

## Original Article

# Salivary levels of interleukin-6 and tumor necrosis factor- $\alpha$ in patients undergoing hemodialysis

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## ABSTRACT

**Background:** Inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF- $\alpha$ ) are elevated in end-stage renal disease (ESRD). IL-6 and TNF- $\alpha$  are toxins which deteriorate renal function, and their pathogenic role has been confirmed in cardiovascular and oral diseases. This study was designed to investigate the salivary levels of IL-6 and TNF- $\alpha$  in patients with ESRD undergoing hemodialysis (HD).

**Materials and Methods:** Twenty patients with ESRD who were treated with 4 h HD sessions, with low flux membrane were included in this cross-sectional study. Average Kt/V index in patients was  $1.19 \pm 0.1$ . Twenty age-sex-matched healthy controls with no infectious diseases during 1 month before saliva sampling were selected. Unstimulated whole saliva was collected and TNF- $\alpha$  and IL-6, concentrations were measured using human IL-6 and TNF- $\alpha$  ELISA kits. Independent *t*-test was used to analyze the data using SPSS ( $\alpha = 0.05$ ).

**Results:** There was a significant difference between dialysis and control groups regarding the salivary levels of TNF- $\alpha$  ( $P = 0.034$ ) and IL-6 ( $P = 0.001$ ).

**Conclusion:** Considering the results of this study and reported role of inflammatory cytokines in the pathogenesis of cardiovascular and oral diseases, measurement of salivary IL-6 and TNF- $\alpha$  in HD patients may help in risk stratification of HD patients and in planning pertinent preventive strategies.

**Key Words:** Cytokine, hemodialysis, renal failure, saliva

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## INTRODUCTION

Chronic kidney disease (CKD) refers to the progressive loss of renal function.<sup>[1]</sup> Unfortunately, large numbers of patients have gone undiagnosed at earlier stages of CKD, which increases the risk for development of end stage renal disease (ESRD).<sup>[2,3]</sup>

Inflammatory response can reflect an underlying systemic disease.<sup>[4]</sup> Patients with CKD demonstrate

activation of inflammatory pathways,<sup>[5,6]</sup> which is accompanied by increased inflammatory markers like cytokines.<sup>[4]</sup> The elevated levels of cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF- $\alpha$ ) have been shown in ESRD.<sup>[7-10]</sup> Furthermore, these inflammatory markers act as toxins<sup>[11]</sup> that predict kidney function deterioration.<sup>[12]</sup>

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Patients with ESRD are at a greater risk of cardiovascular and oral diseases.<sup>[13]</sup> It has been shown that inflammatory markers such as IL-6 and TNF- $\alpha$  enhance the risk of cardiovascular mortality.<sup>[10]</sup> Furthermore, it is confirmed that oral diseases including oral cancer, lichen planus, and periodontal diseases are associated with IL-6 and TNF- $\alpha$  deregulation.<sup>[14]</sup> According to some previous studies, we can consider the merit of IL-6 measurement as a prognostic indicator and provide a clinical perspective on IL-6-blocking therapies in renal disease.<sup>[15]</sup>

To the best of our knowledge, little or no research has been performed so far to compare the salivary levels of IL-6 and TNF- $\alpha$  in patients undergoing hemodialysis (HD) with matched controls. Since, the prognostic value and pathogenic role of IL-6 and TNF- $\alpha$  have been confirmed in publications, the present study aimed to investigate the salivary levels of IL-6 and TNF- $\alpha$  in patients with ESRD undergoing HD.

## MATERIALS AND METHODS

The study subject population consisted of 20 dialysis patients who were diagnosed with end-stage renal disease caused by diabetic nephropathy and admitted at Nephrology Department of Isfahan University of Medical Sciences. The Ethics Committee of the University approved the study protocol and informed consent obtained prior to participation in this cross-sectional study.

### Patient characteristics

The study included 18 years or older patients who were treated with standard 4 h HD sessions, 3 times a week with low flux membrane and were stable by the time the study was conducted. The mean duration of dialysis was  $9 \pm 2.3$  months. The average Kt/V index in patients was  $1.19 \pm 0.1$  and the protein catabolism index was  $1.42 \pm 0.2$  g/kg/day. None of the patients suffered from any symptoms of infection or collagen vascular diseases. In all patients, cardiovascular diseases were assessed on the basis of the history of echocardiographic abnormalities. Patients with previous myocardial infarction, stable angina, stroke, arteriopathy and heavy smokers were excluded. Patients had not received any medications known to affect immune functions and the time period from the last blood transfusion was not shorter than 6 months. Furthermore, patients with unstimulated whole saliva

(UWS) flow rates of  $<0.1$  ml/min were considered as salivary gland hypo-function and excluded from the study.

Twenty age-sex-matched healthy controls with no infectious diseases during 1 month prior to saliva collection were selected from individuals visiting oral medicine clinic of Isfahan University of Medical Sciences.

Two groups were matched for gingival conditions according to modified gingival index (MGI).<sup>[16]</sup> MGI eliminates the use of probing or pressure to establish the presence or absence of bleeding. It also makes it possible to detect and record subtle visual changes in gingival inflammation, permits the intra- and inter-calibration of examiners, and is noninvasive upon repeated evaluations.<sup>[16]</sup>

### Saliva sampling

Participants were asked not to eat, drink, or use saliva stimulators such as chewing gum or mint for at least 1 h before sampling. UWS was collected between 10:00 and 11:00 a.m. using the standard technique.<sup>[9]</sup> Participants were asked to swallow first, and then tilt their head forward and expectorate all saliva into a 50 ml centrifuge tubes for 5 min without swallowing. The saliva samples were frozen at  $-70^{\circ}\text{C}$ , until analysis. All samples were centrifuged at 4500 g for 15 min. Supernatants were drawn off and used in the ELISA cytokine assays. TNF- $\alpha$  and IL-6, concentrations were measured using human IL-6 (sensitivity:  $<0.3$  pg/ml) and TNF- $\alpha$  (sensitivity:  $<1$  pg/ml) ELISA kits (Boster Biological Technology Co, CA, USA) according to manufacturer's recommendation.

### Statistical analysis

SPSS software version 18 (SPSS Inc., Chicago, IL, USA) was used to analyze the data. Distribution of the variables was examined for normality using Kolmogorov-Smirnov test. Since data were normally distributed, parametric independent *t*-test was used ( $\alpha = 0.05$ ).

## RESULTS

Forty patients were enrolled in the study. The mean age of the case and control groups were  $59 \pm 12.4$  and  $53 \pm 10.8$  years, respectively. There were 12 and 8 women in the case and control groups, respectively. Two groups were matched for MGI index (12 edentulous patients and 8 patients with

score 3). There were no significant gender and age differences between case and control groups.

The mean amounts of TNF- $\alpha$  and IL-6 are summarized in Table 1 and Graph 1.

ELISA analysis of whole unstimulated saliva showed that there was a significant difference between two groups regarding the salivary levels of TNF- $\alpha$  ( $P = 0.034$ ) and IL-6 ( $P = 0.001$ ).

## DISCUSSION

Cytokines are soluble proteins that play an important role in the initiation and maintenance of inflammatory and immune responses.<sup>[14]</sup> Many of prior studies have found that inflammatory cytokines such as IL-6 and TNF- $\alpha$  are elevated in ESRD,<sup>[17-19]</sup> and some studies report salivary cytokines and secretion rates are significantly decreased in CKD patients, and recommend further research.<sup>[20]</sup>

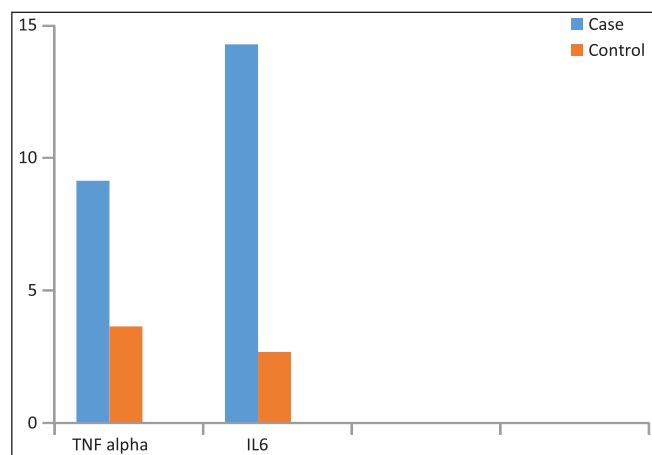
Furthermore, IL-6 and TNF- $\alpha$  are toxins which may deteriorate renal function by triggering endothelial dysfunction and glomerular injury<sup>[4]</sup> and act as independent risk factors for mortality.

We examined the salivary level of IL-6 and TNF- $\alpha$  in patients with ESRD undergoing HD. Our hypothesis

**Table 1: The mean amounts (pg/ml) of TNF- $\alpha$  and IL-6 in HD group and controls**

Salivary cytokine	HD group	Control group
IL-6	14.2 $\pm$ 2.08	2.68 $\pm$ 0.84
TNF- $\alpha$	9.09 $\pm$ 1.78	3.62 $\pm$ 1.19

HD: Hemodialysis; IL-6: Interleukin-6; TNF- $\alpha$ : Tumor necrosis factor-alpha.



**Graph 1:** The mean amounts (pg/ml) of tumor necrosis factor-alpha and interleukin-6 in hemodialysis group and controls.

was that there would be a significant difference in the salivary levels of these markers between HD and control groups. The results of the study confirmed our hypothesis. It was shown that salivary levels of IL-6 and TNF- $\alpha$  are significantly higher in patients with ESRD undergoing HD.

Saliva is a safe and low-cost alternative and as a biofluid, has several advantages over blood supporting its use in diagnosis and assessment of diseases.<sup>[21]</sup> Researches have shown that levels of some serum markers like C-reactive protein, IL-2, IL-6, and TNF- $\alpha$  correlate well with those in saliva.<sup>[22-24]</sup>

Elevated circulating levels of IL-6 in patients with congestive heart failure and active inflammation have been reported.<sup>[25,26]</sup> Therefore, for this study patients with any signs of cardiovascular diseases or infections were excluded. In this study, patients who received dialysis with low-flux polysulfone membrane were included. It is confirmed that the contact of blood with dialyzer membrane leads to degranulation of polymorphonuclear leukocytes and release of inflammatory mediators.<sup>[26,27]</sup> Furthermore, different studies assessed the back-leakage of endotoxin from the contaminated dialysate membrane, and it was found that high-flux dialyzers are the proposed mechanisms of monocyte stimulation and elevated inflammatory cytokines.<sup>[28,29]</sup> Therefore in the present study, patients received dialysis with low-flux polysulfone membrane.

It is now well established that high prevalence of inflammatory cytokines are associated with the high rate of cardiovascular morbidity and mortality in ESRD patients.<sup>[30]</sup> Increased levels of inflammatory biomarkers in ESRD can promote atherosclerosis and thrombosis.<sup>[31]</sup> These mechanisms may explain the high prevalence of cardiovascular disease among persons developing ESRD. Therefore, measurement of salivary cytokines levels may be considered as a noninvasive test for cardiac risk stratification in ESRD patients undergoing HD.

A number of oral diseases including lichen planus, oral cancer, and periodontal diseases have been reported to be associated with IL-6 deregulation.<sup>[14]</sup> Periodontal diseases are prevalent in renal dialysis patients who showed an unacceptable level of oral hygiene and its prevalence increases with the chronicity of the illness.<sup>[32]</sup> Available literature point toward the important role of IL-6 in the loss of periodontal ligament and alveolar bone through the tissue degradation effects of IL-6 on connective

tissue and bone, mediated by metalloproteinase and osteoclasts.<sup>[14]</sup> According to the results of this study, ELISA analysis of whole unstimulated saliva showed that difference between salivary levels of TNF- $\alpha$  and IL-6 was significant ( $P < 0.05$ ) and the elevated salivary levels of inflammatory cytokines may necessitate the need of comprehensive professional oral care and self-care instruction in dialysis patients.

One of our restrictions in this study was sample size and in this study the correlation between salivary and serum IL-6 and TNF- $\alpha$  was not investigated; hence, we suggest comparative analysis of salivary and serum cytokines in a larger group of patients.

We would like to suggest that commenting on the viability of saliva over blood is possible only when you do a comparative analysis for both.

Although our findings demonstrated that salivary inflammatory biomarkers are elevated in patients undergoing HD; however, the relation between salivary cytokine levels and the efficacy of HD has not been assessed. Furthermore, it is recommended to conduct further studies to investigate the prognostic value of salivary inflammatory cytokines in cardiovascular and oral diseases.

## CONCLUSION

Considering the significant elevation of salivary TNF- $\alpha$  and IL-6 in this study and reported role of inflammatory cytokines in the pathogenesis of cardiovascular and oral disease, our findings support the need for additional studies. If confirmed, measurement of salivary IL-6 and TNF- $\alpha$  in HD patient may be considered as a noninvasive test for cardiac risk stratification to help in risk assessment of HD patients, and in planning pertinent preventive strategies.

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### Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

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