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EVALUATION OF BIOMARKER VARIATIONS IN DENTAL CARIES AND TYPE 2 DIABETES MELLITUS: A COMPARATIVE ANALYSIS

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Abstract

Introduction: Diabetes mellitus, which is considered a chronic metabolic disorder, is continuously causing a significant global health challenge. According to recent studies, an estimated 537 million adults aged between 20-79 years have been dealing with diabetes in 2021, and this number is projected to rise to 784 million by 2045 (International Diabetes Federation, 2021). The increasing incidence of type 2 diabetes mellitus (T2DM) epitomizes a substantial global health challenge with profound implications for both individual well-being and healthcare systems worldwide.

Materials and methods: As per the study requirement, 280 patients from DAPM RV Dental College and Hospital, Bangalore aged between 21 to 50 years were included. The 280 patients were divided into 4 groups; Group 1 consisted of 70patients with no dental caries and diabetes and, were regarded as control, group 2 consisted of 70 patients with active dental caries but no diabetes, group 3 consisted of 70 patients with diabetes but no active dental caries, while group 4 consists of 70 patients who are diabetic and have active dental caries.

Results: The salivary pH is a critical factor in the oral cavity's health. The healthy individuals (Group 1), as shown in Table 2, had a mean higher pH (7.69 ± 0.4) compared to patients with dental caries without DM (7.3 ± 0.4; p**** <0.0001) and compared to Type 2 DM without caries. The decrease in the pH in groups 2 and 3 and the shift toward an acidic environment show potential favor toward caries development and indicate metabolic changes in DM. The most significant decrease in pH levels was observed in type 2 DM with active dental caries (7.1 ± 0.2; p**** < 0.0001), suggesting an emphasis on the compound effect of diabetes and dental caries on salivary acidity and highlighting the relationship between systemic health and oral microbiota.

Conclusion: The present study tried to depict detailed insights into the conditions of individuals with diabetes mellitus and dental caries by exploring the serum and salivary biomarkers and inflammatory markers. The present finding suggests a significant change in individuals with a change in systemic and oral parameters; these changes were more evident in individuals with type 2 DM with active dental caries. The present study emphasizes an

integrated approach to managing diabetes mellitus and oral problems by assessing the biomarkers of salivary and serum. Further, exploring the underlying molecular mechanisms in the future would help design effective therapeutic and preventive strategies.

Key Words: Diabetes mellitus, dental caries, serum, salivary biomarkers and inflammatory markers.

INTRODUCTION

Diabetes mellitus, which is considered a chronic metabolic disorder, is continuously causing a significant global health challenge. According to recent studies, an estimated 537 million adults aged between 20-79 years have been dealing with diabetes in 2021, and this number is projected to rise to 784 million by 2045 (International Diabetes Federation, 2021). The increasing incidence of type 2 diabetes mellitus (T2DM) epitomizes a substantial global health challenge with profound implications for both individual well-being and healthcare systems worldwide. T2DM is a chronic metabolic disorder characterized by higher insulin resistance, followed by impaired insulin secretion, causing persistent hyperglycemia and systemic arrangement affecting different organ systems. An increased prevalence of diabetics highlights the urgent need for comprehensive management and prevention strategies to mitigate the burden of this disease.[1]

Diabetes not only causes systemic manifestations but goes far behind causing a spectrum of complications affecting multiple organ systems. Among these, oral health complications are one such condition that constitutes a significant change however it is very quite an overlooked aspect of diabetes management. Individuals with diabetes are predisposed to various oral pathologies, including periodontal diseases, xerostomia, candidiasis, and dental caries.[2]

Dental caries remains one of the most widespread chronic diseases globally, affecting individuals across all age groups and socioeconomic sections. Recent epidemiological data show that dental caries affects approximately 2.4 billion people worldwide (Kassebaum et al., 2017). The association between T2DM and dental caries is a multifactorial role involving complex interactions between systemic factors, oral microbiota, saliva composition, and its host immune responses. Individuals with T2DM usually exhibit changes in salivary composition, such as changes in pH and salivary flow rate, and in various biomolecule levels, such as proteins and electrolytes. These variations may lead to a higher risk of dental caries. Furthermore, the rearranged inflammatory pathways in T2DM are characterized by elevated levels of pro-inflammatory cytokines like IL-6 and TNF-α, which may elevate oral inflammation, leading to the pathogenesis of dental caries.[3]

Saliva, considered a valuable diagnostic fluid, is often called the "mirror of the body," as it reflects the individual's physiological and pathological status. Salivary biomarkers provide a non-invasive and cost-effective means of evaluating both oral and systemic health, including monitoring the progression of T2DM and its related complications. Among the various array of salivary biomarkers, inflammatory markers such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) have been shown as critical indicators for the inflammation in response

to underlying T2DM and its oral manifestations, such as dental caries. Numerous studies have explored the salivary and serum biomarker profiles of individuals with T2DM, focusing on understanding the underlying mechanisms linking diabetes and oral health complications. However, there remains a notable gap in the literature with regards to the comparative analysis of salivary and serum biomarkers, along with inflammatory makers such as IL-6 and TNF-α, in T2DM patients with and without dental caries, as well as their comparison with healthy individuals. Understanding the alterations in biomarker profiles between these groups could provide valuable information into the pathophysiology of T2DM and its association with oral complications, thus aiding in developing targeted preventive and therapeutic strategies.[4]

The present study attempts to address this gap of connection between T2DM and oral health by conducting a comparative analysis of salivary and serum biomarker profiles among healthy individuals and T2DM patients with and without dental caries. By evaluating both saliva and serum samples, the present study aims to comprehensively evaluate the systemic and local inflammatory responses associated with T2DM and dental caries, thereby shedding light on potential biomarkers for early detection, risk stratification, and oral health monitoring in diabetic patients.[5] Moreover, identifying distinct biomarker profiles in T2DM patients with and without dental caries may facilitate the development of personalized treatment approaches tailored to individual patient needs.

METHODOLOGY

Ethical Approval

The present study was performed per the declaration of Helsinki, and the Ethical Review Committee of Chettinad University and DAPM RV Dental College and Hospital, Bangalore after approval for the study (ERC 73/2020). The participants were duly informed about the study and a written consent form was obtained before recruiting them for research.

Study design and Participants:

As per the study requirement, 280 patients from DAPM RV Dental College and Hospital, Bangalore aged between 21 to 50 years were included. The 280 patients were divided into 4 groups; Group 1 consisted of 70patients with no dental caries and diabetes and, were regarded as control, group 2 consisted of 70 patients with active dental caries but no diabetes, group 3 consisted of 70 patients with diabetes but no active dental caries, while group 4 consists of 70 patients who are diabetic and have active dental caries.

Inclusion and exclusion criteria:

Patients clinically diagnosed with type 2 diabetes forat least six months were included in studies. Furthermore, the age, sex, and free of diabetes, or any other metabolic or oral diseases were selected for study.

Patients clinically diagnosed with T2DM at least for 6 months were included as the case population. Furthermore, age, sex, and BMI matched healthy (free of DM, Metabolic diseases, and oral diseases) volunteers were selected for the study as a control population.

While patients with a history of salivary gland surgeries and patients receiving radiotherapy, and long-term local and systemic drug therapy except (oral hypo-glycaemic and insulin). Pregnant/lactating ladies or patients with severe diseases like cancer, AIDS, TB, etc. Patients with a history of systemic illness, and endocrinal and metabolic disorders affecting the salivary glucose levels except DM were excluded.

Clinical examination:

DMFT indexing of the patient's teeth was performed per WHO criteria. The dental caries were thoroughly assessed by a trained dentist, wherein the no. of decayed tooth, missing, and filled teeth were recorded from each patient of respective groups and were added to the patient's DMFT values, and further, their mean DMFT was calculated and highlighted in table 1.

Collection of Samples:

Serum: A fasting blood sample of 2 ml of intravenous blood was collected from the median cubital vein of the forearm from patients suffering from T2DM and healthy volunteers. Blood samples for glucose estimation were collected in a sodium fluoride tube, while the blood was collected in an EDTA tube for other biological parameters. Each blood sample was processed as per the requirement of the estimation of biological parameters. The processed samples were stored at – 80 ˚C till further analysis.

Fasting blood sample was withdrawn from both the patients suffering from DM and healthy volunteers, under aseptic conditions. Only 2 ml of intravenous blood was obtained from the median cubital vein of the forearm with a 5 ml syringe. Each sample was centrifuged at 2000 rpm for 5 minutes and the serum was separated and stored at – 20 ˚C. The serum thus obtained was used for further analysis.

Saliva: In the case of saliva collection, a prior 400 ml of water given to the patient to keep hydrated, later patients were provided with a sterile container and instructed to spit in it. The unstimulated saliva sample was collected from all groups. The samples were stored at – 80 ˚C still the evaluation of parameters was done.

Biochemical Analysis:

Inflammatory markers: A commercially available ELISA kit quantified the concentration of IL-6 & TNF-α in serum and IL-6 in saliva (Abcam, India). The assay was carried out according to the manufacturer's instructions.

The saliva's pH was determined using a pH meter (Lutron^{TM} PH-223). The tip pH meter was dipped in the saliva sample for a few seconds and pH was recorded simultaneously.

Biochemical serum and salivary markers: The serum biochemical parameters such as fasting blood glucose, glycated-Hb, urea, creatinine, total protein, albumin, globulin, alkaline phosphatase, serum calcium, and salivary biochemical parameters such as Salivary Albumin, globulin, alkaline phosphatase were determined photometrically using commercially available reagent kit (Agappe, India & Ecoline by Diasys, India). Further, Salivary proline-

rich protein was evaluated using ELISAkit based method (Abbexa, United Kingdom). Serum and salivary electrolytes such as sodium and potassium were quantified using a commercial kit (Elyte -2 kit, India). At the same time, salivary mucin is estimated by the Alcan method.

Statistical Analysis:

The data were collected, tabulated, and analyzed statistically SPSS ver. 28 (IBM Corporation, Armonk, NY, USA) was used for the statistical analysis. The data were expressed in mean ± SD, and ANOVA was applied to evaluate the difference in biological parameters of saliva and serum among the given groups.

RESULTS

The objective of the present study was to evaluate the variations in biomarker levels of salivary and serum samples collected from different groups: group 1 includes healthy individuals with no dental caries or DM, and group 2 consists of patients with active caries without T2DM. In contrast, group 3 includes patients suffering from DM with no dental caries and Group 4 consists of DM patients with active Caries. Salivary biomarkers include pH, albumin, globulin, mucin, total protein, calcium, sodium, potassium, chloride, alkaline phosphatase, and inflammatory marker IL-6. Serum parameters include Blood glucose, HbA1c, serum calcium, sodium, potassium, and additional relevant biomarkers. Further inflammatory biomarkers in serum were IL-6 and TNF-alpha respectively.

Salivary biomarkers

The salivary pH is a critical factor in the oral cavity's health. The healthy individuals (Group 1), as shown in Table 2, had a mean higher pH (7.69 ± 0.4) compared to patients with dental caries without DM (7.3 ± 0.4; p**** <0.0001) and compared to Type 2 DM without caries. The decrease in the pH in groups 2 and 3 and the shift toward an acidic environment show potential favor toward caries development and indicate metabolic changes in DM. The most significant decrease in pH levels was observed in type 2 DM with active dental caries (7.1 ± 0.2; p**** < 0.0001), suggesting an emphasis on the compound effect of diabetes and dental caries on salivary acidity and highlighting the relationship between systemic health and oral microbiota.

The salivary protein biomarkers reflect indicators of inflammation and immune activity. As highlighted in Table 2, it can be seen that albumin levels significantly decreased their levels. The albumin concentration in healthy individuals was found to be 137 ± 0.56µg/ml, and a significant decrease in albumin level was found in group 2 (135 ± 1.5µg/ml; p**** < 0.0001), group 3 (136.5 ± 0.25µg/ml; p*** < 0.001) and Group 4 (134 ± 2.5µg/ml; p**** < 0.0001) respectively. In the case of globulin, the levels were found to be elevated significantly from group 1 (91.26 ± 0.35 µg/ml) to group 2 (95 ± 0.5 µg/ml; p**** < 0.0001), group 3 (100 ± 1 µg/ml ; p**** < 0.0001) and group 4 (105 ± 2 µg/ml; p**** < 0.0001) respectively. These elevated protein levels suggest an enhanced inflammatory response, possibly as a mechanism to combat the increased oral microbial load or as a systemic reaction to diabetes. Further, in the case of total protein levels, a statistical elevation of total protein was found in the groups compared to the healthy group. The total protein in the healthy

group was found to be 341.7 ± 24.18 µg/ml, while in cases of groups 2, 3, and 4, it was found to be 500 ± 30 µg/ml (p**** < 0.0001), 360 ± 25 µg/ml (p^{ns}< 0.0968), and 550 ± 30 µg/ml (p**** <0.0001) respectively. The levels of mucin proteins level highlighted in Table 2 show a significant change among the groups; the mucin levels in group 2 (1.69 ± 0.2 mg/ml; p**** < 0.0001), group 4 (1.8 ± 0.25 mg/ml; p**** < 0.0001), and group 3 (1.3 ± 0.42 mg/ml; p^{*}<0.05) showed slight significant changes compared to healthy group 1 (1.19 ± 0.15 mg/ml) respectively. The serum calcium levels in healthy individuals were found to show an average calcium level (6.4 ± 0.4 mg/dl), while calcium was decreased in group 2 patients with active caries without DM (5.8 ± 0.5 mg/dl; p**** < 0.0001), while group 3 patients with diabetes without active dental caries (6.2 ± 0.5 mg/dl; p^{*}< 0.05) showed slightly significant changes. Further, group 4, representing diabetic patients with active dental caries, showed a considerable decrease in calcium level (5.6 ± 0.6 mg/ml; p**** < 0.0001). Further serum electrolytes such as salivary sodium showed an increase in values among groups. In the case of group 1, the mean sodium level was 281.6 ± 17.8 mmol/L, which increased in group 2 with 290 ± 18 mmol/L (p^{*}< 0.05). Furthermore, the salivary sodium values showed significant changes in group 3 with type 2 DM without caries (295± 15 mmol/L; p^{****}<0.0001), and in group 4 with diabetes with active dental caries group significantly elevated (300 ± 20 mmol/L; p****<0.0001). As represented in Table 2, in the case of salivary potassium, there was a gradual increase across the group; however, there was no significant finding. In healthy individuals, the mean salivary potassium level was 11.4 ± 3.1 mmol/L, while group 2 patients with dental caries without T2DM showed a slight decrease to 11± 3 (p^{ns}<0.95). Interestingly, both groups 3 and 4 with type 2 DM without caries and type 2 DM with caries exhibited increased potassium with a mean salivary potassium value of 12 ± 2.3 mmol/L (p^{ns} <0.86) and 12.5 ± 3.5 mmol/L (p^{ns} <0.51) but no significant changes. Further, in the case of salivary chloride, there was a significant increase in chloride level in group 2 with a mean chloride level of 50 ± 7 (p****< 0.0001). In contrast, in group 3, the chloride level was found to be 48 ± 3.5 (p^{**}<0.001). There was a significant increase in chloride level in the case of group 4 with a mean of 53 ± 4.5 (p**** < 0.0001). Furthermore, in the case of salivary proline-rich proteins, there was a significant increase in the levels of the proline-rich proteins in group 3 with a value of 235 ± 2.19 (p**** < 0.0001) and in group 4 with a value of 235 ± 2.19 (p**** < 0.0001) respectively, compared to group 1 (230 ± 3.15). In the case ofgroup 3 the proline-rich protein showed slightly elevated with a value of 231 ± 2.18 (p* < 0.05). Further,in the case of the salivary inflammatory marker, IL-6 showed a significant increase in mean values of IL-6 levels in group 2, group 3, and group 4 with a mean value of 45.9 ± 0.8 pg/mL (p**** < 0.0001), 50 ± 0.7 pg/mL (p**** < 0.0001), and 70 ± 2.0 pg/mL (p**** < 0.0001) respectively. Furthermore, the salivary alkaline phosphatase level was found to be significantly increased in groups 4 and 2 with a mean value of 30 ± 6 IU/L(p**** < 0.0001) and 25 ± 5 IU/L(p**** < 0.0001). The ALP level was also seen to be increased in group 3 with a mean ALP value of 18 ± 4 IU/L (p****< 0.0001).

Serum Biomarkers:

The serum ABG levels as highlighted in Table 3, group 4 patients with T2DM with caries showed the highest average blood glucose levels (160 ± 20 mg/dL; p**** < 0.0001), followed by group 3 which represents patients with T2DM without caries (155± 25 mg/dL; p****< 0.0001), and group 2 representing healthy individuals with caries shows ABG levels of 100 ± 15 (p^{**} <0.01). Similarly, in the case of HbA1c, group 3 (patients with DM without caries) and group 4 (DM patients with active caries) showed higher HbA1c levels of 6.8 ± 0.4 (p**** <0.0001 and 7.1 ± 1, (p^{****} <0.0001) followed by group 2 having HbA1c levels of 5.2 ± 0.6 (p^{*} <0.05) compared to group 1. A considerable change in calcium level was observed in group 3 with a mean value of 9.1 ± 0.6 (p****< 0.0001); further, in group 4, the mean calcium values were found to be 9.0 ± 0.3(p****< 0.0001). Further, in the case of serum total proteins, the patients with active caries (group 2) showed considerable change in total protein levels with a mean of 7.2 ± 0.4 g/dL (p^{*} <0.05), the total protein level in the case of group 4 was found to be the lowest with total protein value of 6.8 ± 0.6 g/dl (p^{*} <0.05). In the case of group 3 and group 1, it was found to be 7.1 ± 0.3 g/dl (p^{ns} <0.83) and 7.0 ± 0.5 g/dl, respectively. Although there was a change in protein levels among the groups, however, they were not significant enough.

In the case of serum albumin, group 1 consisted of healthy individuals with no dental caries and showed the highest albumin level of 4.5 ± 0.4 g/dl. In contrast, group 4, representing patients with DM and active caries, showed a significantly lower albumin level of 4.0 ± 0.5 g/dL (p****< 0.0001). In the case of group 3, albumin levels showed significantly lower levels with a mean value of 4.2 ± 0.2 g/dl (p****< 0.0001) respectively. Group 2 also showed a lower level of serum albumin with a mean value of 4.3 ± 0.3g/dl (p**< 0.01).

Further, in the case of serum globulin, groups 2 and 3 showed higher globulin levels with a mean value of 2.9 ± 0.2 g/dl(p****< 0.0001) and 2.9 ± 0.3 g/dl (p****< 0.0001) compared to group 1 respectively. While group 4 with a mean globulin value of 2.8± 0.4 g/dl(p****< 0.0001). Further, in the case of serum electrolytes such as sodium, it can be seen from Table 3 that group 1 showed higher sodium levels with a mean of 140 ± 3 mmol/L. In the case of group 2, there was a significant decrease in the sodium levels with a mean of 138 ± 4 mmol/L (p^{**} < 0.01), followed by group 3 with a mean of139 ± 2 mmol/L(p^{ns} <0.71) and in case group 4 there was a significant decrease in sodium with mean of 137 ± 5 mmol/l (p****<0.0001). In the case of the serum electrolyte potassium, the potassium level in group 4 was slightly higher compared to other groups, with a mean potassium value of 4.3 ± 0.5 mmol/l (p^{ns} < 0.25). In contrast, group 3 exhibited the lowest potassium level of4.0 ± 0.4 mmol/l (p** < 0.01) respectively. With continuation with serum electrolytes, the chloride levels were lower in group 3 with a mean value of 98 ± 5 mmol/L (p** < 0.01). In comparison, the chloride level was found to be slightly elevated in group 2 with a mean value of 101 ± 4 mmol/l (p^{ns} < 0.24); in the case of group 4, it was found to be 99 ± 2 mmol/l (p^{ns} < 0.25) respectively. The serum levels of alkaline phosphatase were highest in group 4 with a mean value of 100 ± 25 IU/L (p^{**} < 0.01). In comparison, group 2 exhibited lower alkaline phosphate levels of 85 ± 15 IU/L (p^{ns} < 0.73). While in the case of group 3, there were no significant changes in ALP levels compared to the control group. In the case of serum levels of creatinine, there were slight changes in group 2 (0.62 ± 0.07; p^{ns} < 0.05) among all four groups, while groups 3 and

4 did not show any specific changes. In the case of serum urea, group 4 showed an elevated level of urea with a mean value of 25.01 ± 6.54 mg/dl ($p^{ns} < 0.99$), followed by group 3 with a mean urea level of 24.17 ± 4.92 mg/dl ($p^{ns} < 0.95$), and group 2 showed a lowest urea level of 23.34 ± 5.07 mg/dl (p*< 0.05).

Inflammatory Markers:

In the case of inflammatory markers such as group 4 (patients with DM and active caries showed the highest levels of IL-6 with a mean value of 16 .55 ± 0.31 pg/ml; p**** < 0.0001, followed by group 3 which showed a serum IL-6 value of 14.56 ± 0.28 pg/ml ; p**** < 0.0001, and group 2 showed IL-6 value of 12.95 ± 0.29 pg/ml, p**** < 0.0001. Further, in the case of the TNF-alpha inflammatory marker, group 4 showed a significantly elevated level of TNF-alpha with a mean of 21.48 ± 0.21 pg/ml ; p**** < 0.0001, while in groups 2 and 3 the TNF-alpha levels were found to be significantly elevated with a mean value of 18.56 ± 0.25 pg/ml ; p**** < 0.0001and 15.96 ± 30 pg/ml; p**** < 0.0001 respectively.

Table 1: DMFT indexing represented in mean with standard deviation of the participants belonging to respective study groups

Groups	Decayed (Mean ± SD)	Missing (Mean ± SD)	Filling (Mean ± SD)	DMFT (Mean ± SD)
Group 1 (Healthy Individual)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Group 2 (Healthy Individual with Dental Caries)	2.29 ± 1.20	0.51 ± 0.5	1.40 ± 1.21	4.2 ±1.74
Group 3 (Type 2 DM patients without caries)	0.0 ± 0.0	1.07 ± 0.83	0.0 ± 0.0	1.07 ± 0.83
Group 4 (Type 2 DM patients with active caries)	3.63 ± 1.75	1.66 ± 1.14	2.63 ± 1.86	7.91 ± 2.48

Table 2: Salivary Biomarkers for different groups are represented in mean with standard deviation.

	Group 1 (Healthy individual)	**Group 2** (Healthy Individual with active Caries)	**Group 3** (Type 2 DM Patients without Caries)	**Group 4** (Type 2 DM Patients with active Caries)
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Salivary pH	7.69 ± 0.4	7.3 ± 0.4	7.2 ± 0.6	7.1 ± 0.2
Salivary Albumin (µg/mL)	137.96 ± 0.47	135 ± 1.5	136.5 ± 0.25	134 ± 2.5

Salivary Globulin (µg/mL)	91.26 ± 0.35	95 ± 0.5	100 ± 1	105 ± 2
Salivary Total Protein (µg/mL)	341.7 ± 24.18	500 ± 30	360 ± 25	550 ± 30
Salivary Mucin (mg/dL)	1.19 ± 0.15	1.69 ± 0.2	1.3 ± 0.42	1.8 ± 0.25
Salivary Calcium (mg/dL)	6.4 ± 0.4	5.8 ± 0.5	6.2 ± 0.5	5.6 ± 0.6
Salivary Potassium (mmol/L)	11.4 ± 3.1	11 ± 3	12 ± 2.3	12.5 ± 3.5
Salivary Sodium (mmol/L)	281.6 ± 17.8	290 ± 18	295 ± 15	300 ± 20
Salivary Chloride (mmol/L)	45.7 ± 6.8	50 ± 7	48 ± 3.5	53 ± 4.5
Salivary proline-rich protein	230± 3.35	235 ± 2.19	231± 2.18	250 ± 1.55
IL-6 (pg/ml)	41.6 ± 0.16	60 ± 1	50 ± 0.75	70 ± 2
Alkaline Phosphatase (AP) (U/L)	13.8 ± 2.8	25 ± 5.3	18 ± 4.2	30 ± 5.5

Table 3: Serum biomarkers for different groups are represented in mean with standard deviation.

	Group 1 (Healthy individual)	**Group 2 (**Healthy Individual with active Caries)	**Group 3 (**Type 2 DM Patients without Caries)	**Group 4 (**Type 2 DM Patients with active Caries)
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Average Blood Glucose (ABG) (mg/dl)	110 ± 10	100 ± 15	155 ± 25	160 ± 20
HbA1c (%)	5.0± 0.5	5.2 ± 0.6	6.8 ± 0.4	7.1 ± 1
Serum calcium (mg/dl)	9.5 ± 0.5	9.2 ± 0.4	9.1 ± 0.6	9.0 ± 0.3
Serum total protein	7.0 ± 0.5	7.2 ± 0.4	7.1 ± 0.3	6.8 ± 0.6

(gm/dl)				
Serum albumin (gm/dL)	4.5 ± 0.4	4.3 ± 0.3	4.2 ± 0.2	4.0 ± 0.5
Serum globulin (gm/dl)	2.5 ± 0.3	2.9 ± 0.2	2.9 ± 0.3	2.8 ± 0.4
Serum Sodium (mmol/l)	140 ± 3	138 ± 4	139 ± 2	137 ± 5
Serum chloride (mmol/l)	100 ± 3	101 ± 4	98 ± 5	99 ± 2
Serum potassium (mmol/l)	4.2 ± 0.3	4.1 ± 0.2	4.0 ± 0.4	4.3 ± 0.5
Serum alkaline phosphate(IU/L)	90 ± 20	85 ± 15	95 ± 10	100 ± 25
Serum creatinine (mg/dl)	0.65 ± 0.06	0.62 ± 0.07	0.65 ± 0.07	0.63 ± 0.08
Serum urea (mg/dl)	24.29 ± 5.20	23.34 ± 5.07	24.17 ± 4.92	25.01 ± 6.54

Table 4: Inflammatory serum biomarkers for different groups are represented in mean with standard deviation.

	Group 1 (Healthy individual)	**Group 2** **(**Healthy Individual with active Caries)	**Group 3** **(**Type 2 DM Patients without Caries)	**Group 4** **(**Type 2 DM Patients with active Caries)
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
IL-6 (pg/ml)	10.15 ± 0.25	12.95 ± 0.294	14.56 ± 0.282	16.55 ± 0.312
TNF-α (pg/ml)	12.82 ± 0.308	15.96 ± 0.312	18.56 ± 0.305	21.48 ± 0.298

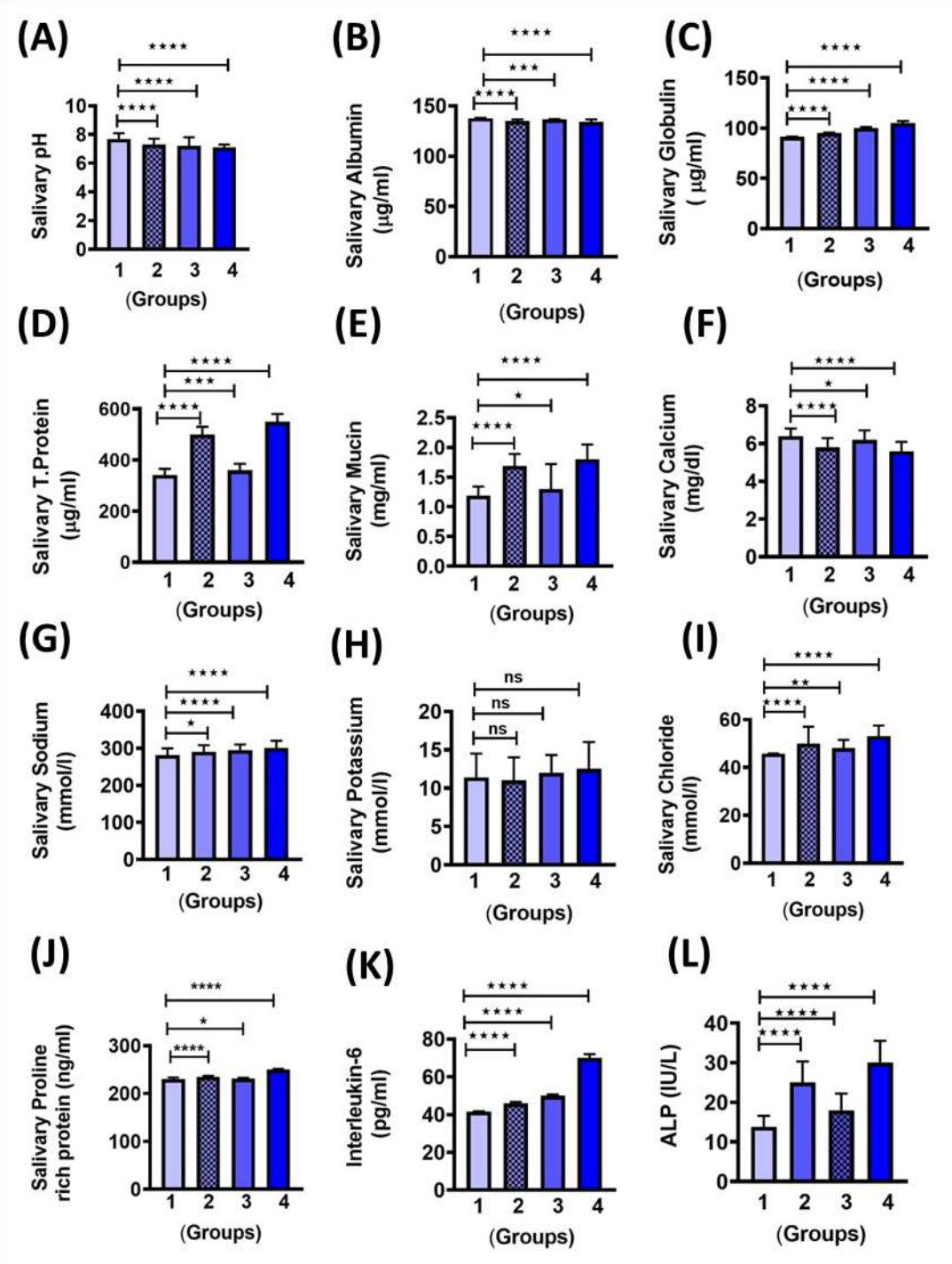

Fig 1 (A-L). The variation of salivary parameters among the groups. Data represents mean ± SD in triplicates; one-way ANOVA with Tukey´s multiple comparison post-test; p* < 0.05, p** < 0.01 and p**** < 0.0001 vs. Healthy patients (Group 1).

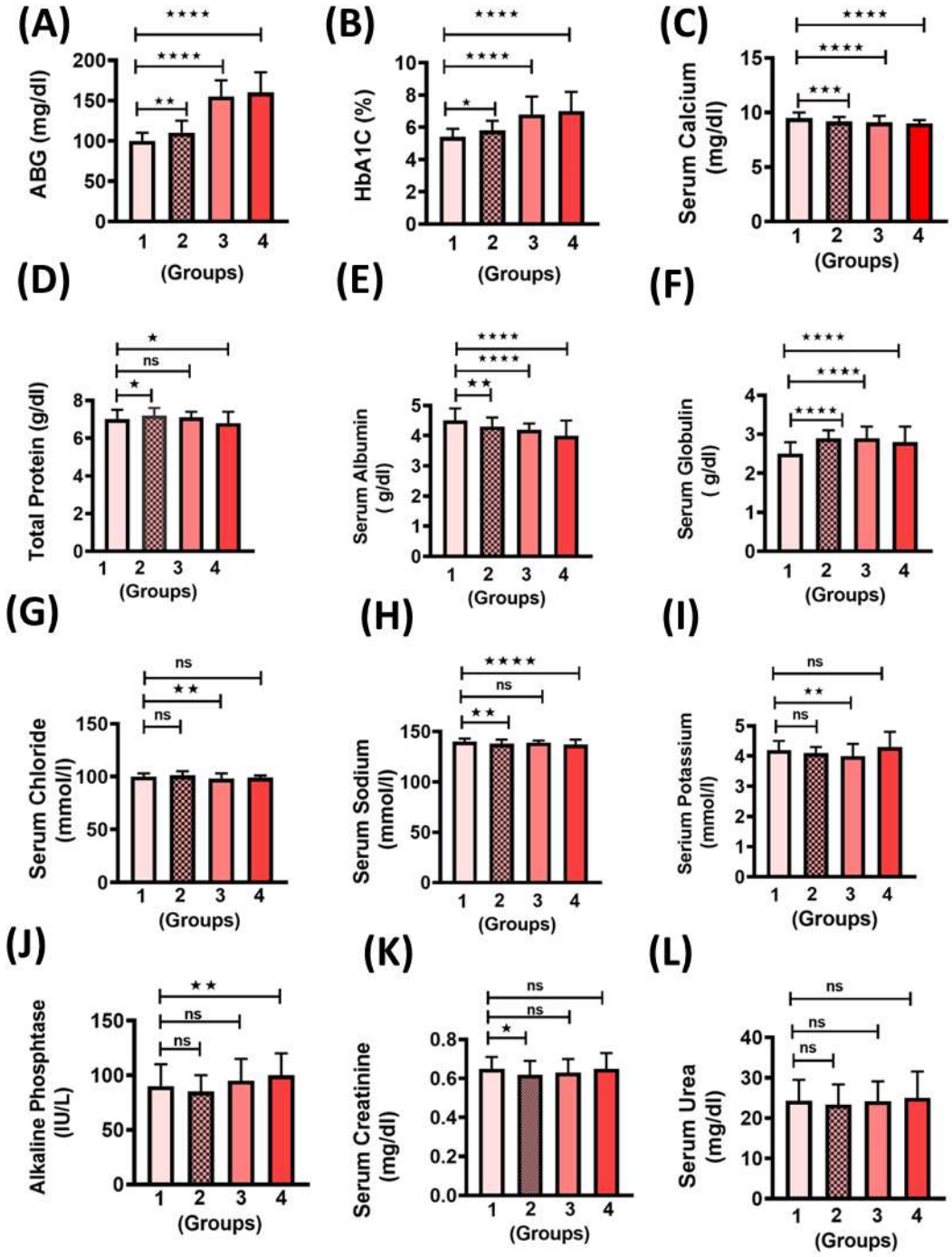

Fig 2 (A-L). The variation of serum parameters among the groups. Data represents mean ± SD in triplicates; one-way ANOVA with Tukey´s multiple comparison post-test; p* < 0.05, p** < 0.01 and p**** < 0.0001 vs. Healthy patients (Group 1).

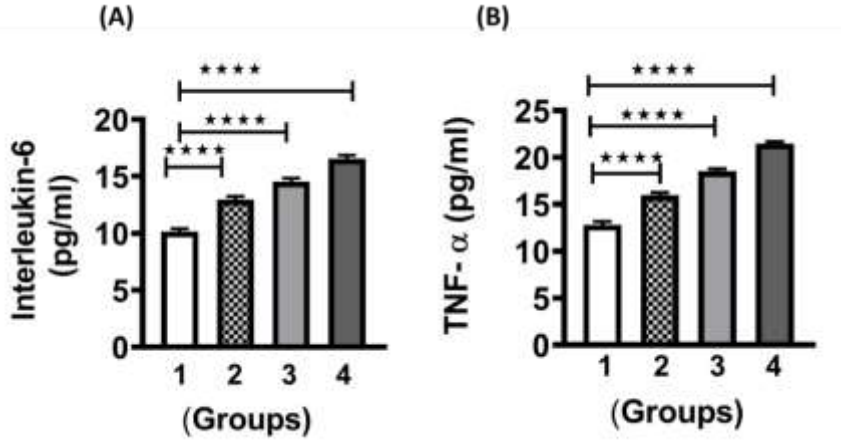

Fig 3 (A-B). The variation of serum Inflammatory markers among the groups. Data represents mean ± SD in triplicates; one-way ANOVA with Tukey´s multiple comparison post-test; p* < 0.05, p** < 0.01 and p**** < 0.0001 vs. Healthy patients (Group 1).

DISCUSSION

The term "diabetes mellitus" is used to identify a group of disorders characterized by elevated levels of glucose in the blood. This elevation is the result of a deficiency in insulin secretion or an increased cellular resistance to the actions of insulin, leading to a variety of metabolic abnormalities involving carbohydrates, fats and proteins.[6]

Diabetes represents an extreme disturbance in glucose metabolism with severe hyperglycemia and insulin deficiency. A number of oral disorders have been associated with DM such as dental caries, gingivitis, periodontitis, salivary dysfunction, altered taste, oral mucosal diseases and infections such as lichen planus, recurrent aphthous stomatitis and candidiasis.[7]

The prevention of periodontal breakdown in diabetic patients is mostly based on the education of the individual. Thus, patients should be informed about the importance of oral health for diabetics, and they should be taught that the main symptom of periodontal disease is gingival bleeding. Candidiasis is a manifestation of an immunocompromised state, and a reduction in salivary flow is another risk factor for oral candidiasis.[8]

The relationship between dental caries and DM is complex. Children with type 1 diabetes often are given diets that restrict their intake of carbohydrate-rich, cariogenic foods, whereas children and adults with type 2 diabetes – which often is associated with obesity and intake of high-calorie and carbohydrate-rich food – can be expected to have a greater exposure to cariogenic foods. Furthermore, a reduction in salivary flow has been reported in people with diabetes who have neuropathy. To know the effect of diabetes on dental caries in diabetic and control group, we used the DMFT index and found that the average score was more in diabetics (10.66) than in the control group and the results were also statistically significant (*P* < 0.01). The literature presents no consistent pattern regarding the relationship between dental caries and diabetes. However, Jones reported an elevated risk of caries due to DM which was in accordance with our study as we found that there was a highly significant

difference between mean values of DMFT score in study and control group (i.e., $P < 0.01$). Thus, dental caries was more in study group as compared to control group. We also found that in the study group, increased blood sugar levels caused increased SM count and were statistically significant ($P < 0.05$). Therefore, higher the SM count more is the caries risk.[9]

Siudikiene *et al*. found that high caries levels in diabetics were significantly associated with age, plaque score and decreased unstimulated salivary flow rate. Reduced salivary secretion increases the likelihood of caries, but good metabolic control prevents the most dangerous salivary changes such as high glucose content and lower pH, while a good diabetic diet, rich in fiber and low in simple carbohydrates, can slow down the production of plaque and the proliferation of acidogenic bacterial microflora.[10]

CONCLUSION

The present study tried to depict detailed insights into the conditions of individuals with diabetes mellitus and dental caries by exploring the serum and salivary biomarkers and inflammatory markers. The present finding suggests a significant change in individuals with a change in systemic and oral parameters; these changes were more evident in individuals with type 2 DM with active dental caries. The present study emphasizes an integrated approach to managing diabetes mellitus and oral problems by assessing the biomarkers of salivary and serum. Further, exploring the underlying molecular mechanisms in the future would help design effective therapeutic and preventive strategies.

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