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Evaluation of Salivary Mucin, Amylase, Protein Profile, and Periodontal Parameters among Hypertensive and Diabetic Patients

Madiha Anwar [1], Beenish F. Alam [1], Saqib Ali [2], Sahibzadi F. Tariq [3], Khalid Aali [4], Eisha Abrar [4], Dalal H. Alotaibi [5], Aljoharah A. Alsinaidi [5], Ali Alrahlah [6,7] and Fahim Vohra [7,8,*]

1 Department of Oral Biology, Bahria University Dental College, BUHS, Karachi 75270, Pakistan; madeeha.anwar@gmail.com (M.A.); nish_alam@yahoo.com (B.F.A.)
2 Department of Biomedical Dental Sciences, College of Dentistry, Imam Abdulrahman Bin Faisal University, Dammam 31441, Saudi Arabia; drsaqiibali@gmail.com
3 Department of Oral Pathology, Rehman College of Dentistry, Peshawar 25000, Pakistan; fatima.tariq1@rmi.edu.pk
4 Department of Restorative Sciences, Dow University Of Health Sciences, Karachi 74200, Pakistan; akhaliid.alia@gmail.com (K.A.); eshaabrar92@gmail.com (E.A.)
5 Department of Periodontics and Community Dentistry, College of Dentistry, King Saud University, Riyadh 11362, Saudi Arabia; dalalotaibi@ksu.edu.sa (D.H.A.); aalsinaidi@ksu.edu.sa (A.A.A.)
6 Department of Restorative Dental Sciences, College of Dentistry, King Saud University, Riyadh 11564, Saudi Arabia; aalrahlah@ksu.edu.sa
7 Engineer Abdullah Bugshan Research Chair for Dental and Oral Rehabilitation, College of Dentistry, King Saud University, Riyadh 11564, Saudi Arabia
8 Department of Prosthetic Dental Science, College of Dentistry, King Saud University, Riyadh 11545, Saudi Arabia
* Correspondence: fvohra@ksu.edu.sa; Tel.: +966-1434555

Abstract: Systemic and localized oral disease instigates alterations in salivary composition and content. The objective of the study was to evaluate the levels of salivary amylase, mucin, total protein levels, and periodontal inflammatory parameters in Type 2 Diabetes mellitus (T2DM), hypertensive (HTN) patients, and healthy controls. The study sample was divided into three groups: healthy, T2DM, and HTN. Salivary samples were collected from the included participants and salivary flow rate (SFR) and pH were measured. The salivary levels of amylase, mucin, and total protein concentration were analyzed using an enzyme-linked immunosorbent assay. The effect of anti-hypertensive and hypoglycemic drugs on the salivary flow rate, salivary pH, mucin, amylase, and total protein concentrations was evaluated. The results were analyzed with Chi-squared and analysis of variance to compare the means and standard deviations of variables among the study groups. SFR was significantly ($p < 0.01$) lower among diabetics (0.78 ± 0.45 mL/min) in comparison to healthy (1.52 ± 0.62 mL/min) and hypertensive (1.07 ± 0.7 mL/min) subjects. PISA values were significantly higher in T2DM (1029 ± 234.6 mm^2) and HTN (799.4 ± 155.05 mm^2) subjects when compared to controls, indicating a high inflammatory burden of oral cavity caused by these conditions, and showed statistically significant difference between the groups (p-value < 0.001). Mucin levels were significantly higher (p-value < 0.05) in hypertensive patients (4.6 ± 1.17 units) compared to diabetics (3.59 ± 1.03 unit/mL) and healthy (2.26 ± 1.09 units/mL) subjects. Amylase levels were significantly higher among healthy subjects (1.76 ± 0.75 mg/mL) compared to both hypertensive (1.33 ± 1.0 mg/mL) and diabetic (0.88 ± 0.57 mg/mL) patients. Total protein concentration was significantly raised (p-value < 0.001) in diabetics (37.67 ± 3.12 mg/mL) compared to healthy (29.3 ± 3.22 mg/mL) subjects. Significant differences in BOP, CAL, and PPD was observed (p-value < 0.001). Use of antihypertensive and hypoglycemic drugs showed a significant influence on salivary flow rate, protein, mucin, and amylase levels. T2DM and HTN induced irregularities in salivary flow rate, pH, amylase, and mucin levels and showed an increased incidence of moderate to severe periodontitis in patients. UWS levels of SFR, mucin, amylase, and total protein can be used as diagnostic and therapeutic biomarkers in patients with T2DM and HTN with oral disease.

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1. Introduction

Saliva is a complex biofluid which lubricates, nourishes, and maintains the health of the oral cavity. Saliva is comprised of a wide variety of enzymes, proteins, chemokines, cytokines, and electrolytes, which are important for performing different functions such as digestion, lubrication, taste, swallowing, and immune response [1]. On average, 300 mL of unstimulated whole saliva (UWS) is produced in a day and the salivary flow rate (SFR) is 0.1–0.3 mL/min. At rest or during sleep the SFR is estimated to be zero. Nearly 80–90% of daily secretion is stimulated and the average stimulated SFR is 1.5–7 mL/min [2,3]. Salivary components are reported to vary due to oral conditions such as periodontitis, lichen planus, and oral submucous fibrosis, and salivary flow, pH, and composition are critical for maintaining the health of the oral cavity [4–7]. Systemic conditions such as Diabetes Mellitus, hypertension, Grave's disease, and osteoporosis are also reported to influence salivary flow and constituents [8].

Diabetes Mellitus (DM) is caused by poor glycaemic control, and it is a risk factor for the multi-system disorder. According to the International Diabetes Foundation, Diabetes Atlas 9th Edition, Pakistan has reached a diabetic prevalence of 17.1%. It has been estimated that 19 million adults in Pakistan will be suffering from diabetes by 2025, which will increase the risk of life-threatening complications [9]. Diabetes Mellitus type I (T1DM) is due to the primary failure of the pancreatic beta cells to produce insulin, while Diabetes Mellitus type II (T2DM) is caused by a failure of the insulin signaling mechanism in the target cells of the body. A reduction in salivary flow rate, pH, and xerostomia has been reported in patients with T2DM, which is a potential cause of increased incidence of caries and oral infections [10]. Periodontitis is an inflammatory condition of periodontal tissues and its incidence in the Pakistani population is reported to be 25.9% [11]. Poor glycemic control in T2DM increases the risk of periodontitis by approximately three-fold when compared to non-diabetic patients [12]. It has been reported that patients with T2DM have increased levels of advanced glycation end products (AEGs) and inflammatory cytokines in their serum and periodontal tissues, which influences the periodontal tissues and exacerbates the process of periodontitis [13]. With an increase in studies on salivary diagnostic procedures, changes in the levels of amylase and glucose have been studied, showing increased salivary amylase levels among T2DM people [14–16]. Hypertension (HTN) is another chronic condition, which is a risk factor for cardiovascular disorders, cerebrovascular disease, and renal disorders with a high mortality rate [17]. HTN is a cause of the deteriorated periodontal condition of the oral cavity and is one of the potential causes of gingival inflammation, bleeding, and chronic periodontitis leading to tooth loss [18]. A recent systematic review confirmed a positive relationship between HTN and periodontitis and reported an increased incidence of severe periodontitis in patients with HTN [14]. Similarly, an improvement in periodontal health leads to a reduction in blood pressure [19].

Alterations within the salivary components have also been reported in hypertensive patients, and Strahler et al. suggested a significant influence of antihypertensive drugs on the salivary amylase levels [20]. Mucins are an important component of saliva and perform the function of lubrication as well as microbial binding. The mucins line the oral mucosa, prevent direct damage to the mucosa, and play a role in caries reduction [21]. Patients suffering from T2DM and hypertension have reported an increased incidence of caries and oral infections [22]. It has been reported that periodontitis decreases the salivary flow rate and causes an increased output in protein levels, particularly mucins, which is believed to impart a protective influence to prevent oral infections [23].

Total protein levels (TPL) in saliva are greatly influenced by T2DM; however, the influence of hypertension on the TPL in saliva is contradictory. There is not enough

literature to report a difference in salivary TPL in patients suffering from hypertension. Iqbal et al. conducted a study on total protein concentrations of diabetic patients and found considerable variations in the levels when compared to healthy subjects [24]. Studies have also shown that providing periodontal therapy significantly reduces the increased protein levels and supports managing DM and HTN [25].

Periodontal parameters such as periodontal pocket depth (PPD), clinical attachment loss, and bleeding on probing (BOP) were also checked in the patients. Moreover, periodontal epithelial surface area (PESA) and periodontal inflamed surface area (PISA) were also calculated to quantify the inflammatory burden on oral cavity caused by the two systemic diseases. PISA has emerged as a novel and quantifiable indicator of periodontal inflammatory burden and its association with systemic diseases [26].

To our knowledge from indexed literature, data pertaining to changes in the levels of salivary components due to T2DM and HTN is limited. The null hypothesis was that there is no significant difference in the levels of amylase, mucin, and total proteins in patients suffering from T2DM and HTN. The aim of the present study was to compare the salivary flow rate, salivary pH, levels of amylase, mucin, total protein concentration, and periodontal parameters in T2DM and HTN patients compared to healthy controls.

2. Materials and Methods

2.1. Ethical Considerations

The current study was performed in accordance with the declaration of Helsinki, and the Ethical Review Committee of the Bahria University provided ethical approval for the study (ERC 73/2020). The participants were informed of the steps involved in the study and written consent was obtained prior to recruiting them for research.

2.2. Study Participants and Grouping

The sample size was calculated (OpenEpi, v3) at a 5% margin of error, 95% confidence interval, and 80% expected levels of amylase compared with known levels in saliva from a previous study in diabetic patients. The total sample size was calculated to be 48 for both case and controls. The total sample was raised to 60 in order to compensate for dropouts [27]. The participants who fulfilled the inclusion criteria were recruited using consecutive sampling techniques from the dental outpatient department of Bahria University Dental College, Karachi, Pakistan. The participants were age-matched to rule out the influence of age on the study variables. The study population was divided into three groups, with 20 subjects in each group ($n = 20$): healthy controls and diabetic and hypertensive patients. The patients were assessed by the PI and SA and were recruited for the study after explaining the procedures and requirements. Informed consent was obtained, and the participants were then allotted to each group.

Healthy controls: Healthy patients above 40 years of age with no history of chronic illness (such as diabetes mellitus, hypertension, cardiac problems, and prolonged use of anti-inflammatory medications) or use of antibiotics within the last six months. Those who had had recent periodontal treatments, orthodontic treatments, implants, or other dental diseases were also excluded. The age and gender were kept similar between the other two groups to maintain the uniformity of the data. After taking a complete history of the subjects, a HbA1C test was conducted for T2DM. Furthermore, chairside evaluation of blood pressure was conducted for 3 days at different timings to rule out hypertension.

Diabetic patients: According to the prevalence data of Pakistani Population, T2DM is most often seen in middle-aged and older patients greater than 40 years old [28]. Therefore, the inclusion criteria were patients above 40 years of age with a history of T2DM for more than 3 years with HBA1C values $\geq$7% and $\leq$10%. This was done to maintain the homogeneity of the sample. The patients may or may not have been taking hypoglycemic medicines for controlling T2DM. Subjects with a recent history of use of antibiotics, anti-inflammatory medicines, or periodontal or orthodontic treatment were excluded from the study. The patients who were suffering from any complication related to T2DM or any

other chronic diseases such as hypertension or metabolic or nutritional disorders were excluded. Chairside evaluation of blood pressure using a sphygmomanometer was done on three different days to rule out hypertension.

Hypertensive patients: Hypertension is also prevalent in patients above 40 years of age in Pakistan [29]. Therefore, the inclusion criteria for the subjects were patients above 40 years of age with a history of hypertension for more than 3 years. Subjects with systolic pressure $\geq$ 140 mmHg and diastolic pressure $\geq$ 90 were included. Subjects who had a recent history of hospitalization due to any complications related to hypertension and cardiac issues, use of antibiotics, anti-inflammatory medications, blood thinners, or periodontal or orthodontic treatment were excluded. They were age-matched with the control and the diabetic group to maintain the homogeneity of the data. HbA1C was tested to rule out T2DM. Chairside measurement of the blood pressure was also performed on three different days and times using a sphygmomanometer.

The patients who were suffering from any other chronic illness, such as cardiac or renal disorders, were excluded from the study. After the consent of the patients was received, the oral examination was performed, and oral hygiene status and periodontal status were checked using the probe and recorded in the data collection proforma.

2.3. Unstimulated Whole Salivary (UWS) Collection

The UWS was collected in the early morning hours. The patients were advised not to eat or drink anything at least two hours before the saliva collection. Before collecting the saliva, participants were asked to rinse their mouth thoroughly using tap water to remove any debris or food particles. Saliva was collected in the container after 10 min of rinsing. The patients were then seated on the dental chair and were asked to allow the UWS to collect in their mouth for 5 min without swallowing. The UWS was expectorated in the graded measuring cylinder. The unstimulated salivary flow rate (UWSFR) was determined by dividing the amount of saliva expectorated (mm) and taking an average. The container was immediately placed in an icebox to preserve the samples before freezing at -80 °C. The samples were analyzed within 2 months of collection.

2.4. Assessment of pH, Amylase, Total Protein, and Mucin Concentration

The pH of the UWS was assessed by using a pH meter (Lutron^{TM} PH-223). The pH meter was dipped in the container for a few seconds and the reading was recorded in the data collection proforma. The estimation of salivary amylase was performed after making a slight modification to the method described by Ligtenberg et al., using ELISA [30]. All the salivary samples were thawed and diluted with normal saline. Preparation of buffered starch solution was performed by melting 0.4 g of soluble starch with a hot solution of 30 mM sodium chloride, 70 mM of sodium benzoate, and 200 mM disodium hydrogen phosphate. A volume of 1 mL of the buffered starch was placed within the tubes, placed in a water bath for 5 min at 37 °C. Twenty microliters of diluted saliva samples was placed in the test tube inside a vortex mixer and further incubated in a water bath (37 °C). Subsequently, 8 mL of distilled water and 1 mL of iodine solution (5 mM) was added, the contents were mixed carefully, and 250 mL was placed in a flat-bottomed ELISA plate reader (Biotech Synerygy) and its absorbance was assessed at 660 nm.

Lowry's method (1951) was used for the calculation of total protein concentration [31]. A working standard was prepared by adding 0.2 mL of Bovine serum albumin and distilled water within five test tubes, using one test tube containing distilled water as a blank. Subsequently, 4.5 mL of reagent 1 containing 1 mL of 0.5% CuSO4.5 H_2O in water, 48 mL of 2% Na_2CO_3 in 0.1 N NaOH, and 1 mL of 1% NaK Tartarate in water was supplemented, and incubation for 10 min was performed. A volume of 0.5 mL of reagent 2 encompassing one part of Folin–Ciocalteu's phenol reagent and water was incorporated and re-incubated for a further 30 min. The absorbance was analyzed at 660 nm, while the standard graphs were strategized by means of an ELISA plate reader.

Mucin was estimated using Alcian Blue technique [32]. The saliva samples were placed in the tubes and diluted with distilled water. Alcian blue solution (Sigma-Aldrich Corp, St Louis, USA) was added to 50 mM sodium acetate buffer and incubated at room temperature for 30 min. Subsequently, samples were placed for centrifugation for 20 min and 1 mL of 95% ethanol was vortexed for 10 s. One part Aerosol OT (Sigma-Aldrich Corp, St. Louis, MO, USA) and 2 parts distilled water were mixed, followed by adding an equivalent amount of ethyl ether. Saliva samples were centrifuged for 15 min and analyzed on an ELISA plate reader at a wavelength of 605 nm. Concentration levels of the salivary mucin were then calculated using the standard graph.

2.5. Clinical Periodontal Inflammatory Parameters

Intra-oral examination was performed by a calibrated examiner (SA) to evaluate the periodontal parameters PPD, CAL, and BOP at six sites per tooth except for the 3rd molars. The examiner was calibrated with a trained periodontist on 10 patients, the readings were taken by both the examiners, and inter-examiner reliability was calculated using Kappa Statistics and the agreement was found to be excellent (94.3%). PPD (rounded off to the nearest mm) was measured as the distance from the cementoenamel junction to the bottom of the pocket (mm). BOP was assessed as present or absent. These measurements were performed in all the quadrants using a UNC 15 probe in six sites per tooth (mesiobuccal, mid-buccal, distobuccal, distolingual/distopalatal, mid-lingual/mid-palatal, mesiolingual/mesiopalatal). For classification of periodontitis, the criteria proposed by the Centers for Disease Control and Prevention and American Academy of Periodontology (CDC/AAP) was used [33,34]. Severe periodontitis was defined as at least 2 sites with CAL of $\geq$6 mm (on 2 different teeth) or at least 1 site with PPD $\geq$ 5 mm. Moderate periodontitis was indicated by the presence of CAL of $\geq$4 mm or at least 2 sites with PPD of $\geq$5 mm (on 2 different teeth). Mild periodontitis was indicated in cases with at least 2 sites with a CAL $\geq$ 3 mm and PPD $\geq$ 5 mm (on 2 different teeth). Healthy patients were categorized by the presence of BOP in less than 6 sites or completely absent, PPD less than 4 mm, and CAL 0–2 mm at less than 6 sites. Fractured teeth or broken-down roots were not assessed.

PESA was calculated using equations for each tooth type with CAL and PPD. PISA is the sum of PPD on BOP positive sites. Both parameters were calculated using MS Excel Spreadsheet from a previous study (www.parsprototo.info) (accessed on 20 June 2021) [35,36] and the mean values were then entered in SPSS (Version 22, IBM, NY, USA) for analysis.

2.6. Statistical Analysis

Statistical Analysis was performed using statistical program for social sciences (SPSS version 21). ANOVA was applied to evaluate the difference in mean salivary flow rates, pH, mucin, amylase, total protein concentrations and the effect of medications among the groups. The Chi-squared test was applied to calculate the differences in periodontal parameters among the groups.

3. Results

Gender distribution among the healthy, T2DM, and HTN groups ranged from 40% to 60% (Table 1). The mean age among healthy, T2DM, and HTN patients were 54 $\pm$ 4.8, 54.4 $\pm$ 4.1, and 52.4 $\pm$ 6.2 respectively. The mean unstimulated salivary flow rate among healthy, T2DM, and HTN subjects was significantly different (p = 0.002). The mean salivary flow rate was significantly (p-value = 0.002) lower among T2DM patients (0.78 $\pm$ 0.45) in comparison to healthy (1.52 $\pm$ 0.62) and HTN (1.07 $\pm$ 0.7) subjects. The salivary flow rate among HTN was lower ($p < 0.05$) than healthy subjects but higher ($p < 0.05$) than T2DM subjects. Diabetics (5.9 $\pm$ 0.5) had the lowest salivary pH when compared to healthy (7.2 $\pm$ 0.15) and HTN (6.3 $\pm$ 0.27) patients. Among the subjects, 75% of the HTN subjects used anti-hypertensive drugs while 80% of the T2DM individuals used hypoglycemic drugs to control their blood sugar levels.

Table 1. Descriptive statistics, mean salivary flow rate, and salivary pH among the groups.

	Healthy	T2DM	HTN	*p*-Value
Gender				
Males	10 (50%)	12 (60%)	11 (55%)	
Females	10 (50%)	8 (40%)	9 (45%)	
Mean age (in years)	54 ± 4.8	54.4 ± 4.1	52.4 ± 6.2	
Salivary flow rate (mL/min)	1.52 ± 0.62	0.78 ± 0.45	1.07 ± 0.7	0.002 *
Salivary pH	7.2 ± 0.15	5.9 ± 0.5	6.3 ± 0.27	<0.001 **
Drugs				
None	0 (100%)	5 (25%)	4 (20%)	
Anti-hypertensive	0 (100%)	15 (75%)	0 (0%)	<0.001 **
Hypoglycemic	0 (100%)	0 (0%)	16 (80%)	

T2DM—Type II diabetes mellitus; HTN—Hypertension; * level of significance at 5%; ** level of significance at 1%.

A significant difference was observed in salivary mucin levels between the groups ($p < 0.001$) (Table 2). Mucin levels were significantly higher ($p < 0.05$) in HTN patients (4.6 ± 1.17) compared to T2DM (3.59 ± 1.03) and healthy (2.26 ± 1.09) subjects. Amylase levels were significantly higher among healthy subjects (1.76 ± 0.75) compared to both HTN (1.33 ± 1.0) and T2DM (0.88 ± 0.57) patients. T2DM patients showed the lowest amylase levels compared to HTN and healthy patients. There was a statistically significant difference for total protein concentration between the study groups ($p < 0.001$). Total protein concentration was significantly raised (*p*-value < 0.001) in T2DM (37.67 ± 3.12) compared to healthy (29.3 ± 3.22) subjects (Table 2).

Table 2. Levels of mucin, amylase, and total proteins among the study groups.

Parameters	Healthy	HTN	T2DM	*p*-Value
Mucin (units/mL)	2.26 ± 1.09	4.6 ± 1.17	3.59 ± 1.03	<0.001 **
Amylase (mg/mL)	1.76 ± 0.75	1.33 ± 1.0	0.88 ± 0.57	0.004 *
Total Proteins (mg/mL)	29.3 ± 3.22	31.3 ± 5.28	37.67 ± 3.12	<0.001 **

T2DM—Type II diabetes mellitus; HTN—Hypertension; * level of significance at 5%; ** level of significance at 1%.

Table 3 presents the USWFR, mucin, amylase, and total protein concentration among the patients on hypoglycemic and antihypertensive drugs. It was observed that the hypoglycemic drugs significantly reduced the salivary flow ($p = 0.026$) and salivary pH levels ($p < 0.001$). Mucin levels were significantly raised in the subjects taking anti-hypertensives ($p = 0.007$), while amylase levels were significantly reduced with the use of hypoglycemic drugs ($p = 0.006$). The total protein concentration was significantly raised in subjects taking hypoglycemic drugs ($p < 0.001$).

Table 3. Effect of anti-hypertensive and hypoglycemic drugs on salivary flow rate, salivary pH, levels of mucin, amylase, and total protein concentrations.

Medication	No Medication	Anti-Hypertensive	Hypoglycemic	*p*-Value
Salivary flow rate (mL/min)	1.36 ± 0.72	1.03 ± 0.7	0.8 ± 0.46	0.026 *
Salivary pH	6.98 ± 0.5	5.6 ± 0.28	5.3 ± 0.42	<0.001 **
Mucin (units/mL)	3.08 ± 1.67	4.6 ± 1.11	3.14 ± 1.86	0.007 *
Amylase (mg/mL)	1.67 ± 0.93	1.13 ± 0.7	0.88 ± 0.57	0.006 *
Total proteins (mg/mL)	30.7 ± 4.6	31.5 ± 5.2	37.7 ± 3.24	<0.001 **

* level of significance at 5%, ** level of significance at 1%.

Significant differences in BOP, CAL, and PPD were observed (*p*-value < 0.001), as shown in Table 4. The values of PESA and PISA are significantly different between the three groups (*p*-value < 0.001) and indicate a high inflammatory burden in diabetic patients compared to other groups.

Table 4. Severity of periodontitis based on CDC/AAP classification, PESA, and PISA values.

Parameters	Control	Hypertensive	Diabetic	*p*-Value
Periodontitis severity				
None	18 (90%)	2 (10%)	1 (5%)	
Slight	2 (10%)	5 (25%)	6 (30%)	<0.001 **
Moderate	0 (0%)	8 (40%)	9 (45%)	
Severe	0 (0%)	5 (25%)	4 (20%)	
PESA (mm^2)	1722.5 ± 135.7	3157.09 ± 242.33	2789.3 ± 201.7	<0.001 **
PISA (mm^2)	96.9 ± 14.91	1029 ± 234.6	799.4 ± 155.05	<0.001 **

** level of significance at 1%. Values of PISA and PESA computed using One-Way ANOVA test. Severity of periodontitis calculated on the basis of CDC/AAP classification; PESA—Periodontal epithelial surface area; PISA—Periodontal inflammatory surface area.

4. Discussion

Saliva is a dynamic fluid that reflects oral and systemic health. This investigation aimed to compare the salivary flow rate; salivary pH; levels of amylase, mucin, and total protein concentration; and periodontal parameters in T2DM and HTN patients with healthy controls. Significant differences were observed in salivary mucin, amylase, total protein, and periodontal parameters in T2DM and HTN patients. Therefore, the null hypothesis was rejected.

Reduced SFR is associated with multiple systemic disorders, including T2DM. It has been reported that persistent hyperglycemia adversely affects the function of salivary glands, causing a reduction in the production of saliva [37]. This was further confirmed in this study, as the SFR of diabetic subjects was significantly lower than the flow rates of healthy and HTN subjects. The findings of this study are similar to the findings of previous studies which assessed SFR in diabetics [38]. Medication has also been reported to affect SFR and pH. In the present study, it was observed that the use of hypoglycemic drugs had significantly reduced the UWSFR and the salivary pH. These findings are contradictory to the study conducted by Fyhaa et al., reporting that hypoglycemic drugs do not produce any significant effect on the SFR and TPL [39]. The levels of mucin in subjects using antihypertensive medicines were significantly raised when compared to other groups. However, levels of amylase were reduced in T2DM patients when compared to the healthy controls. The mucin levels of subjects using hypoglycemic drugs were slightly raised in comparison to healthy subjects. The TPL was significantly raised in subjects taking hypoglycemic drugs. Literature related to the influence of hypoglycemic drugs on SFR and salivary composition is limited.

HTN and antihypertensive medicines have also been associated with a decreased SFR [40]. In this study, the reduced SFR was also associated with hypertension, and a significant reduction was observed in subjects using anti-hypertensive drugs. Similar findings were observed in the studies conducted by Merlin et al. [41]. Mohiti et al. also reported that SFR and pH of saliva are significantly affected in hypertensive patients [42]. Decreased salivary flow rate causes hyposalivation, which increases the acidity of saliva [43]. As the quantity and pH of saliva is reduced, the saliva turns viscous and adversely affects the oral health and quality of life of the patients [44]. These findings are in agreement with the studies conducted by Villa et al. [45] and Risdiana et al. [46], who reported that reduction in SFR is associated with a compromise in the buffering capacity of saliva, which is associated with inflammation of the oral cavity, rampant caries, and difficulty in swallowing. It is important to educate such patients about oral care to prevent the negative impacts of these diseases on the oral cavity.

The negative impact of hyposalivation and acidic pH was confirmed in this study by further investigations of the periodontal perimeters. The periodontal inflammatory parameters significantly differed from the healthy group in both diabetics and hypertensives. Diabetes is a risk factor for periodontitis [47]. BOP and chronic periodontitis are associated with hypertension according to many studies, and this was further confirmed in the results

of the present study [14]. Periodontitis has also been implicated as a possible contributing factor to hypertension. The CAL and PPD significantly differed among the groups. The hypertensive and diabetic groups showed an increased prevalence of CAL and PPD, which is indicative of chronic periodontitis. These findings are similar to previous investigations showing an increased association of diabetes and hypertensives with increased incidence of periodontitis [48]. Furthermore, the values of PISA indicate that oral health was significantly hampered due to T2DM and HTN. The values were higher in T2DM subjects, which shows that DM has a significant negative impact on the oral health of a person. These findings are similar to a recent study that determined that a greater estimate of PISA was significantly linked with DM [33]. PISA values were higher in HTN patients as well, which is also similar to the study conducted by Pietropaoli et al., who determined high PISA values in HTN patients [49]. These inflammatory conditions produce a change in salivary inflammatory markers and affect the function of several other components. This was confirmed by the changes produced in mucin, amylase, and TPL levels in the saliva of T2DM and HTN subjects in this study.

Mucins in saliva are a heterogeneous group of glycoproteins produced by the salivary glands. They play an important role in maintaining the viscoelastic properties and help in the clearance of microbes. Mucin levels were raised in both diabetic and hypertensive patients. The highest levels of mucin were observed in the hypertensive group. High mucin levels are indicative of increased viscosity of saliva with low SFR. The study conducted by Menicagli et al. reported that mucin levels can be used to predict the hereditary predisposition for the development of diabetes [48]. The study further reported that although there is an increase in levels of mucins in diabetic patients, instead of imparting a protective action on oral cavity, it causes a strong feeling of dry mouth. This is mainly due to increased formation of MUC5B and MUC7 proteins, which overcomes the effects of MUC1 in the oral cavity, which forms the protective layer within the oral cavity.

The levels of TPL were also raised in T2DM and HTN subjects; however, diabetics showed the highest levels among the groups. Contradictory results have been observed in previously conducted studies. Some studies reported a higher level while some stated a reduction in the levels of TPL in diabetics [50,51]. It has also been suggested that there is no significant difference in TPL among diabetics and healthy individuals. The TPL levels indicate the presence of chronic inflammation, and raised levels might be responsible for the presence of chronic inflammation of the periodontium in T2DM and HTN patients [52]. In their research, Hasan et al. reported that the TPL levels were increased in the saliva of the diabetics, but their levels were significantly decreased in serum [53]; however, periodontal health of the patients was not evaluated. In addition, increased protein levels have been associated with an increase in the permeability of the basement membrane due to the presence of DM and HTN, also associated with periodontal disease [54]. This enhances the leakage of proteins through the gingival crevice, causing an increase in TPL levels.

In contrast to mucins and TPL, the amylase levels were reduced in the study groups when compared to the healthy group. Amylase performs the hydrolyzation of 1–4 glycosidic bonds and produces maltose and maltotriose [55]. It has been reported that higher levels of salivary amylase are helpful in maintaining blood glucose levels, thus reducing the risk for the development of DM [16]. It is pertinent to mention that a reduction in amylase levels among T2DM subjects was observed in the current study. Mandel et al. reported that patients with lower salivary amylase levels have a greater risk of insulin resistance and the development of T2DM [56]. They also identified that amylase levels vary with dietary habits. Unfortunately, the literature on assessing the amylase levels in hypertensive patients and the effects of antihypertensive drugs on amylase is limited. In a study conducted by Strahler et al., an increase in salivary amylase levels in hypertensive patients was observed. This is contrary to the findings of the present study, probably due to heterogeneity in the methodology of the compared investigations [20].

The current study was a cross-sectional, single-center study conducted on a limited number of subjects. Moreover, periodontitis itself manifests several changes in SFR, pH,

and levels of proteins; therefore, it is difficult to establish whether T2DM and HTN are the cause of changes in SFR, pH, and levels of proteins. Therefore, future investigations, including multi-center randomized controlled trials with an increased sample size and different but comprehensive study groups should be conducted to further confirm the findings of the present study.

5. Conclusions

The study showed a significant reduction in salivary flow rate, pH, and amylase levels in T2DM and HTN patients. Mucins were significantly raised in both T2DM and HTN patients, while amylase was higher among healthy controls compared to HTN and T2DM patients. The incidence of moderate to severe periodontitis was observed in the HTN and T2DM groups.

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