Salivary flow rate and oral health status in type 2 diabetics

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SUMMARY
Introduction Decreased salivary flow is frequently associated with numerous diseases such as diabetes mellitus and may lead to numerous oral diseases. The aim of this study was to compare salivary flow rate and oral health status in type 2 diabetics and healthy controls.

Material and methods The study involved 90 patients, divided into the three groups: 30 with well controlled (HbA1c<9%), 30 with poorly controlled (HbA1c≥9%) diabetes and 30 healthy subjects. The following clinical parameters were determined: decayed, missing and filled teeth (DMFT); plaque index (PI), sulcus bleeding index (SBI), probing pocket depth (PPD) and clinical attachment level (CAL). Culture of Candida spp. specimens were obtained from tongue dorsum and inoculated into Sabouraud Dextrose Agar. Saliva was collected using “a spit technique”.

Results Highest mean of unstimulated salivary flow was in healthy subjects; however significant difference between groups was not observed. Stimulated salivary flow results indicate significant reduction in diabetics as well as significant relation between metabolic control and salivary flow. Unstimulated and stimulated salivary flows were negatively and significantly correlated with periodontal parameters and DMFT.

Conclusion The present findings indicate that decreased salivary flow rate could have a significant impact on oral health status in type 2 diabetics.

Keywords: diabetes mellitus; salivary flow; dental caries; periodontitis; candidiasis

INTRODUCTION
Diabetes mellitus (DM) is metabolic syndrome characterized by chronic hyperglycemia caused by absolute or relative lack of insulin. Chronic hyperglycemia leads to many complications, which underlines the importance of adequate metabolic control. Glucose metabolism control significantly impacts the extent and severity of diseases associated with diabetes including those in oral cavity.

Saliva is biological fluid of fundamental importance for the preservation of oral health. Consequently, decreased salivary flow is frequently associated with numerous oral diseases. There is clear evidence that the prevalence, severity and progression of periodontal disease are higher in diabetics, although mechanisms for such association are not clearly understood [1, 2]. The main etiological factor for the development of periodontal disease is dental plaque (biofilm). Increased amount of plaque in patients with diabetes is a result of increased salivary glucose and decreased salivary secretion. Bacteria from biofilm appear to act directly or indirectly, via cell and humoral components of specific and non-specific host responses [3]. It is well known that periodontal disease can have negative impact on metabolic control and the incidence of diabetes complications, but also the treatment of periodontal disease can favorably affect glycemic regulation [4, 5]. The role of saliva in the maintenance of tooth integrity is also of great importance, as confirmed by Leone et al. [6]. The authors examined the influence of saliva on the occurrence as well as development of dental caries and concluded that the flow of saliva, undoubtedly, presents most important factor for the development of cavities. Due to reduced secretion of saliva, caries lesions develop rapidly affecting even the places that are not caries susceptible. A review of the literature reveals reduced salivary flow rate in patients with diabetes [7], which could explain increased dental caries incidence in this population. Some of the early, nonspecific signs of poorly-controlled diabetes include oral candidiasis and other opportunistic infections [8]. Oral candidiasis is frequently a sign of systemic immunosuppression. In fact, reduced salivary secretion combined with high concentration of glucose in saliva can accelerate the growth of fungi and their adherence to oral mucosal epithelial cells. Oral candidiasis is reported to be more prevalent especially in diabetic denture wearers [9], who do smoke and have poor glycemic control [10].

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The aim of this study was to compare the salivary flow rate and oral health status in type 2 diabetics and healthy controls.

**METHODS**

**Study design and participants**

The study involved 90 patients, 60 with type 2 diabetes and 30 without diabetes (control subjects), aged 45-65 years. With respect to level of HbA1c diabetic subjects were divided into the two groups: 30 better-controlled (HbA1c<9%) and 30 poorly-controlled (HbA1c≥9%), recruited from the Department of Endocrinology University Hospital Foca, Bosnia and Herzegovina. The 9% of HbA1c cut-off point has been suggested to represent an indicator for ineffective blood glucose management in type 2 diabetes [11]. The control group consisted of 30 healthy subjects who visited Dental Clinic, Faculty of Medicine Foca, University East Sarajevo, for regular checkups. The study was approved by the institutional committee of ethics (No. 01-8/140) and was conducted in accordance with the Helsinki Declaration of 1975, as revised 1983. The presence of severe mental or systemic disorder, pregnancy, signs or symptoms of AIDS and antibiotic administration during the last 6 months were exclusion criteria in this study. After the study was explained to the patients, written informed consent was obtained from all patients recruited in the study.

Oral clinical examination was performed at the Dental clinic, Faculty of Medicine Foca, University of East Sarajevo, Bosnia and Herzegovina according to WHO criteria [12]. The examination was conducted using a dental mirror and both dental and periodontal probes. The following was determined: decayed, missing and filled teeth (DMFT); plaque index (PI), sulcus bleeding index (SBI), probing pocket depth (PPD) and clinical attachment level (CAL). Periodontal parameters were assessed at four sites around each tooth (mesiobuccal, distobuccal, mesiolingual and distolingual locations). Culture specimens to *Candida* spp. were obtained from dorsum of the tongue using a sterile cotton-tipped swab and inoculated into Sabouraud Dextrose Agar for 48 hours.

All patients were asked to abstain from eating for 2 hours before saliva collection [13]. Both unstimulated and stimulated saliva were collected using “a spit technique”. Stimulated saliva was collected using 10% citric acid that was dropped onto the tongue [14]. Each patient was instructed to sit in dental chair with head tilted forward and instructed not to speak, do any head movements or swallow any saliva if present in the mouth during the procedure. After that, the patients were asked to spit in a sterile cup every minute for 5 minutes. Salivary flow was calculated in ml/min.

**Statistical analyses**

All statistical analyses were performed using SPSS version 19.0 for Windows. Results were expressed as mean values ± standard deviation (SD). The differences between the groups were assessed by ANOVA or chi-square test. Relationships between variables were evaluated by Pearson correlation coefficient. The value of p<0.05 was considered statistically significant.

**RESULTS**

Sociodemographic and clinical characteristics of all subjects are presented in Table 1. Study included 64 (57.8%) female and 26 (42.2%) male subjects. The mean age of the study population was 57.58±7.19. The healthy subjects had slightly more unstimulated salivary flow rate than well-controlled diabetics, although the difference was not statistically significant. Poorly-controlled diabetics had statistically significantly lower unstimulated and stimulated salivary flow rate than healthy control (Table 2).

Table 1. Sociodemographic and clinical characteristics of patients

| Parameter | HbA1c < 9% | HbA1c ≥ 9% | HS | p |
|-----------|------------|------------|----|---|
| Age (years) (X±SD) | 59.50 ± 6.64 | 60.73 ± 5.89 | 52.50 ± 6.27 | *p* < 0.05 |
| Gender (%) | Male | 53.3 | 43.3 | 30 | p*** > 0.05 |
| | Female | 46.7 | 56.7 | 70 |
| Diabetes duration (years) (X±SD) | 8.57 ± 7.44 | 9.18 ± 6.97 | - | p** > 0.05 |
| HbA1c (X±SD) | 7.67 ± 1.02 | 10.54 ± 1.59 | - | p** < 0.05 |

*ANOVA; **t-test; ***χ² test
HS – healthy subjects
ZO – zdrave osobe
teeth and higher mean value of DMFT index than healthy control, but statistically significant differences between the groups were not observed. Also, the difference in prevalence of missing and filled teeth was statistically significant between the groups.

Regarding the frequency of oral candidiasis, there was statistically significant difference between poorly controlled diabetics and healthy subjects. No statistically significant difference was found between well-controlled diabetics and healthy subjects in regards to candida isolation (Table 3).

Pearson correlation analyses revealed statistically significant negative correlation between unstimulated salivary flow rate (USFR) as well as stimulated salivary flow rate (SFR) and DMFT index, plaque index (PI), sulcus bleeding index (SBI), clinical attachment loss (CAL) and probing pocket depth (PPD). Negative but non-significant correlation was observed between USFR as well as SFR and oral candidiasis (Table 4).

Table 3. Mean values of USFR and SFR in subjects with different metabolic control of diabetes mellitus type 2 and healthy patients

|           | HbA1c < 9% | HbA1c ≥ 9% | HS | ZI | p*            |
|-----------|------------|------------|----|----|---------------|
| USFR (ml/min) | 0.23 ± 0.14 | 0.19 ± 0.11 | 0.30 ± 0.16 | 1:3 > 0.05         |
| PNSP (ml/min) |             |            |    |    | 2:3 > 0.05    |
| SFR (ml/min)  | 0.70 ± 0.34 | 0.60 ± 0.33 | 0.85 ± 0.33 | 1:3 < 0.01         |
| ZI – zdravi ispitanici |

Table 4. Correlation of USFR and SFR with parameters of oral health

|           | USFR | SFR |
|-----------|------|-----|
| PNSP      | 0.239** | 0.329** |
| SFR       | 0.202** | 0.271** |

DISCUSSION

Several studies reported reduced salivary secretion of both unstimulated and stimulated saliva in diabetics [15, 16]. The pathogenic mechanisms linking diabetes and hyposalivation are not fully understood. Dehydration as a result of prolonged hyperglycemia and resultant polyuria is considered to be the main cause of salivary glands hypo-function. However, dehydration by itself cannot explain functional changes in salivary glands. It is believed that the two most common degenerative complications of diabetes, neuropathy and microangiopathy are crucial for pathologic changes in the structure of salivary glands [17]. Increased concentration of calcium in parotid and submandibular saliva can explain higher prevalence of sialolithiasis in diabetics and consequently oligosalia. Also, influence of glycemic control on salivary flow is still controversy [11, 16]. The present findings show that the mean USFR was highest in healthy subjects, but there was no significant difference between groups. Similar results were presented in the study of Panchbhai et al. [19]. Results of our study indicate statistically significant SFR reduction in diabetics and significant corelation between metabolic control and salivary flow. Our results are in agreement with the study of Chavez et al. that also confirmed this relationship [20].

Recent studies clearly indicated that diabetes is an important risk factor for periodontitis [21, 22]. Diabetes is considered to promote periodontal disease through an exaggerated inflammatory response to the periodontal pathogens [23]. Some studies indicate that although diabetes presents a risk factor for periodontitis, periodontitis may, on the other hand, have a negative effect on the metabolic control of diabetes. [4, 5, 24]. Results of our study demonstrated
deeper periodontal pockets, more attachment loss, more bleeding on probing and higher mean value of plaque index in poorly-controlled diabetics. These results are in accordance with the findings of Mohamed et al. [25]. Moreover, our findings indicated that both, unstimulated and stimulated salivary flow rate, were negatively and significantly correlated with periodontal parameters. It has been shown that diabetic patients with xerostomia are more susceptible to periodontal infection [26]. Some studies confirmed that periodontal disease was strongly related to salivary flow rate [27].

Previous studies reported contradictory results about relationship between dental caries and diabetes mellitus. Our results showed significantly increased number of decayed teeth in poorly controlled diabetics. Similar results were obtained in study by Bakhshandeh et al. where subjects with better glycemic control had significantly lower number of decayed teeth compared to those with poor glycemic control [28]. In contrast, Syrjälä et al. revealed no association between the HbA1c level and dental caries [29]. Apart from metabolic control of diabetes, development of dental caries is affected by many other factors, among which dental plaque presence is the most important. It is well established that development of caries is a result of metabolic events in dental plaque over time and that it can be increased in terms of impaired function of saliva. High glucose level in saliva and gingival crevicular fluid can cause increase of saliva cariogenic organisms in both supragingival and subgingival plaque in diabetics [30]. Salivary flow reduction leads to impaired antimicrobial actions of saliva as well. In our study, salivary flow rate was negatively and significantly correlated with DMFT, in contrast to the results of study by Karjalainen et al. [31]. Moore et al. reported an association between a low salivary flow rate and slightly increased incidence of dental caries [32]. As a contradictory result, Collin et al. reported higher prevalence of dental caries among those with higher salivary flow rate [33].

According to the literature, prevalence of oral candidiasis was reported to be higher in patients with diabetes type 2 compared to healthy persons [34, 35]. In addition to changes in the composition and quantity of saliva, the presence of infection with Candida spp. in type 2 diabetics is also associated with impaired cellular immunity. The high prevalence of Candida spp. is especially pronounced in diabetic denture wearers. Candida spp. can co-aggregate with bacteria in biofilm of denture surface that than become a reservoir of aforementioned microorganisms with further potential to colonize oral mucosa. It has been estimated that 33.3% of diabetics in our study were diagnosed with oral candidiasis and that is in accordance with the study by Shenoy et al. [36]. Similar results were reported by Guggenheimer et al. [37]. All those findings support the role of diabetes mellitus as a predisposing factor for increased Candida spp colonization of oral mucosa. In accordance with the study of Navazesh et al. [38], results of our study also showed negative correlation between salivary flow rate and oral candidiasis although without statistical significance.

CONCLUSION

In conclusion, within the limitation of the cross-sectional study design, our study demonstrated that decreased salivary flow rate could have a significant impact on oral health status in patients with diabetes mellitus type 2. Due to the importance of saliva in the maintenance of tooth integrity and oral health in general, management of oral diseases in diabetics should include a comprehensive evaluation of salivary function.

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UVOD
Dijabetes melitus (DM) metabolički je sindrom koji se karakteriše hroničnom hiperglikemijom nastalom zbog relativnog ili apsolutnog nedostatka insulina. Hronična hiperglikemija vodi mnogim komplikacijama, što ukazuje na važnost adekvate metaboličke kontrole. Kontrola metabolizma glukoze značajno utiče na obim i težinu bolesti udruženih sa dijabetesom.

Pljuvačka je biološka tečnost koja je od fundamentalne važnosti za očuvanje oralnog zdravlja. Smanjen protok pljuvačke je često dovodi u vezu sa brojnim oboljenjima, kao što je dijabetes melitus, i može dovesti do sistemske imunsupresije. Zapravo, smanjeno lučenje pljuvačke može učestalost dijabetesa, kao i značajnu vezu između metaboličke kontrole i protoka pljuvačke. Protok nestimulisane i stimulisane pljuvačke je pokazao značajnu negativnu korelaciju sa parodontalnim parametrima i KEP-om.

Zaključak
Rezultati ove studije ukazuju da smanjen protok pljuvačke može imati značajan uticaj na status oralnog zdravlja kod obolelih od dijabetesa.

MATERIJAL I METODE RADA
Dizajn studije i učesnici
U studiju je uključeno 90 pacijenata, 60 obolelih od dijabetesa tipa 2 (kontrolnih ispitanika) i 60 obolelih od dijabetesa tipa 1 (kontrolnih ispitanika). U odnosu na nivo HbA1c ispitanici sa dijabetesom su podeljeni na dve grupe: 30 bolje kontrolisanih (HbA1c < 9%) i 30 loše kontrolisanih (HbA1c ≥ 9%).

Materijal i metode rada
U studiji je uključeno 90 pacijenata, 60 obolelih od dijabetesa tipa 2 (kontrolnih ispitanika) i 60 obolelih od dijabetesa tipa 1 (kontrolnih ispitanika). U odnosu na nivo HbA1c ispitanici sa dijabetesom su podeljeni na dve grupe: 30 bolje kontrolisanih (HbA1c < 9%) i 30 loše kontrolisanih (HbA1c ≥ 9%).
fakulteta u Foć, Univerziteta u Istočnom Sarajevu radi redovnih kontrolnih pregleda. Studija je odobrena od strane institucional-
og etičkog komiteta (No. 01-8/140) i sprovedena je u skladu sa Helsinškom deklaracijom iz 1975, revidiranom 1983. Prisustvo
tekših mentalnih ili sistemskih poremećaja, trudnoća, znaci ili
symptomi AIDS-a i primena antibiotika tokom poslednjih šest
meseći su bili kriterijumi za isključenje u ovoj studiji.

Nakon što je studija objašnjena pacijentima, pacijenti uključeni
u ovo istraživanje su potpisali saglasnost za učešće u studiji.

Oralni klinički pregled je obavljen na Stomatološkoj klinici
Medicinskog fakulteta u Foć, Univerziteta u Istočnom Sarajevu,
Bosna i Hercegovina, u skladu sa kriterijumima SZO [12]. Pregled
je obavljen uz korišćenje stomatološkog ogledala kao i obe,
and stomatološke i parodontalne, sonde. Određeni su sledeći parametri:
kariorzii, ekskrahosvani plombirani zubi (KEP), indeks plaka (IP),
indeks krvarenja gingive (IKG), dubina parodontalnog džepa
(DPDŽ) i nivo pripojnog epitela (NPE). Parodontalni parametri
su procenjeni na četiri mesta oko svakog zuba (meziobukalno,
distobukalno, meziolingvalno i distolingvalno lokalizaciji). Kulture
uzoraka Candida spp. su prikupljene sa dorzuma je-
zika, oralni klinički pregled je obavljen na Stomatološkoj klinici
u ustima tokom procedure. Posle toga, pacijenti su zamoljeni da
pljačku prisutnu
kiseline na jezik [14]. Svaki pacijent je upućen da se suzdrže od jela dva sata pre

Sociodemografske i kliničke karakteristike svih ispitanika su
predstavljene u Tabeli 1. Studija je obuhvatila 64 (57,8%) žen-
ska i 26 (42,2%) muških ispitanika. Srednja vrednost starosti
populacije studije je bila 57,58 ± 7,19.

Zdravi ispitanici su imali nešto veći protok nestimulisane
pljuvačke nego dobro kontrolisani dijabetičari, iako razlika nije
bila statistički značajna. Loše kontrolisani dijabetičari su imali
značajno niži protok nestimulisane i stimulisane pljuvačke nego
dvrede kontrolisanih. Bolesnici sa dijabetesom tipa 2 i lošom metabo-
likom kontrolom su imali značajno više karioznih zuba i veće
vrednosti indeksa KEP od zdravih ispitanika. Dobo kontrolosani
dijabetičari su imali više karioznih zuba i veće vrednosti indeksa
KEP od zdravih ispitanika, ali nisu primećene statistički značajne
razlike između grupa. Takođe, razlike u učestalosti ekstrahovanih
i plombiranih zuba je bila statistički značajna između grupa.

Posmatrajući učestalost oralne kandidijaze, uočena je stati-
tički značajna razlika razlike između loše kontrolisanih dijabetičara
i zdravih ispitanika. Statistički značajna razlika nije nađena
između dobro kontrolisanih dijabetičara i zdravih ispitanika u
odnosu na izolaciju kandide (Tabela 3).

Analiza Pinsonove korelacije je otkrila statistički značajnu
negativnu korelaciju između PNSP, kao i PSP i indeksa KEP,
indeksa plaka (IP), indeksa krvarenja gingive (IKG), nivoa
pripojnog epitela (NPE) i dubine parodontalnog džepa (DPDŽ).
Negativna korelacija je uočena između PNSP kao i PSP i oralne
kandidijaze, ali nije bila statistički značajna (Tabela 4).

DISKUSIJA

Nekoliko studija je objavilo prisustvo smanjene sekrecije
i nestimulisane i stimulisane pljuvačke kod dijabetičara [15,
16]. Patološki mehanizmi koji povezuju dijabetes i hiposali-
vaciju nisu potpuno razjašnjeni. Dehidratacija koja nastaje
kao rezultat produžene hiperglikemije i posledična poliurija
se smatraju glavnim uzrokom smanjenje funkcije pljuvačnih
žlezda. Međutim, dehidratacija sama sebe ne može objasniti
funkcionalne promene u pljuvačnim žlездama. Veruje se da su
dve najčešće degenerativne komplikacije dijabetesa. neuropatija
i mikroangiopatija, od presudnog značaja za patološke promene
ne u strukturi pljuvačnih žlезд [17]. Povećana koncentracija
kalcijuma u parotidnoj i submandibularnoj pljuvačkoj može
objasniti veću učestalost sialoljitijaze kod dijabetičara i posle-
dičnu oligosijaliju. Takođe, uticaj glikemijeske kontrolе je još
uvek nerazjašnjen [11, 18]. Rezultati ove studije pokazuju da je
vrednost nivoa nestimulisane pljuvačke bila najveća kod zdravih
ispitanika, ali značajna razlika između grupa nije primećena.

Slične rezultate su u svojoj studiji objavili Panchbhai i sar. [19].
Rezultati naše studije pokazuju statistički značajno smanjenje
protoka stimulisane pljuvačke kod dijabetičara i značajnu vezu
između metaboličke kontrolе i protoka pljuvačke. Naši rezultati
su u skladu sa rezultatima studije koju su objavili Chavez i sar.,
koji takođe potvrđuju ovaj odnos [20].

Nedavne studije nedvosmisleno upućuju na to da je dijabetes
važan faktor rizika u nastanku parodontopatije [21, 22]. Smatra se da
dijabetes doprinosi razvoju parodontopatije kroz izražen
inflamatorni odgovor na parodontalne patogene [23]. Neke

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studije potvrđuju da je parodontopatija snažno povezana sa protokom pljuvačke [27].

Prethodne studije izveštavaju kotradiktorne rezultate o vezi odnosa zubnog karijesa i dijabetesa melitusa. Rezultati naše studije su pokazali značajno povećan broj karioznih zuba kod loše kontrolisanih dijabetičara. Slične rezultate su prikazali Bakhshandeh i sar. u svojoj studiji, gde su ispitani sa boljom glikemijskom kontrolom imali manje karioznih zuba u poređenju sa ispitnicima sa lošom glikemijskom kontrolom [28]. Suprotno navedenom, Syräjlä i sar. nisu našli povezanost između nivoa HbA1c i zubnog karijesa [29]. Pored metaboličke kontrole dijabetesa, na razvoj karijesa utiču i mnogi drugi faktori, od kojih prisustvo dentalnog plaka ima najveći značaj. Poznato je da je razvoj karijesa posledica metaboličkih dešavanja u dentalnom plaku u funkciji vremena i da može biti povezan u uslovima poremećene funkcije pljuvačke. Visoki nivoi glukoze u pljuvački i gingivalnoj tečnosti mogu dovesti do povećanja salivarnih kariogenih mikroorganizama u supragingivalnom i subgingivalnom plaku kod dijabetičara [30]. Smanjenje protoka pljuvačke takođe vodi ka slabljenju antimikrobne aktivnosti pljuvačke. U našoj studiji protok pljuvačke je bio u negativnoj i značajnoj korelaciji sa KEP-om, nasuprot rezultatima koje su u svojoj studiji prikazali Karjalainen i sar. [31]. Suprotno tome, Collin i sar. su objavili veću učestalost karijesa među ispitnicima sa većim protokom pljuvačke [33].

U okviru ograničenja dizajna studije preseka, naša studija pokazala su značajnu većinu dijabetičara obolelih od dijabetesa melitusa 2. Uz promene u sastavu i količini pljuvačke, prisustvo infekcije gljivicama iz roda kandida kod obolelih od dijabetesa melitusa tipa 2 dovodi u vezu sa poremećenim cellularnim imunitetom. Velika učestalost Candida spp. je posebno izražena kod dijabetičara koji nose mobilne protetske nadoknade. Candida spp. može koegzistirati sa bakterijama iz biofilma na površini proteza, koje tako postaju rezervoar gorepomenutih mikroorganizama sa daljim potencijalom za koloniziranje oralne mukoze. Oralna kandidijaza je potvrđena kod 33,3% dijabetičara naše studije, što je u skladu sa navodima koje su u svojoj studiji daju Shenoy i sar. [36]. Slične rezultate su objavili Guggenheimer i sar. u svojoj studiji [37]. Svi ovi navodi podržavaju ulogu dijabetesa melitusa kao predisponirajućeg faktora za povećanou kolonizaciju oralne mukoze gljivicama iz roda kandida. Kao što su objavili Navazesh i sar. [38], rezultati naše studije su pokazali negativnu korelaciju između protoka pljuvačke i oralne kandidijaze, iako bez statističke značajnosti.

ZAKLJUČAK

U zaključku, u okviru ograničenja dizajna studije preseka, naša studija pokazuje da smanjen protok pljuvačke može imati značajn uticaj na status oralnog zdravlja kod bolesnika obolelih od dijabetesa melitusa tipa 2. Dbog važnosti pljuvačke u očuvanju integriteta zuba i oralnog zdravlja uopšte, treman oralnih bolesti kod dijabetičara bi trebalo da uključi i sveobuhvatnu evaluaciju salivarne funkcije.

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