

Salivary cytokine signatures and their association with glycaemic markers in adults with type 2 diabetes

Assinaturas de citocinas salivares e sua associação com marcadores glicêmicos em adultos com diabetes tipo 2

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ABSTRACT

Objective: To investigate salivary pro- and anti-inflammatory cytokine profiles in adults with type 2 diabetes (T2D) and their associations with glycaemic markers. **Material and Methods:** A cross-sectional study was conducted including 20 adults with T2D and 20 age-matched controls. Unstimulated saliva was collected, and concentrations of IL-1 β , IL-4, IL-6, IL-8, IL-10, IL-17A, IL-22, TGF- α , and TNF- α were quantified using a multiplex immunoassay. Fasting blood glucose (FBG) and glycated haemoglobin (HbA1c) were obtained from clinical records. **Results:** T2D group exhibited higher salivary IL-6 and IL-10 levels compared with controls. Several pro-/anti-inflammatory cytokine ratios, including IL-1 β /IL-10, IL-8/IL-10, TGF- α /IL-10, and TNF- α /IL-10, were significantly altered in the T2D group. In the T2D group, IL-1 β , IL-6, IL-10, IL-22, TGF- α , and TNF- α were positively correlated with FBG, whereas specific ratios, such as IL-22/IL-4 and TNF- α /IL-4, were positively associated with HbA1c. **Conclusion:** Adults with T2D display a distinct salivary cytokine profile characterised by elevated IL-6 and IL-10 and altered pro-/anti-inflammatory ratios. Salivary cytokine levels are associated with both short- and long-term glycaemic control, suggesting that salivary biomarkers can reflect systemic metabolic and inflammatory status.

KEYWORDS

Cytokines; Glycaemic control; Oral health, Saliva; Type 2 diabetes.

RESUMO

Objetivo: Investigar os perfis de citocinas pró-inflamatórias e anti-inflamatórias salivares em adultos com diabetes tipo 2 (DT2) e suas associações com marcadores glicêmicos. **Material e Métodos:** Foi realizado um estudo transversal incluindo 20 adultos com DT2 e 20 controles com a mesma faixa etária. Foi coletada saliva não estimulada e as concentrações de IL-1 β , IL-4, IL-6, IL-8, IL-10, IL-17A, IL-22, TGF- α e TNF- α foram quantificadas usando um imunoensaio multiplex. A glicemia de jejum (FBG) e a hemoglobina glicada (HbA1c) foram obtidas a partir de registros clínicos. **Resultados:** O grupo T2D apresentou níveis salivares mais elevados de IL-6 e IL-10 em comparação com os controles. Várias relações de citocinas pró/anti-inflamatórias, incluindo IL-1 β /IL-10, IL-8/IL-10, TGF- α /IL-10 e TNF- α /IL-10, foram significativamente alteradas no grupo T2D. No grupo T2D, IL-1 β , IL-6, IL-10, IL-22, TGF- α e TNF- α foram positivamente correlacionados com FBG, enquanto proporções específicas, como IL-22/IL-4 e TNF- α /IL-4, foram positivamente associadas com HbA1c. **Conclusão:** Adultos com DT2 apresentam um perfil distinto de citocinas salivares, caracterizado por níveis elevados de IL-6 e IL-10 e proporções pró-/anti-inflamatórias

alteradas. Os níveis de citocinas salivares estão associados ao controle glicêmico de curto e longo prazo, sugerindo que biomarcadores salivares podem refletir o estado metabólico e inflamatório sistêmico.

PALAVRAS-CHAVE

Citocinas; Controle glicêmico; Saúde bucal; Saliva; Diabetes tipo 2.

INTRODUCTION

Estimates have indicated that approximately 529 million people worldwide were living with diabetes in 2021, corresponding to a global age-standardised prevalence of 6.1%. Type 2 diabetes (T2D) accounts for more than 96% of all cases and is expected to affect an estimated 1.31 billion people by 2050 [1]. T2D is characterised by impaired insulin secretion from pancreatic β -cells and resistance to insulin action, resulting in persistent hyperglycaemia and ranks as the tenth leading cause of death and disability worldwide [2].

Chronic hyperglycaemia is directly associated with microvascular and macrovascular complications, including retinopathy, neuropathy, nephropathy, and cardiovascular disease [3]. Disorders of bone healing and periodontitis have also been associated with inflammation induced by hyperglycaemia in T2D [4,5]. Therefore, maintaining glycaemic control is essential to prevent these complications and to reduce the morbidity and mortality associated with T2D [6]. Monitoring of T2D typically relies on serological parameters such as fasting blood glucose (FBG) and glycated haemoglobin (HbA1c) [7].

Beyond metabolic dysregulation, T2D is increasingly recognised as a chronic low-grade inflammatory condition characterised by elevated circulating levels of pro-inflammatory cytokines such as interleukin (IL)-1 β , IL-6, IL-17, tumour necrosis factor alpha (TNF- α) [8]. These cytokines contribute to the development of insulin resistance and β -cell dysfunction by interfering with insulin signalling pathways and promoting oxidative stress [9,10]. Elevated serum cytokine levels have been correlated with glycaemic markers and disease progression, reinforcing the role of systemic inflammation in both the onset and complications of T2D [9]. Therefore, profiling serum cytokines provides valuable insights into the inflammatory status of patients with diabetes and may help identify those at higher risk for complications or poor glycaemic control.

Although these serum-based assessments are widely used, they are invasive and often associated with pain and discomfort. Consequently, there has been growing interest in using saliva as an alternative tool for monitoring glycaemic and inflammatory parameters in people with diabetes [11]. Saliva collection is non-invasive, cost-effective, and easy to perform and store [12]. As a biofluid, saliva can reflect an individual's systemic health status through the presence of diverse biomarkers [13]. Several inflammatory biomarkers, including cytokines, have been identified in saliva as potential indicators of subclinical inflammation. Their presence may arise from serum transudation through the highly vascularised salivary glands, as well as from epithelial and inflammatory cells in the gingival crevicular fluid [11]. Since cytokine levels reflect systemic inflammatory status, their analysis in saliva may serve as a valuable approach for monitoring inflammatory diseases [14].

Previous studies have explored the relationship between salivary cytokines and glycaemic markers in diabetes [15-17]. However, the interplay between salivary pro- and anti-inflammatory cytokines and their potential association with blood glucose regulation remains unclear. It is plausible that alterations in glycaemic status may influence the balance between these cytokine groups, resulting in a distinct salivary inflammatory profile among individuals with T2D. Therefore, this study aimed to identify salivary pro- and anti-inflammatory cytokines associated with markers of glycaemic control in adults with T2D.

MATERIALS AND METHODS

Study design and sample

A cross-sectional study was conducted involving two groups: 20 individuals with type 2 diabetes (T2D group) and 20 individuals without diabetes (Control group). The study protocol was submitted to and approved by the Research Ethics Committee of the University Hospital of the Federal University of Maranhão

(CAAE: 60673322.0.0000.5086). All participants were fully informed about the objectives and procedures of the study, and those who agreed to participate signed a written informed consent form. In addition, all participants were provided with guidance on disease prevention, oral hygiene procedures, and treatment for oral diseases, and were referred for dental care at the university clinics when necessary.

The T2D group consisted of male and female volunteers aged 18 years or older, with a confirmed medical diagnosis of type 2 diabetes for at least one year, who were admitted to the medical clinic of the University Hospital of the Federal University of Maranhão. The control group included volunteers without type 2 diabetes, recruited from the dental clinics of the same university. Exclusion criteria for both groups included: hospitalisation in an intensive care unit; unconsciousness; a diagnosis of hypothyroidism, Parkinson's disease, or Alzheimer's disease; current or past chemotherapy or radiotherapy; recent cancer diagnosis; graft-versus-host disease; pregnancy or lactation; presence of nodules; and clinical signs of infectious and/or inflammatory salivary gland disease.

The sample size was estimated using G*Power software version 3.1.9.6 (Universität Kiel, Germany), based on a correlation analysis. An expected effect size (ρ) of 0.56 was assumed for the correlation between salivary cytokine levels and glycaemic markers, with a significance level of 0.05 and a statistical power of 0.80. This calculation indicated that a minimum of 20 participants per group would be sufficient, resulting in a total sample of 40 individuals. To minimise potential bias, the control group was age-matched with the diabetes group within a ± 4 -year range.

Data collection

A semi-structured, verbally administered questionnaire was used to collect sociodemographic data, including age, sex, educational level, and monthly household income. Body weight and height were measured to calculate body mass index (BMI, kg/m^2). For the T2D group, the duration of the diabetes diagnosis was obtained from hospital electronic medical records. Additionally, blood samples were collected by the hospital medical team for analysis of glycated haemoglobin (HbA1c) and fasting

blood glucose (FBG) within ± 48 hours of saliva sample collection.

Saliva collection

Saliva samples were collected in the morning, between 8:00 and 9:00 a.m. Participants were instructed to abstain from solid food for at least 60 minutes prior to collection. After rinsing the mouth with water, unstimulated saliva was collected using the passive drool technique. Participants were seated with their head tilted slightly forward and lips parted, allowing saliva to drain into a sterile 50 mL graduated polypropylene tube over a timed period of five minutes [18]. Samples were then frozen and stored at -40°C until cytokine analysis.

Salivary cytokine levels

The salivary concentrations (pg/mL) of interleukin (IL)-1 β , IL-4, IL-6, IL-8, IL-10, IL-17A, IL-22, transforming growth factor alpha (TGF- α), and tumour necrosis factor alpha (TNF- α) were measured in triplicate using a multiplex assay. This technique is based on immunoassay principles coupled with flow cytometry, whereby each analyte is captured by a colour-coded bead conjugated with specific antibodies. A customised MILLIPLEX® Human Cytokine/Chemokine/Growth Factor Magnetic Bead Panel (HCYTA-60K, Merck, Darmstadt, Germany) was used for simultaneous quantification of the nine cytokines. Assays were performed in accordance with the manufacturer's instructions.

Statistical analysis

Data analysis was performed using R software version 4.5.1 (R Foundation for Statistical Computing, Vienna, Austria) and GraphPad Prism version 10.6.1 (GraphPad Software, San Diego, USA). Descriptive statistics included absolute and relative frequencies, means, medians, interquartile range (IQR), and standard deviation ($\pm$ SD). The chi-square test was used to compare categorical variables between groups.

The normality of continuous data was assessed using the Shapiro–Wilk test. Given the non-normal distribution of the data, the Mann–Whitney U test was employed for between-group comparisons of salivary cytokine levels, and the effect size was estimated using the rank-biserial correlation. Associations between

continuous variables, including salivary cytokine concentrations, pro-/anti-inflammatory ratios, and serum glycaemic markers (HbA1c and FBG), were examined using Spearman's rank correlation coefficient (ρ). A significance level of 5% was adopted for all analyses ($p < 0.05$).

RESULTS

General description

The T2D group comprised 60% males and 40% females, with a mean age of 57.6 ± 6.8 years, while the Control Group included an equal distribution of males (50%) and females (50%), with a mean age of 51.6 ± 12.7 years. Educational level and monthly family income were also similar between groups. The prevalence of arterial hypertension and mean body mass index (BMI) did not differ significantly between groups ($p = 0.507$ and $p = 0.357$, respectively). Overall, the study groups were comparable in general characteristics (Table I).

Salivary cytokine signature in T2D

Figure 1 presents the univariate comparative analysis of salivary cytokine levels between the diabetes and control groups. The results showed that the T2D group had significantly higher levels of IL-6 ($p = 0.003$) and IL-10 ($p = 0.021$) than the control group.

Figure 2 summarises the correlation analysis among salivary cytokines. In the type 2 diabetes (T2D) group, a strong pattern of positive correlations

was observed among several salivary cytokines, particularly between IL-4, IL-6, IL-10, IL-17A, IL-22, TGF- α , and TNF- α . IL-6 showed significant positive correlations with IL-4 ($\rho = 0.764$, $p < 0.001$), IL-10 ($\rho = 0.871$, $p < 0.001$), IL-17A ($\rho = 0.739$, $p < 0.001$), IL-22 ($\rho = 0.540$, $p = 0.014$), TGF- α ($\rho = 0.873$, $p < 0.001$), and TNF- α ($\rho = 0.804$, $p < 0.001$). Similarly, IL-10 exhibited strong positive correlations with IL-4 ($\rho = 0.828$, $p < 0.001$), IL-17A ($\rho = 0.797$, $p < 0.001$), IL-22 ($\rho = 0.669$, $p < 0.001$), TGF- α ($\rho = 0.752$, $p < 0.001$), and TNF- α ($\rho = 0.696$, $p < 0.001$). TNF- α was also strongly correlated with TGF- α ($\rho = 0.891$, $p < 0.001$). In contrast, the control group showed weaker correlations among cytokines. The most notable associations were observed between IL-22 and IL-4 ($\rho = 0.732$, $p < 0.001$), TGF- α and IL-10 ($\rho = 0.841$, $p < 0.001$), and TNF- α with IL-10 ($\rho = 0.669$, $p = 0.001$) and TGF- α ($\rho = 0.758$, $p < 0.001$).

Correlation between salivary cytokine levels and serum glycaemic markers

Table II shows the correlations between salivary cytokine levels and serum glycaemic markers in T2D group. No significant correlations were found between individual salivary cytokines and HbA1c levels ($p > 0.05$). However, several cytokines showed significant positive correlations with FBG. Specifically, IL-1 β ($\rho = 0.732$, $p = 0.032$), IL-6 ($\rho = 0.535$, $p = 0.022$), IL-10 ($\rho = 0.453$, $p = 0.025$), and IL-22 ($\rho = 0.589$, $p = 0.011$) were positively correlated with FBG. Stronger associations were

Table I - Comparative analysis of general data between study groups

Variables	T2D Group (n=20)		Control Group (n=20)		<i>p</i>
Sex [n (%)]					0.525
Female	8	(40.0)	10	(50.0)	
Male	12	(60.0)	10	(50.0)	
Age [mean $\pm$ SD]	57.6	± 6.8	51.6	± 12.7	0.088
Education Level [n (%)]					0.327
Up to 8 years	9	(45.0)	6	(30.0)	
More than 8 years	11	(55.0)	14	(70.0)	
Monthly Family Income [mean $\pm$ SD]	3.401	± 2.466	2.769	± 1.979	0.436
Arterial Hypertension [n (%)]					0.507
No	12	(60.0)	14	(70.0)	
Yes	8	(40.0)	6	(30.0)	
BMI [mean $\pm$ SD]	26.9	± 6.5	28.0	± 4.7	0.357

T2D = type 2 diabetes; SD = Standard Deviation; BMI = Body Mass Index. Monthly family income is presented in Brazilian Reais (BRL, R\$).

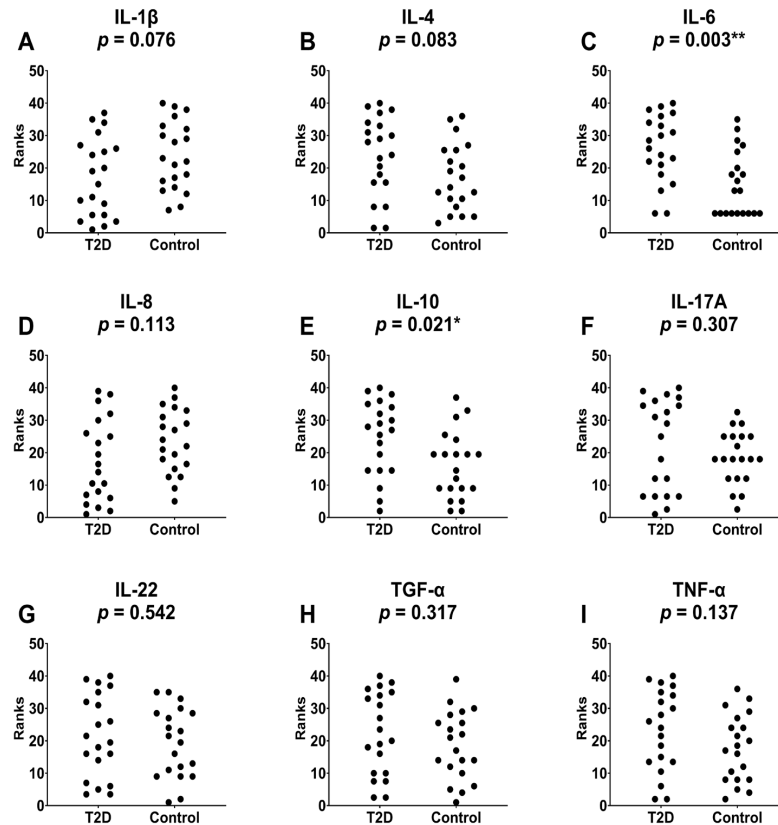


Figure 1 - Rank plots comparing salivary levels of IL-1 β (A), IL-4 (B), IL-6 (C), IL-8 (D), IL-10 (E), IL-17A (F), IL-22 (G), TGF- α (H), and TNF- α (I) between the type 2 diabetes and control groups. ** $p < 0.05$; * $p < 0.01$.

Table II - Correlation between salivary cytokine levels and serum HbA1c and FBG in the type 2 diabetes group

Salivary cytokine	HbA1c	FBG
	Rho	Rho
	<i>p</i>	<i>p</i>
IL-1 β	-0.090	0.505
	0.732	0.032*
IL-4	-0.454	0.415
	0.067	0.087
IL-6	-0.387	0.535
	0.125	0.022*
IL-8	0.253	0.271
	0.327	0.277
IL-10	-0.195	0.525
	0.453	0.025*
IL-17A	-0.247	0.285
	0.338	0.252
IL-22	-0.141	0.585
	0.589	0.011*
TGF- α	-0.167	0.632
	0.522	0.005**
TNF- α	-0.21	0.624
	0.418	0.006**

Rho = Spearman's correlation coefficient; HbA1c = glycated haemoglobin; FBG = fasting blood glucose. * $p < 0.05$; ** $p < 0.01$.

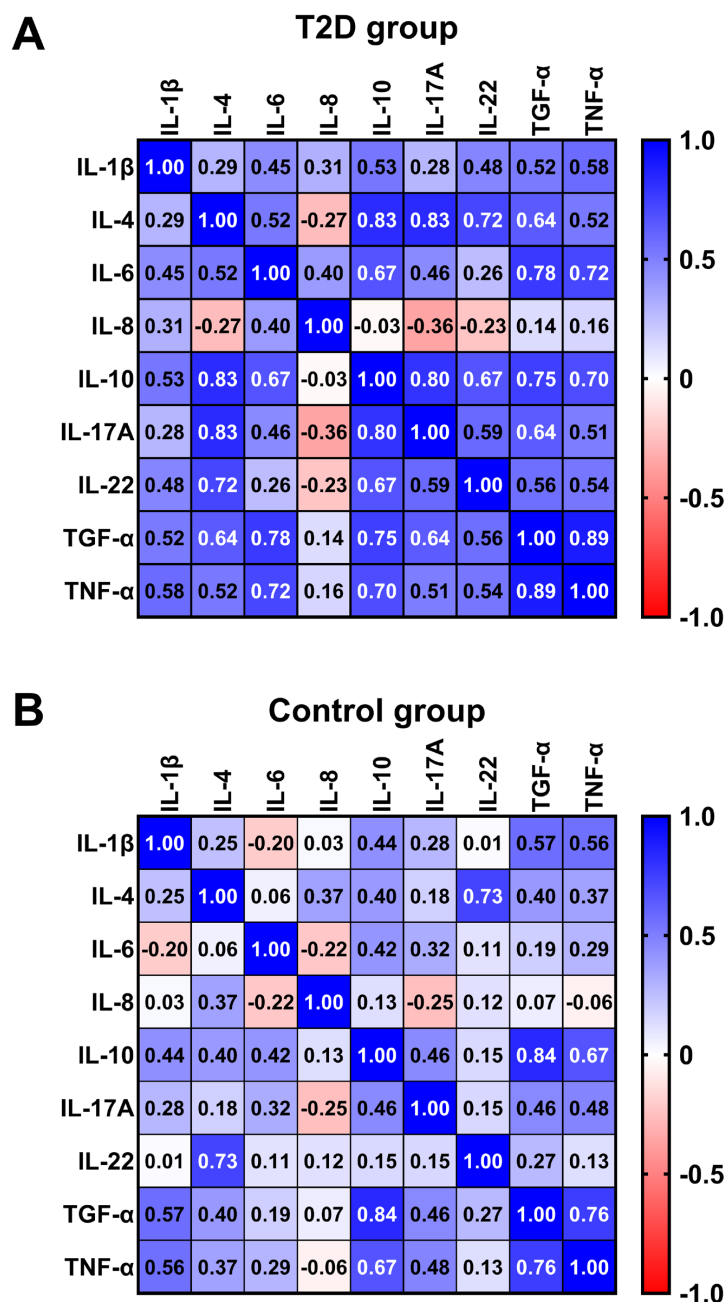


Figure 2 - Heat map of Spearman's correlation coefficients between salivary marker levels in the (A) type 2 diabetes group and (B) control group.

observed for TGF- α ($\rho = 0.522$, $p = 0.005$) and TNF- α ($\rho = 0.418$, $p = 0.006$).

Pro-/anti-inflammatory cytokine ratio analyses

Table III presents the comparison of pro- to anti-inflammatory cytokine ratios between T2D and control groups. Significant differences were observed for several ratios, indicating an altered inflammatory balance in the T2D group. The IL-1 β /IL-4 ratio was significantly

lower in individuals with T2D compared with controls ($p = 0.008$), as well as the IL-1 β /IL-10 ($p < 0.001$), IL-8/IL-10 ($p = 0.004$), IL-22/IL-10 ($p = 0.032$), TGF- α /IL-10 ($p = 0.005$), and TNF- α /IL-10 ($p = 0.011$) ratios, suggesting a compensatory anti-inflammatory response or a dysregulated cytokine network associated with chronic metabolic inflammation.

Table IV presents the correlations between salivary pro- to anti-inflammatory cytokine ratios and serum glycaemic markers. Significant

Table III - Comparative analysis of pro- to anti-inflammatory cytokine ratios between type 2 diabetes and control groups

pro-/anti-inflammatory ratio	T2D group		Control group		Effect size	<i>p</i>
	Median	IQR	Median	IQR		
IL-1 β /IL-4	4.16	9.96	12.8	29.5	0.495	0.008**
IL-6/IL-4	0.625	0.919	1.17	2.56	0.15	0.425
IL-8/IL-4	35.5	216	121	174	0.33	0.076
IL-17A/IL-4	0.0835	0.413	0.024	0.0341	0.205	0.273
IL-22/IL-4	17.9	23	29.2	40.4	0.265	0.156
TGF- α /IL-4	2.16	4.04	2.19	3.4	0.005	0.989
TNF- α /IL-4	8.16	16.2	9.39	16.2	0.065	0.735
IL-1 β /IL-10	3	11.5	46.5	133	0.705	<0.001***
IL-6/IL-10	0.665	1.18	2.1	8.12	0.305	0.102
IL-8/IL-10	52.2	199	487	430	0.535	0.004**
IL-17A/IL-10	0.0882	0.625	0.109	0.131	0.0725	0.705
IL-22/IL-10	27.2	107	140	146	0.4	0.032*
TGF- α /IL-10	2.7	3.2	7.78	8.63	0.5175	0.005**
TNF- α /IL-10	6.63	17.2	38.7	53.8	0.47	0.011*

IQR = Interquartile Range. Effect size was estimated using the rank-biserial correlation. **p* < 0.05. ***p* < 0.01. ****p* < 0.001.

Table IV - Correlation between pro- to anti-inflammatory cytokine ratios and serum HbA1c and FBG in the type 2 diabetes group

pro-/anti-inflammatory ratio	HbA1c	FBG
	Rho <i>p</i>	Rho <i>p</i>
IL-1 β /IL-4	0.475	0.163
	0.054	0.518
IL-6/IL-4	-0.188	0.407
	0.470	0.094
IL-8/IL-4	0.455	-0.145
	0.066	0.567
IL-17A/IL-4	-0.083	0.035
	0.750	0.890
IL-22/IL-4	0.605	0.165
	0.010	0.512
TGF- α /IL-4	0.199	0.244
	0.444	0.330
TNF- α /IL-4	0.492	0.136
	0.045	0.590
IL-1 β /IL-10	0.318	0.107
	0.214	0.671
IL-6/IL-10	-0.511	-0.083
	0.036	0.744
IL-8/IL-10	0.317	-0.068
	0.215	0.788
IL-17A/IL-10	-0.262	-0.021
	0.311	0.935
IL-22/IL-10	0.070	-0.417
	0.790	0.085
TGF- α /IL-10	-0.195	-0.126
	0.453	0.618
TNF- α /IL-10	0.118	-0.192
	0.652	0.445

Rho = Spearman's correlation coefficient.

positive correlations were found between HbA1c and the IL-22/IL-4 ($\rho = 0.605$, $p = 0.010$) and TNF- α /IL-4 ($\rho = 0.492$, $p = 0.045$)

ratios. In contrast, the IL-6/IL-10 ratio showed a significant negative correlation with HbA1c ($\rho = -0.511$, $p = 0.036$). No significant

correlations were observed between any cytokine ratios and FBG levels. These findings suggest a potential longitudinal effect of glycaemic control on the imbalance between pro- and anti-inflammatory cytokines.

DISCUSSION

The main findings of this study suggest that adults with type 2 diabetes exhibited a distinct salivary cytokine profile compared with non-diabetic controls. Significant positive correlations were observed between several salivary cytokines and FBG. Specific pro-/anti-inflammatory ratios, such as IL-22/IL-4 and TNF- α /IL-4, were also found to be positively associated with HbA1c. These results suggest coordinated alteration of pro- and anti-inflammatory mediators in the oral environment of people with T2D. Furthermore, the dysregulation of individual cytokines appeared to be associated with immediate glycaemic levels, whereas long-term glycaemic control, as reflected by HbA1c, influenced the interactions among cytokines. These findings may indicate that the salivary signature of inflammatory cytokines can be modified by altered glycaemic profiles in adults with T2D.

Biologically, chronic hyperglycaemia can induce oxidative stress and activate innate immune pathways, promoting the secretion of pro-inflammatory cytokines [19], while anti-inflammatory cytokines such as IL-4 and IL-10 may increase as a compensatory mechanism to counterbalance systemic and local inflammation [20]. The observed correlations support the hypothesis that salivary biomarkers may mirror systemic metabolic status, providing a non-invasive window into the chronic low-grade inflammation characteristic of T2D.

The present study demonstrated significant associations between FBG and several salivary cytokines, including IL-1 β , IL-6, IL-10, IL-22, TGF- α , and TNF- α . These findings suggest that short-term fluctuations in glycaemic levels can rapidly influence the inflammatory profile within the oral environment. Acute hyperglycaemia has been shown to induce oxidative stress and activate inflammatory signaling pathways, particularly through NF- κ B signalling, leading to increased secretion of pro-inflammatory mediators such as IL-1 β , IL-6, and TNF- α [21–23].

Defective regulation of IL-6 may exacerbate inflammatory immune reactions, contributing to a state of chronic low-grade inflammation and the consequent heightened expression of inflammatory factors [24]. Furthermore, the concurrent rise in IL-10 levels may reflect a compensatory anti-inflammatory response aimed at limiting tissue damage [25]. However, IL-10 appears to be less effective at suppressing inflammation in individuals with T2D, as high glucose concentrations can induce IL-10 resistance in macrophages, resulting in reduced anti-inflammatory responsiveness [26]. Therefore, these results support the concept that salivary cytokine concentrations are sensitive to immediate metabolic alterations and may serve as dynamic, non-invasive indicators of short-term glycaemic control in individuals with T2D.

In contrast, long-term glycaemic control, represented by HbA1c levels, was associated with specific pro-/anti-inflammatory cytokine ratios, particularly salivary levels of IL-22/IL-4, TNF- α /IL-4 and IL-6/IL-10. These findings can highlight the cumulative effect of chronic hyperglycaemia on immune regulation and cytokine network balance. Sustained high glucose exposure promotes persistent low-grade inflammation, endothelial dysfunction, and immune dysregulation [27], which may explain the altered pro-/anti-inflammatory ratios observed in the present study. IL-22 plays a dual role, contributing to tissue repair while amplifying inflammatory responses under chronic metabolic stress [28,29], whereas TNF- α is a key mediator of insulin resistance and β -cell dysfunction [30,31]. The relationship between these cytokine ratios and HbA1c levels suggests that prolonged poor glycaemic control may shift the salivary cytokine environment towards a pro-inflammatory state. These findings further support the potential utility of saliva as a source of biomarkers for monitoring the metabolic and inflammatory status of T2D progression.

Salivary levels of TNF- α and TGF- α showed the strongest positive correlations with FBG levels. The correlation between TNF- α and blood glucose levels can be explained by its action on inhibition peripheral insulin receptors by inducing serine phosphorylation of the insulin receptor substrate-1, leading to insulin resistance [32]. The relationship between TGF- α and FBG can be explained by a shared underlying pathophysiology. Chronic hyperglycaemia

creates a state of systemic and oral inflammation and tissue injury, which in turn can lead to a compensatory and persistent overexpression of the wound-healing growth factors. As a member of the epithelial growth factor family, TGF- α participates of regulation of several cellular responses, including cell proliferation, inflammatory processes, and extracellular matrix regulation [33]. Furthermore, elevated TGF- α levels have been associated with an increased risk of several diabetic complications, including ketoacidosis, and neurological and ocular disorders [34].

This study has limitations that should be considered. Firstly, although the sample size was determined by an a priori power calculation based on a high expected effect size to ensure the detection of clinically meaningful correlations, the relatively small number of participants limits the generalisability of our findings. Therefore, larger-scale studies are needed to support these results and enable subgroup analyses based on other health-related variables. Secondly, the absence of an assessment of periodontal status is also a limitation. Periodontal disease is prevalent in individuals with T2D and can increase salivary cytokine levels [35], potentially acting as a confounding factor. Although evidence has shown that systemic inflammatory diseases present increased levels of salivary cytokines even in the absence of periodontitis [36], the lack of a periodontal assessment in this study limits our ability to determine the relative contribution of local oral inflammation versus systemic metabolic dysregulation to the observed salivary cytokine profile. Future studies should include detailed periodontal examinations, including probing depth, clinical attachment loss, and bleeding on probing, as well as assessments of other oral conditions such as xerostomia and dental caries, to adjust for these variables and elucidate the specific contribution of T2D to the salivary inflammatory signature.

The present study has several notable strengths and clinical implications. The simultaneous quantification of multiple inflammatory cytokines and their ratios enabled a comprehensive characterisation of the salivary inflammatory landscape in individuals with T2D. The use of saliva, a non-invasive and easily collectible biofluid, highlights its potential as an accessible and non-invasive monitoring tool [37,38]. The present results suggest that a

panel of salivary cytokines, particularly TNF- α and TGF- α for short-term glycaemic status and cytokine ratios such as IL-22/IL-4 and TNF- α /IL-4 for long-term control, could serve as complementary tools to traditional serological markers. These findings suggest that salivary cytokine analysis could be a promising way to non-invasively track the inflammatory burden and metabolic status of people with T2D.

CONCLUSION

In conclusion, this study suggests that T2D is associated with a dysregulated salivary cytokine profile. Short-term glucose levels were associated with the concentration of specific inflammatory mediators, while long-term glycaemic control was associated with a broader imbalance in the pro- and anti-inflammatory cytokine network. Salivary levels of TNF- α and TGF- α emerged as particularly sensitive indicators of immediate glycaemic status.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Author's Contributions

JRR, MRLA, MCFNC, VPR: Conceptualization. JRR, TRAP, ESL, VPR: Formal Analysis. JRR, MRLA, GCN, MCFNC: Investigation. JRR, GCN, RSSA, VPR: Methodology. GCN, RSSA, MCFNC, VPR: Validation. JRR, MRLA, TRAP, ESL: Writing – Original Draft Preparation. GCN, RSDSA, MCFC, VPR: Writing – Review & Editing.

Conflict of Interest

The authors declare that they have no conflicts of interest.

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Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the Research Ethics Committee of the University Hospital of the Federal University of Maranhão (CAAE: 60673322.0.0000.5086).

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