects were incorporated in this study; they were divided into two groups

1- Fifty two SLE patients.

2- Thirty one control subjects (without signs and symptoms of any systemic disease).

Regarding oral ulceration in SLE patients, it has been shown that: Twenty four (46.15%) SLE patients were with oral ulceration (P+OU). Twenty eight (53.84%) SLE patients were without oral ulceration (P-OU). (table 1).

<table>
<thead>
<tr>
<th>Age range</th>
<th>P+OU(24)</th>
<th>P–OU (28)</th>
<th>χ²</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;20 years</td>
<td>5(20.83%)</td>
<td>0</td>
<td>6.92*</td>
<td>0.03</td>
</tr>
<tr>
<td>20-40 years</td>
<td>14(58.33%)</td>
<td>23(82.14%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;40 years</td>
<td>5(20.83%)</td>
<td>5(17.85%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*significant at p<0.05

According to the age categories the highest number and percentage of SLE patients with oral ulcer (P+OU) 14(58.33%) and 23 (82.14%) of patients without oral ulceration (P-OU) was found at age range of (20-40) years old. Statistical analysis showed a significant difference within SLE subgroup at (p value 0.03) table (2).
In the current study the total number of SLE patients was 52, 48(92.3%) females and 4 (7.69%) male. Table (3) showed that there was a highly significant difference between females and male (p value 0.003) and female to male ratio was 12:1.

Table 3: The percentage male and female SLE patients

<table>
<thead>
<tr>
<th>Gender</th>
<th>Male</th>
<th>Female</th>
<th>χ²</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLE patients</td>
<td>4(7.69%)</td>
<td>48(92.31%)</td>
<td>8.99*</td>
<td>0.003</td>
</tr>
</tbody>
</table>

*significant at p<0.05

Clinical findings in control subjects and SLE patients according to gender, malar rash and oral ulcerations (table 4).

Table 4: Clinical finding of study groups

<table>
<thead>
<tr>
<th>Categories</th>
<th>Controls</th>
<th>P+OU</th>
<th>P-OU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>5(16.12%)</td>
<td>3(12.5%)</td>
<td>1(3.57%)</td>
</tr>
<tr>
<td>Female</td>
<td>26(83.87%)</td>
<td>21(87.5%)</td>
<td>27(96.42%)</td>
</tr>
<tr>
<td>Single oral ulcer</td>
<td>N/P</td>
<td>11(45.83%)</td>
<td>N/P</td>
</tr>
<tr>
<td>Multiple oral ulcer</td>
<td>N/P</td>
<td>13(54.17%)</td>
<td>N/P</td>
</tr>
<tr>
<td>Painful oral ulcers</td>
<td>N/P</td>
<td>9 (37.50%)</td>
<td>N/P</td>
</tr>
<tr>
<td>Non painful oral ulcers</td>
<td>N/P</td>
<td>15(62.50%)</td>
<td>N/P</td>
</tr>
<tr>
<td>Malar Rash</td>
<td>N/P</td>
<td>5(20.83%)</td>
<td>2(7.14%)</td>
</tr>
</tbody>
</table>

NP: Not present

The OU was observed in different sites; more than one site was recorded in the present study. Figure (1) explains the distribution of patients according to site of oral ulcer; the tongue was more frequent than other sites (figure 1).
Regarding serum VEGF-A levels among study groups, it was significantly higher (p≤0.001) in SLE patients than in controls, and was significantly higher (p≤0.05) in patients with malar rash and in those with non-painful ulcers (table 5).

Table 5: Means differences of VEGF-A levels in study groups

<table>
<thead>
<tr>
<th>Category</th>
<th>VEGF-A  pg/ml mean±SD</th>
<th>T test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control subjects</td>
<td>79.61±12.71 142.89±24.73</td>
<td>3.04**</td>
<td>0.001</td>
</tr>
<tr>
<td>SLE patients</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SLE males</td>
<td>136.15±50.05 143.46±128.56</td>
<td>0.22</td>
<td>0.41</td>
</tr>
<tr>
<td>SLE females</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Regarding VEGF-A levels between P+OU and P-OU showed no significant differences (table6).

Table 6: Means differences of VEGF-A level between P+OU and P-OU.

<table>
<thead>
<tr>
<th>Groups</th>
<th>P+OU mean±SD</th>
<th>P-OU mean±SD</th>
<th>T test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGF-A pg/ml</td>
<td>144.86±20.22</td>
<td>140.67±29.25</td>
<td>0.11 (NS)</td>
<td>0.45</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Oral ulcer (OU) may be one of the important symptoms in SLE that changes with disease activity [6] mentioned that ulcerations of the oral cavity in the long setting of SLE have been considered as predictors of systemic vasculitis and worse prognosis, while [19,20] stated that oral features of SLE are frequently encountered and may include oral ulceration, raised keratotic plaque, honeycomb plaque, and nonspecific erythema, finally the involvement of oral mucosa is frequently seen in (CLE) and (SLE) regarding the prevalence, a wide range of lesions has been reported [21].

The results of this study showed that female to male ratio was 12:1 in SLE, these results agree with others [22] in a large cohorts study conducted in UK with a total of 924 SLE patients were involved found the ratio was 13:1, A study done by [23] in Cairo Egypt of 60 SLE patients male was 4(6.66%) and female was 56(93.33%), other found women are estimated to be six to ten times more likely to develop SLE than man [24], finally a study done in Algeria found female to male ratio: 10: 1 [25]. These differences between female to male in the incidence of disease may be related to the hormonal role of estrogen and other gonadal hormones which have complex effects on the immune system, also the disease activity increases during pregnancy, a role for female hormones has been proposed. The gene dose effect of the X chromosome that is the presence of two X

**Highly significant at p≤0.001**

**Significant at p≤0.05**
chromosomes in the female while one X chromosome present in males. As the X chromosome carries immunological related genes, which could be mutated and contribute to the onset of SLE, while the Y chromosome has no identified mutations associated with autoimmune disease \[26\].

The results of present study showed that oral ulceration was found in 46.15\% of SLE patients encountered in this study while 53.84\% without oral ulcer and this agree with \[10\] they found the prevalent of oral ulceration was (28.1\%) from 188 SLE patients, There were more than one sites in the oral cavity affected by ulceration including palate, buccal mucosa, tongue, labial mucosa and lip, these finding agree with \[27\] the classical lesions in SLE patients were ulcers of the hard palate and with cutaneous lupus erythematosus lesions of the buccal mucosa, lips, or soft palate, While other nonspecific oral ulcers comprise aphthous ulcers, lupus cheilitis and other types of oral lesions \[22\], another author mentioned that from 26 SLE patients with oral ulcerations there is one case with ulcer plaques of lower lip and buccal mucosa and two cases with ulcer lesions of tongue, lips and buccal mucosa \[26\].

Our preliminary results indicate that serum VEGF-A was increased in SLE patients, the study reveals that there was highly significant difference (p ≤0.001) was found between control subjects and SLE patients with and without oral ulcer (144.86±20.22 pg/ml and 140.67±29.25 pg/ml) respectively, these findings agree with other studies also showed elevation in the level of VGEF-A occur in SLE patients compared to normal, a study conducted in Egypt reported that the VEGF-A was significantly higher in the SLE patients than control with (p value 0.0001) \[26\], also \[28\] they mentioned that VEGF-A in SLE was found to be significantly increased compared to control. Over all, the VEGF-A is a key modulator of angiogenesis, endothelial cell proliferation and migration, capillary permeability, and it has been reported that elevated serum levels are found in chronic inflammatory rheumatic diseases, including SLE, compared to healthy controls, as well as a direct correlation between disease activity and its serum level \[29\].

Up to the beast of our knowledge these differences in the level of VGEF-A may not specifically related to the site of oral ulcer rather than may related to the general condition of the patient (age, gender) or SLE activity at time of investigation.

**CONCLUSION**

Oral ulceration associated with SLE usually painless, multiple, nonspecific, and most common sites were tongue and labial mucosa. Serum VEGF-A in SLE patients was significantly higher than in than control.

**ETHICAL CLEARANCE**

The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

**CONFLICT OF INTEREST**

The authors declare that they have no conflict of interest.
FUNDING: Self-funding

REFERENCES


