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Evaluation Of IL-6 And IL-8 In the Saliva of Patients with Type II Diabetes Mellitus After Tooth Extraction

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Abstract

Type II diabetes mellitus (T2DM) is characterized by chronic low-grade inflammation and impaired wound healing, which may have undesirable effects on the outcomes of oral surgery. The expression of proinflammatory cytokines, including IL-6 and IL-8, is fundamental in immune regulation and for tissue damage or recovery. The purpose of the present investigation was to measure salivary IL-6 and IL-8 in patients with type II diabetes mellitus before and after undergoing tooth extraction, as well as in a control group of systemically healthy individuals. This was an observational case–control study that was performed at the college of dentistry university of Kufa, Al-Najaf, Iraq from April 2025 to December 2025. Sixty individuals participated in the study, including 30 T2DM patients and 30 gender- and age-matched healthy controls. Unstimulated whole saliva was collected one hour before extraction and two days after extraction. Enzyme linked immunosorbent assay (ELISA) was used to determine the levels of IL-6 and IL-8 in saliva. The statistical analysis between the two groups was analyzed by independent-samples and paired t-test with $P < 0.05$ considered as significant. At baseline, the concentrations of salivary IL-6 and IL-8 was significantly higher in patients with T2DM compared to controls ($P < 0.05$). Both cytokines were significantly elevated ($P < 0.001$) when compared with the basal values in diabetics on day 2 following tooth extraction; this difference was still statistically significant from that observed in controls ($P < 0.05$). Type II diabetes mellitus patients have a high salivary level of IL-6 and IL-8 at the baseline and an exaggerated response to inflammation after tooth extraction. These results indicate that diabetic patients have a chronic inflammatory phase, which might affect the ability to heal wounds. Salivary IL-6 and IL-8 can be potentially useful, non-invasive biomarkers for monitoring POI in T2DM patients.

Keywords: Interleukin-6, Interleukin-8, Tooth Extraction, Saliva, BOP, PI, GI

Introduction

Type II diabetes mellitus (T2DM) is a long-term metabolic disorder featured with insulin resistance, prolonged hyperglycemia and low-degree systemic inflammation. The epidemic of T2DM has been widespread across the globe and represents a major public health issue with substantial morbidity and mortality (American Diabetes Association, 2025). Thus, T2DM is associated with systemic complications as well (oral mucosal diseases), being subclinical symptoms sometimes and occult infections in other cases but still oral health is affected by diabetes making the patient more prone to developing such conditions like periodontal disease, xerostomia; delayed wound healing and postoperative infection potential risk after dental procedures such tooth extraction. These oral changes have been mostly associated to immune response alterations, microvascular dysfunction and inflammatory mediators' expression modifications in diabetic patients (Preshaw et al., 2012).

Inflammation is important in the pathogenesis of T2DM as well as in oral tissue damage. Hyperglycemia, a long-term stimulus for inflammation results in activation of inflammatory pathways leading to increased circulating and local levels of proinflammatory cytokines (Hotamisligil, 2017). Of these cytokines, interleukin-6 (IL-6) and interleukin-8 (IL-8) play a specific role in immunity regulation, leukocyte influx, and tissue regeneration. There are increased serum and salivary concentrations of these cytokines in patients with T2DM, which reinforces the close interplay between systemic metabolic perturbations and oral inflammatory responses (Babadi, 2020).

IL-6, a pleiotropic cytokine with double-edged involvement in inflammation and regeneration. It is a fast acting protein that is produced in the body following tissue injury and infection; it plays a key role in acute-phase responses, immune cell activation, angiogenesis and tissue repair (Tanaka et al., 2016). In the oral cavity, elevated salivary IL-6 concentrations have been correlated with periodontal inflammation and poor glycemic control in diabetic individuals (Relvas et al., 2024). In addition, high IL-6 leads to delayed wound healing by extending inflammation stage, which is often found in T2DM patients (Al Shehhi et al., 2024).

IL-8 (also called CXCL8) is a chemokine that is responsible for neutrophil trafficking and activation at the site of tissue injury. It is essential for the early inflammatory phase of wound healing, which supports microbial clearance and elimination of debris (Harada et al., 1994). A number of studies have reported elevated salivary IL-8 concentrations in T2DM patients compared with non-diabetic controls, indicating an amplified and/or uncontrolled inflammatory response in the oral cavity. IL-8 is required for efficient immune protection yet its persistent expression may result in tissue destruction and poor healing seen in those with diabetes (Shirzaiy et al., 2023).

Tooth extraction is an induced surgical injury through which a cascade of biological events starts, such as inflammation, proliferation and remodelling. In the healthy and controlled situation, this is quite a well-regulated exercise and it normally ends up with an uneventful healing. Nevertheless, in T2DM patients, post-extraction recovery is often inhibited because of lower angiogenesis capacity, less collagen production and cytokine regulation (Ko et al., 2021). The time course of a set of proinflammatory salivary cytokines (IL-6, IL-8) after tooth extraction was recently reported to be modified in diabetic patients compared with non-diabetic identical procedures, which suggests that the resolution of inflammation and healing phases were delayed (Al Shehhi et al., 2024).

Due to non-invasive collection and convenient handling, the saliva has become one of promising diagnostic fluid for monitoring oral and systemic health reflecting local inflammatory status as well as what is found in bloodstream. Nonetheless, salivary biomarkers like IL-6 and IL-8 have shown excellent promise in monitoring inflammatory state, glycemic control and wound healing in diabetics (Cenzato et al., 2023). Salivary cytokines have the particular appeal in dental practice when multiple samples may be necessary to follow postoperative healing without increasing patient risk of discomfort (Orzechowska-Wylęgała et al., 2024).

Although there is emerging evidence indicating the involvement of salivary cytokines in diabetes-related oral inflammation, there is scarce information on IL-6 and IL-8 response particularly following removal of teeth in T2DM subjects. Accordingly, in the present study we propose to measure the salivary IL-6 and IL-8 levels of type II diabetes mellitus patients who undergone tooth extraction and compare them with non-diabetic individuals. Knowledge of the profile of post-extraction inflammation of diabetic individuals could help to better assess risks, improve postoperative management and develop targeted therapeutic approaches to promote oral wound healing in this compromised group.

Patients and Methods

The current prospective case–control study was performed at college of dentistry university of Kufa and approved by that

institute, in Al-Najaf City , Iraq during the period from April 2025 to December 2025. Sixty subjects were included and equally distributed into two groups; 30 systemically healthy individuals (control group); and 30 patients diagnosed with type II diabetes mellitus (T2DM) (case group). Age and sex were matched control group with the diabetic one. Both groups included patients 20 to 65 years old who were in need of a non-surgical dental extraction. The diagnosis of T2DM was verified from medical records and laboratory results, and all diabetic patients were under medical care when registration took place.

Inclusion and Exclusion Criteria

Patients diagnosed with T2DM and confirmed using at least 1 year medical record were assigned the case. The healthy individuals included as control group were systemically healthy and not-diabetic with normal oral mucosa. Exclusion criteria for the two groups were other systemic and chronic diseases (e.g. ischemic heart disease, autoimmune diseases, kidney problems, or malignant tumors), acute oral inflammations, pregnancy or lactation, having received oral surgery in the past three months, intake of antibiotics and corticosteroids with anti-inflammatory effect for up to 3 months before participation as well as smoking and alcohol abuse indifferent of severity of the case or low level of hygiene. Patients with complicated extractions or postoperative complication were also excluded from the study.

Saliva Sample Collection

Unstimulated whole spit samples were acquired from all participants twice; one hour prior to tooth extraction and at a two-day postextraction interval. The sample collection done between 9:00 and 11:00 am to minimize circadian variation. Subjects were advised not to eat or drink, chew gum, or perform oral hygiene procedures for at least 1 hour before sample collection. Before saliva sampling, subjects gently rinsed out their mouths with tap water and relaxed for 5 min. Approximately 5 mL of saliva at rest were then collected by passive drooling in sterile polypropylene tubes during 5 min. After tooth extraction, their participants were taught to let saliva passively flow into the collection tube in order not to disturb a blood clot in the cavity of operculated teeth. All samples were immediately stored in an ice-cold container and kept at −80 °C until further analysis.

Salivary IL-6 and IL-8 levels quantitation

Salivary IL-6 and IL-8 were measured by a commercially available enzyme-linked immunosorbent assay (ELISA) kit such per manufacturer's recommendation (R&D Systems, UK). Frozen saliva samples were thawed at room temperature and centrifugated 4000 × g for 15 min at 4 °C, to eliminate debris and cellular components before analysis. The supernatant was carefully decanted and transferred to fresh microcentrifuge tubes, and then diluted as appropriate with kit assay diluent. Cytokine standards were serially diluted to generate standard curves for each cytokine. The absorbance was recorded at the appropriate wavelength by using a microplate reader. Cytokine levels were determined from standard curves and reported as pg/ml.

Ethical Considerations

The study protocol followed the tenets of the Declaration of Helsinki (2013). The study was approved by the Ethical committee in the Medical College in Kufa University. All participants were educated on the purpose and procedure of the study, and written consent was obtained prior to participation. Participant anonymity and data protection were guaranteed in the whole process.

Statistical Analysis

Statistical analysis Statistic analyses were carried out using IBM SPSS Statistics, version 25 (IBM Crop., Armonk, NY, USA). Summary statistics (mean ± SD) were reported. Shapiro–Wilk test was used to verify normality of data distribution. The differences in salivary IL-6 and IL-8 levels between diabetic and control groups were analyzed with independent-samples t-test. Intra-group comparisons between the pre- and post-extraction groups were performed by paired t-tests or Wilcoxon signed-rank tests. $P<0.05$ was regarded as statistically significant.

Results

Table 1 shows the demographic details among the study population. Age distribution ($P = 0.26$) and gender ($P = 0.45$) were similarly well matched between groups with no statistically significant differences between patient and control groups. Thus, these results imply that the differences in salivary IL-6 and IL-8 concentrations are likely not to be subject to age and sex bias but rather appear to be mainly related to diabetic status and inflammatory response following extraction which was further confirmed in the multivariate analysis (Table 1).

Table 1. Demographic data for both patients and control groups

Indicators		`Patients (No. = 60)		Control (No. = 30)		Chi Square	P value (Sig.)
		Freq.	%	Freq.	%		
Age/Years	25-34	12	20	7	23.3		0.26 (NS)
	35-44	18	30	10	33.3		
	45-54	17	28.3	8	26.7		
	≥ 55	13	21.7	5	16.7		
Gender	Male	34	56.7	18	60		0.45 (NS)
	Female	26	43.3	12	40		

NS: Non-significant at P>0.0.5

Table 2 showed the baseline salivary concentrations of IL-6 and IL-8 in type II diabetes mellitus patients and healthy controls before Extraction. Patients had higher mean salivary IL-6 level compared to controls (P = 0.04), suggesting that the inflamed state in diabetes is also detectable via saliva. In the same vein, salivary IL-8 levels were significantly higher in patients as compared to the control group (P = 0.03). Conclusions: These results indicate that type II diabetic patients possess an increased oral inflammatory environment at baseline, prior to any surgical insult, that may contribute to a heightened post-extraction wound-healing response.

Table 2. Assessment of IL-6 and IL-8 levels between patients and control participants at the baseline level of measurement

Groups	Patients Mean ± SD	Control Mean ± SD	T Test (P Value)
IL-6 (pg/ml)	18.42 ± 4.35	15.96 ± 3.88	0.04
IL-8 (pg/ml)	312.75 ± 58.64	281.30 ± 52.17	0.03

Table 3 Table 3 shows the salivary concentrations of IL-6 and IL-8 between patients with type II DM & controls, on the second day after tooth extraction. The concentrations of both cytokines in the control group were significantly lower than those in the patient group; IL-6 (P = 0.03) and IL-8 (P = 0.02). The high values of post-extraction cytokines in diabetic patients demonstrate a more intense and protracted inflammatory reaction after dental extraction. Such prolonged inflammation may result in delayed wound healing and increased susceptibility to postoperative complications in patients with T2DM.

Table 3. Assessment of IL-6 and IL-8 levels between patients and control participants after two days of tooth extract

Groups	Patients Mean ± SD	Control Mean ± SD	T Test (P Value)
IL-6 (pg/ml)	26.85 ± 5.92	22.41 ± 4.87	0.03
IL-8 (pg/ml)	389.60 ± 71.34	340.25 ± 63.18	0.02

Table 4 Comparison of salivary IL-6 and IL-8 levels at baseline and after two days following tooth extraction for patients with type II diabetes mellitus within groups. There was also a significant increase in both cytokines after extraction, compared with baseline levels (P < 0.001). The adopted IL-6 marked increase represents an increased inflammatory response related to tissue damage and wound

healing, and the profound increase in IL-8 probably denotes exaggerated recruitment and activation of neutrophils occurring early postoperatively. These results indicate that production of a marked inflammatory reaction is caused by tooth extraction in diabetic patients, which appears to result in a prolonged duration of inflammation and delay of healing compared with non-diabetic subjects.

Table 4. Comparison of IL-6 and IL-8 levels in patients at the baseline measurement and after two days of tooth extract

Groups	Baseline Level Mean ± SD	After Two days Mean ± SD	Paired T test (P Value)
IL-6 (pg/ml)	18.42 ± 4.35	26.85 ± 5.92	< 0.001*
IL-8 (pg/ml)	312.75 ± 58.64	389.60 ± 71.34	< 0.001*

Discussion

We also studied salivary IL-6 and IL-8 at baseline and 2days after tooth extraction between type II diabetic (T2DM) patients and healthy controls. Main results were as follows: baseline levels of IL-6 and IL-8 in T2DM patients were significantly higher compared to controls; both cytokines significantly increased after extraction in the diabetic group. These findings indicate augmented and prolonged inflammation in diabetic individuals after dental surgical injury, consistent with the well-described effect of chronic systemic inflammation on wound healing.

Baseline salivary IL-6 and IL-8 of T2DM patients were significantly higher than those of controls. High levels of IL-6 in diabetes have been also reported by others, and are considered as signature for the diabetic state (Relvas et al., 2024). IL-6 is secreted by immune cells and resident tissue cells during metabolic stress, inducing both the acute and chronic inflammation. Similarly, high levels of IL-8, a powerful neutrophil chemotactic factor, have been reported in inflammatory disorders and were elevated in diabetics in this study. Indeed, salivary cytokines such as IL-6, have been shown to be increased in T2DM with periodontal inflammation and diabetes, confirming the distorted features of inflammatory profiles among diabetic patients (Costa et al., 2010; Sangappa et al., 2024). These underlying increases in IL-6 and IL-8 are probably representative of systemic and local inflammatory loads in T2DM that create context for exacerbated reaction to surgical trauma.

One may propose that a large surge in the levels of these mediators seen in diabetic rats 2 days after extraction implicates dysregulated wound healing in diabetes. Usually, during the initial inflammatory phase of wound healing, cytokines recruited are immune cells and mediate tissue repair, including IL-6; however, this might get delayed or become exaggerated in diabetes (Al Shehhi et al., 2024). Moderate elevations after extraction in IL-6 and IL-8 observed in our study however, indicate a prolonged inflammatory phase. Comparable results have also been documented in response to tooth extraction, indicating a slow resolution of inflammation with higher salivary IL-6 levels in diabetes subjects as opposed to non-diabetic individuals (Saim et al., 2025). Salivary IL-8 also increases during wound healing as a result of its function in entraining the migration and activity of neutrophils, and post-extraction higher levels among diabetics could be indicative of a compensatory increase in leukocyte activity or impaired cytokine resolution (Hotamisligil, 2017; Relvas et al., 2024).

Several researches examined the level of salivary cytokines in oral inflammatory diseases. For example, high biomarkers such as IL-6 in chronic periodontitis and diabetes have been associated with disease severity and glycemic condition (Costa et al., 2010; ; Sangappa et al., 2024). While periodontitis and diabetes have common pathways of inflammation, the issue is in the context of wound healing post-surgery, which include temporal coordinated inflammatory initiation and then subsequent proliferative phases followed by remodeling stages. Diabetes appears to hinder the various stages of wound healing which includes functioning of leukocyte, angiogenesis and cytokine signalling process, resulting in often delayed healing (Asoka et al., 2022). Neutrophil chemotaxis, and macrophage function are altered by hyperglycemia itself leading to an inefficient inflammatory response which could perpetuate a high concentration of proinflammatory cytokines including IL- 6 and IL-8 (Thimmappa et al., 2023).

Although we anticipated diabetes to patients with elevated basal IL-6 and IL-8 levels due to chronic inflammation, the extent of rise post-extraction illuminates how further surgical insult escalated inflammatory signaling in this population. IL-6, in

addition to its role in acute inflammation, affects the proliferation of fibroblasts and endothelial cells, both of which are important for wound healing. Yet, chronically elevated levels can paradoxically have the opposite effect by extending inflammation and tissue destruction (Hotamisligil, 2017; Al Shehhi et al., 2024). Increased IL-8 levels recruit neutrophils to the site of injury that is necessary for early defense, but excessive or prolonged recruitment results in tissue damage and impaired transition to the proliferative phase (Adnan et al., 2025).

Our results highlight the potential of saliva as a non-invasive biofluid for assessing inflammatory responses. The saliva carries biologically active molecules of both local oral and systemic inflammatory states, delivering more valuable information than serum in some cases (Al Shehhi et al., 2024). With the simplicity of sampling and cost-beneficial properties, salivary biomarkers like IL-6 and IL-8 might become the basis for simple risk stratification and post-surgical monitoring in dental surgery, in particular, with comorbidities such as diabetes (Sahibzada et al., 2017).

These studies further extend earlier findings that inflammatory responses in organ injury are not only maximally increased at baseline, but sustained postinjury. This is of clinical importance as the failure to resolve inflammation can lead to complications such as infection, delayed tissue regeneration and chronic non-healing wounds (Thimmappa et al., 2023). The identification of temporal alterations in salivary inflammatory profiles could enable clinicians to individualize postoperative management—e.g., by focusing on glycemic improvement or adjunctive anti-inflammatory approaches in patients with high risk (Injeyan et al., 2025).

Nevertheless, there are some limitations that should be considered. While informative, to focus exclusively on IL-6 and IL-8 may not include the full richness of the wound healing milieu; molecules such as TNF-α, IL-1β, and growth factors also importantly contribute to inflammation and repair. Further studies are warranted to extend the number of biomarkers tested, and correlate with clinical measures of healing as well as glycemic control such as HbA1c. A similar approach in longitudinal studies either beyond the 2-day immediate postoperative period or later during proliferative and remodeling periods of healing could elucidate changes that occur over time in cytokine levels.

Conclusion

The current study showed that IL-6 and IL-8 in salivary level were higher at baseline T2DM patients than in control group and after tooth extraction increased significantly which means T2DM have an increasing excessive and longer inflammation. These results are in agreement with the available evidence that associates diabetes with modified inflammatory pattern of cytokines, as well as expedited wound healing. Saliva is a readily accessible non-invasive medium to measure these responses that could aid in better clinical management of diabetic subjects requiring dental surgeries.

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