Salivary and serum levels of tumor necrosis factor-alpha in oral lichen planus: a systematic review and meta-analysis study

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Objective. Tumor necrosis factor-α (TNF-α) has a role in the progression of the oral lichen planus (OLP). The aim of this meta-analysis study was to evaluate the salivary and serum TNF-α levels in patients with OLP.

Study Design. We searched in the databases of PubMed/Medline, Science direct, Scopus, Web of Science, and Cochrane Library for studies reported from 1983 to 2016. All studies were checked for evaluation of salivary and serum levels of TNF-α in patients with OLP compared with healthy controls.

Results. Twelve studies were included in the meta-analysis. The mean difference of 7 studies reporting salivary TNF-α levels in patients with OLP versus healthy controls was 25.90 pg/mL (95% confidence interval [CI] 15.31-36.49; \(P < .00001\)) and 7 studies reporting serum TNF-α levels was 1.65 pg/mL (95% CI = -0.82 to 4.11; \(P = .19\)).

Conclusions. In patients with OLP, the higher levels of TNF-α in saliva compared with serum suggest that measurement of this marker in saliva may be more useful than in serum for determining diagnostic and therapeutic aims. (Oral Surg Oral Med Oral Pathol Oral Radiol 2017;124:e183-e189)

Oral lichen planus (OLP) is a chronic inflammatory disease with a greater incidence in women and a different age range in around the world.1 The prevalence of OLP is around 1% to 2% in the general population.1 The exact etiology of OLP is unclear, but the immunologic system plays a significant role.2 Nuclear factor-kB (NF-kB) as a primary transcription factor controls the expression of a series of cytokines, including tumor necrosis factor-α (TNF-α),3 and as a major mediator of inflammatory actions directed toward both tissue destruction and recovery. TNF-α also stimulates fibroblast growth while inducing death of diseased cells at the site of inflammation.4 TNF-α has multiple stimulatory activities on activated T cells, including increasing the proliferation, interleukin-2 receptor (IL-2R) expression, and the response to IL-2 stimulus. These effects on T cells should upregulate T-cell activation.5 Therefore, tissue TNF-α expression increases with activation of the innate immune response.6 The high concentration of TNF-α has a role in the progression of the pathologic events in OLP,7 and the serum level of this proinflammatory cytokine is associated with various risk factors, but little is known about the genetic background and the complex interactions.8 A number of studies reported in the English language literature that have measured TNF-α levels in the saliva and serum of patients with OLP have shown different results. Hence, the aim of this meta-analysis was to evaluate the salivary and serum TNF-α levels in patients with OLP and to determine whether saliva can be used as an alternative to serum in evaluating TNF-α levels in these patients.

MATERIALS AND METHODS

Search strategies
We used the keywords “oral lichen planus” and “OLP” combined with “TNF-α” and “tumor necrosis factor” in our search of databases including PubMed/Medline, Science direct, Scopus, Web of Science, and Cochrane Library for English language publications from 1983 to November 24, 2016.

Study selection
Two reviewers (MS and HRM) independently assessed the studies to determine if they met the inclusion criteria, and where there was disagreement, a third
reviewer (MR) resolved the disagreement. Studies were included if they (1) were case-control studies reported in the English language literature; (2) assessed TNF-\(\alpha\) levels in the serum or saliva of patients with OLP; (3) diagnosed OLP on the basis of clinical methods, histologic methods, or both according to the criteria set forth by the World Health Organization\(^9\); (4) reported that the healthy controls did not have OLP and any other cutaneous dermatologic or systematic disease; and (5) reported that the patients with OLP had no other dermatologic or systematic disease.

**Data extraction**

The relevant data extracted from all the studies included the name of the author(s), year of publication, country, number of patients with OLP, number of healthy controls, male/female ratio for both groups, age range of both groups (mean \(\pm\) SD), and levels of TNF-\(\alpha\) in two groups (mean \(\pm\) SD). TNF-\(\alpha\) levels in serum and saliva were measured using enzyme-linked immunosorbent assay performed with a human TNF-\(\alpha\) high-sensitivity kit, and different units of measurement were used across studies (pg/mL or ng/L), except for the study by Gallab et al.\(^{10}\) in which BD Cytometric Bead Array Th1/Th2 Human kit (BD BioSciences, Franklin Lakes, NJ) was used. If the data were presented using the standard error (SE), then the following formula was used to calculate SD:

\[
SE = \frac{SD}{\sqrt{N}}
\]

where \(N\) = sample size.\(^{11}\)

**Quality evaluation**

Two reviewers (MS and MR) independently evaluated the quality of each included study using the Newcastle-Ottawa Quality Assessment Scale to determine the quality of selection of study participants, comparability, exposure, and outcome, with a maximum score of 9 points. The quality of studies was divided into 3 categories: (1) high quality (total score: 7-9); (2) moderate quality (total score: 4-6); and (3) low quality (total score: 0-3). Disagreements were resolved through bilateral discussion.\(^{12}\)

**Statistical analyses**

We analyzed the data (a random-effects model) with Review Manager 5.3 (RevMan 5.3, The Cochrane Collaboration, Oxford, UK) with mean difference (MD), weighted MD, and 95% CIs. We also used Comprehensive Meta-Analysis software version 2.0 (CMA 2.0) with a random effects model of bias. The MD of the studies was calculated for to estimate of TNF-\(\alpha\) levels in the serum or saliva of patients with OLP compared with that of healthy controls in the meta-analysis. \(Q\) and \(I^2\) statistics were used for heterogeneity between estimations; for the \(Q\) statistic, heterogeneity was considered for \(P < .1\). \(P\) value (2-tailed) < .05 was considered statistically significant in this meta-analysis. Publication bias was assessed through funnel plot analysis with Begg’s and Egger’s tests based on difference in means.

**RESULTS**

A total of 348 studies were identified using the database search strategy. Of these, 35 studies were eligible for evaluation, and 23 of those were excluded from the study for various reasons (Figure 1). Thus, 12 studies were included in the meta-analysis.

**Study characteristics**

The characteristics of the studies included in the meta-analysis are presented in Table 1. The studies were published between 1994 and 2012. Five studies reported TNF-\(\alpha\) levels in serum,\(^{13-17}\) 5 studies in saliva,\(^{10,18-21}\) and 2 studies in both samples.\(^{22,23}\) Three studies were from the United States,\(^{18,19,21}\) 2 studies from China,\(^{20,23}\) and 1 study each from Taiwan,\(^{15}\) Egypt,\(^{10}\) Turkey,\(^{13}\) Greece,\(^{14}\) Iran,\(^{16}\) Japan,\(^{17}\) and Belgium.\(^{22}\) The reported mean age of patients with OLP in the studies was 45 to 61.2 years (range 18-78 years), and the mean age of controls was 34.9 to 59 years (range 18-74 years). The male/female ratio of patients with OLP\(^{10,13,14,17-19,21}\) and controls\(^{10,13,14,17-19,21}\) was \(\leq 1\). One study\(^{20}\) had Chinese full text, which could not be translated fully, and therefore, we did not have information about gender and age.

**Meta-analysis of TNF-\(\alpha\) levels in patients with OLP and in healthy controls**

The results of pooled estimates of 7 studies reporting TNF-\(\alpha\) levels in the saliva of patients with OLP compared with that of healthy controls were MD = 25.90 pg/mL with 95% CI 15.31-36.49 and \(P < .00001\). However, significant statistical heterogeneity was found across studies \((I^2 = 98\%;\ P < .00001)\) (Figure 2). Results of the pooled estimates of all studies reporting TNF-\(\alpha\) levels in serum patients with OLP compared with that in healthy controls were MD = 1.65 pg/mL with 95% CI –0.82 to 4.11 and \(P = .19\). However, significant statistical heterogeneity was found across studies \((I^2 = 94\%;\ P < .00001)\). The overall results of pooled estimates of all studies were MD = 11.89 pg/mL; 95% CI 8.50-15.29; \(P < .00001\), and statistical heterogeneity was found across all studies \((I^2 = 98\%;\ P < .00001)\).
Number of patients and controls in meta-analysis
This meta-analysis included TNF-α levels in the saliva of 164 patients with OLP and 182 controls and also TNF-α levels in the serum of 356 patients with OLP and 242 controls.

Quality evaluation
Table II shows the quality score for each study included in the meta-analysis. There was agreement on the elements of the quality scores between the 2 examiners. The mean quality score of 2 reviewers of 12 studies was 6.25 (moderate quality). The study by Liu et al.20 had Chinese full text and could not be translated, and therefore, we used minimum total score (total score = 4).

Publication bias
Figure 3 shows the symmetric funnel plot for all studies. Begg’s and Egger’s tests did not reveal a significant evidence of publication bias among the included studies (Begg’s test: $P = .273$; Egger’s test: $P = .050$).

DISCUSSION
The immunologic system plays a significant role in OLP.24 Therefore, this study evaluated the levels of TNF-α as a factor in immune and inflammatory responses. A random-effects model was used in this meta-analysis to calculate the pooled estimates and the heterogeneity between studies. Saliva10,18,19,21-23 and serum TNF-α levels14-16,22,23 were significantly

Fig. 1. The flow chart of study.
increased in patients with OLP compared with healthy controls; however, the study by Liu et al. did not show a significant correlation for saliva levels, and the study by Pekiner et al. did not show a significant correlation for serum levels. Furthermore, Yamamoto et al. found serum TNF-α levels significantly decreased in patients with OLP compared with healthy controls. The pooled MD showed that TNF-α levels in the saliva and serum of patients with OLP were higher compared with those in healthy controls and that this difference in saliva levels was significant (saliva: 25.90 pg/mL; P < .00001 vs serum: 1.65 pg/mL; P = .19; TNF-α levels in saliva were around 24.25 pg/mL more than in serum).

In this meta-analysis, the higher levels of TNF-α in saliva compared with those in serum suggest that measurement of this marker in saliva for treatment purposes may be more useful than measurements of this marker in serum. Saliva offers some advantages over serum because it can be collected noninvasively by individuals with modest training, and its analysis has demonstrated values of various biochemical and immunologic parameters comparable with those that are routinely assessed in serum. Saliva, as a body fluid, has important diagnostic value and potential to be used for tests measuring biologic markers that aid in the diagnosis of systemic and especially oral pathologies.

### Table 1. Characteristics of 12 studies included in meta-analysis

<table>
<thead>
<tr>
<th>First author of study, year</th>
<th>Country</th>
<th>Mean age/range of patients with OLP, years</th>
<th>Gender (M/F) of patients with OLP</th>
<th>Mean age/range of controls, years</th>
<th>Gender (M/F) of controls</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kaur, 2015</td>
<td>Belgium</td>
<td>NA/41-65</td>
<td>NA</td>
<td>NA/42-65</td>
<td>NA Sal, Ser</td>
<td></td>
</tr>
<tr>
<td>Liu, 2011</td>
<td>China</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA Sal</td>
<td></td>
</tr>
<tr>
<td>Pekiner, 2012</td>
<td>Turkey</td>
<td>51.1/NA</td>
<td>9/21</td>
<td>48.1/NA</td>
<td>12/18</td>
<td>Ser</td>
</tr>
<tr>
<td>Rhodus, 2005</td>
<td>USA</td>
<td>57/42-68</td>
<td>0/13</td>
<td>58/40-74</td>
<td>0/13</td>
<td>Sal</td>
</tr>
<tr>
<td>Rhodus, 2005</td>
<td>USA</td>
<td>57/NA</td>
<td>4/9</td>
<td>59/NA</td>
<td>3/10</td>
<td>Sal</td>
</tr>
<tr>
<td>Rhodus, 2006</td>
<td>USA</td>
<td>57.2/28-78</td>
<td>0/13</td>
<td>Matched</td>
<td>0/13</td>
<td>Sal</td>
</tr>
<tr>
<td>Sklavounou-Andrikopoulou, 2004</td>
<td>Greece</td>
<td>47.5/NA</td>
<td>8/18</td>
<td>37.8/NA</td>
<td>11/15</td>
<td>Ser</td>
</tr>
<tr>
<td>Sun, 2007</td>
<td>Taiwan</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA Ser</td>
<td></td>
</tr>
<tr>
<td>Taghavi Zenouz, 2012</td>
<td>Iran</td>
<td>Matched/18-60</td>
<td>NA</td>
<td>Matched/18-60</td>
<td>NA Ser</td>
<td></td>
</tr>
<tr>
<td>Yamamoto, 1994</td>
<td>Japan</td>
<td>61.2/25-82</td>
<td>6/20</td>
<td>34.9/25-48</td>
<td>10/10</td>
<td>Ser</td>
</tr>
<tr>
<td>Zhang, 2008</td>
<td>China</td>
<td>45/28-62</td>
<td>16/14</td>
<td>40/24-56</td>
<td>15/15</td>
<td>Sal, Ser</td>
</tr>
</tbody>
</table>

NA, not applicable; OLP, oral lichen planus; Sal, saliva; Ser, serum.
Malarkodi et al.\textsuperscript{27} evaluated salivary and serum TNF-\(\alpha\) levels in patients with OLP and in healthy controls. Although the mean value of salivary and serum TNF-\(\alpha\) in patients with OLP was higher than that of the control group, with statistical significance, expressions of TNF-\(\alpha\) in both test specimens were almost equal, suggesting that saliva can be a good alternative to serum to analyze TNF-\(\alpha\) levels in patients with OLP. Simark et al.\textsuperscript{28} detected augmented messenger RNA levels of TNF-\(\alpha\) in OLP tissues using polymerase chain reaction and showed that TNF-\(\alpha\) could play a crucial role in the pathogenesis of OLP leading to more specific and effective therapies. Al-Mohaya et al.\textsuperscript{29} concluded the polymorphisms of the TNF-\(\alpha\) gene were significantly associated with the risk of OLP susceptibility.

Tanni et al.\textsuperscript{30} investigated the association between smoking status and TNF-\(\alpha\) in patients with chronic obstructive pulmonary disease and showed that smoking may influence TNF-\(\alpha\) level—mediated systemic inflammation. Singh et al.\textsuperscript{31} evaluated the effect of smoking on salivary TNF-\(\alpha\) levels in patients with chronic periodontitis and concluded that smoking affected salivary TNF-\(\alpha\) levels. Another study by Petrescu et al.\textsuperscript{32} reported that serum TNF-\(\alpha\) levels were significantly higher in healthy heavy smokers compared with healthy nonsmokers.

Gavala et al.\textsuperscript{33} reported that alcohol can inhibit the levels of TNF-\(\alpha\) in serum. Their data showed that salivary TNF-\(\alpha\) concentration varied in different clinical types of OLP, being particularly elevated in the erosive/atrophic form of the disease. It is obvious that enhanced TNF-\(\alpha\) production of saliva reflects clinical changes and correlates with the severity of OLP. Chandrashekara et al.\textsuperscript{34} explained that psychological stress could influence the immune system and reduce TNF-\(\alpha\) levels significantly. Schulz et al.\textsuperscript{3} suggested that age was significantly associated with plasma levels of circulating TNF-\(\alpha\).

Therefore, saliva and serum TNF-\(\alpha\) levels can be affected by age, type of OLP, stress, alcohol consumption, smoking, and genetics. Because these factors cannot be easily matched in both patients with OLP and healthy controls, there is heterogeneity among the study populations of the studies used in this meta-analysis.

Limitations of this meta-analysis include variable timing of saliva collection, different kits and methods of TNF-\(\alpha\) assays, variation in criteria for selection of healthy controls, varying type and severity of OLP across studies, lack of uniform matching of age and gender between patients with OLP and healthy controls.
and need to estimate mean value and SD in several studies.

CONCLUSIONS
In patients with OLP, the higher levels of TNF-α in saliva compared with serum suggest that measurement of this marker in saliva may be more useful than serum measurements for determining diagnostic and therapeutic aims. Confounding factors, such as age, type of OLP, stress, alcohol consumption, smoking and genetics, must be taken into consideration while interpreting results.

REFERENCES
30. Tanni SE, Pelegnino NR, Angeleli AY, Coorea C, Godoy I. Smoking status and tumor necrosis factor-alpha mediated


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