Original Article

Quantitative estimation of sodium, potassium and total protein in saliva of diabetic smokers and nonsmokers: A novel study

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Abstract

Aims: The aim of the study was to evaluate the difference in sodium, potassium, total protein in whole saliva in diabetic smokers, diabetic nonsmokers and healthy controls. **Materials and Methods:** Nonstimulated saliva samples were collected from a group of diabetic smokers, diabetic nonsmokers, and controls. Supernatant after centrifugation was used to determine the levels of sodium, potassium, and total protein by using semiautomatic analyzer. **Results:** There exists a statistical difference in the levels of potassium and total protein between diabetic smokers, nondiabetic smokers, and controls. Difference in the levels of sodium is only significant with nondiabetic smokers and controls. **Conclusion:** Diabetes mellitus is known to alter the composition of saliva. The purpose of this study was to estimate and compare the levels of salivary potassium, sodium, and total protein in smoker diabetic patients and nondiabetic smokers and controls, and to explore potential of salivary electrolytes [Na+, K+] and total proteins as markers. The estimated values of salivary constituents add to the data already recorded in Indian population. However, further studies using large samples are required to evaluate the findings in our study.

Key words: Diabetes, potassium, saliva, smoking, total protein

INTRODUCTION

Human saliva contains a large number of proteins and peptides that are easily accessible and may serve as a potential source of biomarkers to monitor changes that occur under pathological conditions. The value of saliva as a biological fluid for the detection of diagnostic and prognostic biomarkers has become increasingly well established. Collection of human saliva is a simple, noninvasive, and cost-effective approach for screening large populations. It is easy to handle and may be repeated without inflicting much discomfort to the subjects.[1]

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Diabetes mellitus is a major global health problem and it has an increasing prevalence due to several factors, such as the population growth, aging, urbanization, and increasing prevalence of obesity or lack of physical exercise. The number of people diagnosed with diabetes is increasing at an alarming rate. It is estimated that by the year 2030, 366 million people worldwide will have the disease.[2]

Worldwide, tobacco-related diseases cause about 5 million premature deaths per year. Most of these deaths occur in smokers. A widely used approach for measuring exposure is determination of tobacco-derived biomarkers in biologic fluids.[3]

The oral cavity is the first organ in the human body to be exposed to the cigarette smoke. The tobacco smoke alters normal homeostasis of the oral cavity, including the saliva's antioxidant and other protective systems. The mucosal changes in smokers may also arise from the drying effects

of the mucosa, high intraoral temperatures, intraoral pH changes, local alteration of membrane barriers and immune responses, or altered resistance to bacteria, fungal, and viral infections. Smoking-related cell damage may leave molecular footprints in the saliva, offering the potential for noninvasive early diagnosis of tobacco-related oral diseases.[1]

Saliva, and not blood, was chosen as the sample used in the study, as many reports have suggested that saliva can be an alternative to blood.[4] Saliva contains a large number of proteins that have metabolic, immune response, transporting, and several other cellular functions. Its collection is noninvasive compared to the collection of other body fluids, and hence has a great potential for use in the diagnosis of systemic and localized diseases.[5,6]

There is very limited literature about salivary changes in diabetic smokers. Hence, we have made an attempt to analyze the levels of sodium, potassium, and total protein in diabetic smokers and nonsmokers in comparison with healthy controls.

MATERIALS AND METHODS

Subjects of either sex aged 30 years and above attending the Department of Oral Pathology and Microbiology, Bapuji Dental College and Hospital, Davangere was considered for inclusion in the study. This study was categorized into three different groups. Group I comprised of 25 known diabetic, nonsmoking patients, Group II comprised of 25 known diabetic smoking patients, and Group III comprised of 25 nondiabetic and nonsmoking controls.

The complete history was taken on a proforma devised for the study. The details of their habits, especially of smoking were specifically sought. A thorough general and oral examination was carried out and blood samples were collected for random blood sugar. Nonstimulated saliva samples were collected[7] and centrifuged for 30 min at 3000 rpm to obtain a clear supernatant fluid. The clear supernatant saliva was analyzed for sodium, potassium, and total protein using a semiautoanalyzer.[8-10]

Correlation between sialochemistry of Na+, K+, and the total protein levels and glycemic status in diabetics was determined. Salivary Na+, K+, and total protein levels between the groups were compared for statistical significance. Descriptive data that included mean, standard deviation, and percentages was calculated for each group. Multiple group comparisons were made by one-way ANOVA followed by unpaired *t*-test for pairwise comparisons. Categorical data were analyzed by Fisher's Exact Test. For all the tests, a *P* value of 0.05 or less was considered for statistical significance.

RESULTS

Total of 75 subjects were analyzed, out of which 25 were diabetic without smokers, 25 were diabetic smokers and 25 were healthy controls.

Group I comprised of 25 cases out of which 14 were males and 11 were females, age range varied from 32-86 years, mean and standard deviation of 51.3 ± 12.2. Group II comprised of 25 subjects were only males, age range varied from 30-76 years, mean and standard deviation of 50.2 ± 11.4. Group III is a control group with 13 males and 12 female subjects. The age range varied from 33-86 years, mean and standard deviation of 50.0 ± 13.1. The mean age in all groups was found to be statistically nonsignificant [Table 1].

In Group I diabetic nonsmokers, the random blood sugar levels ranged from 143-428 mg% with mean and standard deviation of 278.9 ± 91.6 mg%. Salivary sodium levels ranged from 23.9-271.9 mmol/L with mean and standard deviation of 118.7 ± 80.8 mmol/L. Salivary potassium ranged from 7.2-24.5 mmol/L, mean and standard deviation of 13.6 ± 4.8 mmol/L. Similarly, Salivary total protein ranged from 0.74-1.39 with mean and standard deviation of 1.00 ± 0.19 mmol/L. In Group II diabetic smokers, the random blood sugar levels ranged from 149-522 mg% with mean and standard deviation of 296.7 ± 91.1mg%. Salivary sodium levels ranged from 27.0-217.3 mmol/L with mean and standard deviation of 92.1 ± 47.8 mmol/L. Salivary potassium levels ranged from 11.4-27.4 mmol/L with mean and standard deviation of 20.5 ± 5.7 mmol/L. Similarly, salivary total protein ranged from 0.91-1.92 mmol/L with the mean and standard deviation of 1.37 ± 0.35 mmol/L. In controls, the random blood sugar levels ranged from 65-116 mg% with the mean and standard deviation of 94.4 ± 13.3 mg%. Salivary sodium ranged from 11.5-217.3 mmol/L with mean and standard deviation of 80.0 ± 51.1 mmol/L. The salivary potassium range is from 2.6-18.3 mmol/L with mean and standard deviation of 8.9 ± 4.2 mmol/L and salivary total protein ranged from 0.01-0.94 mmol/L with mean and standard deviation of 0.47 ± 0.28 mmol/L [Table 2].

On statistical analysis by unpaired "*t*" test, on comparison of salivary sodium [Graph 1] between Groups I and III, ["*P*" <0.05 with *t* = 2.02] statistical significance values were obtained. But there was no significance between Groups II and III, as well as Groups I and II. The value for the salivary potassium [Graph 2] showed high significance on comparison of Group I and Group III, Group II and Group III, and Group I and II. Total protein [Graph 3] also showed high significance between the groups, that is,

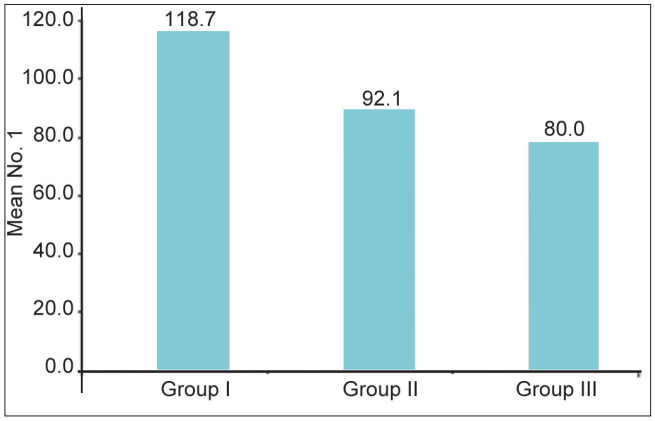
Table 1: Age and sex distribution between control and diabetics.

Groups	No. of Cases	Age [Years]		Sex	
		Range	Mean±SD	Male	Female
I. Diabetics [Non-Smokers]	25	32-86	51.3±2.2	14	11
II. Diabetics [Smokers]	25	30-76	50.2±11.4	25	-
III. Healthy Controls	25	33-86	50.0±13.1	13	12

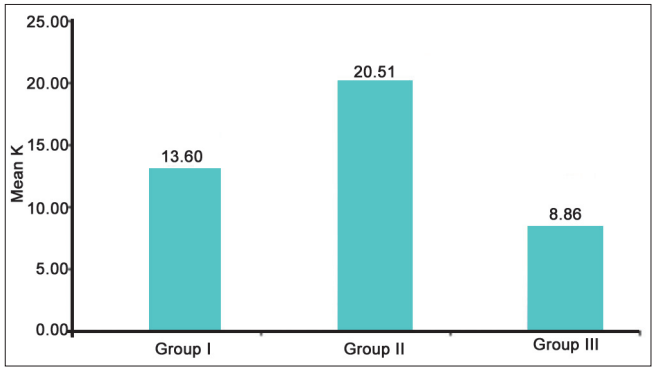
Table 2: Groupwise Comparisons of Serum Glucose and Saliva Components.

		RBS [mg%]		Sodium[mmol/l]		Potassium[mmol/l]		Total Protein[mmol/l]	
		Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD
Group I		143-428	278.9 ± 91.6	23.9-271.9	118.7 ± 80.8	7.2-24.5	13.6 ± 4.8	0.74-1.39	1.00 ± 0.19
Group II		149-522	296.7 ± 91.1	27.0-217.3	92.1 ± 47.8	11.4-27.4	20.5 ± 5.7	0.91-1.92	1.37 ± 0.35
Group III		65-116	94.4 ± 13.3	11.5-217.3	80.0 ± 51.1	2.6-18.3	8.9 ± 4.2	0.01-0.94	0.47 ± 0.28
ANOVA	F	55.8		2.57		35.0		64.7	
	P	<.001, HS		0.08, NS		<.001, HS		<.001, HS	
*Significance of difference	I – III	t=9.97 *P*<.001, HS		t=2.02 *P*<.05, S		t=3.68 *P*<.001, HS		t=7.69 *P*<.001, HS	
	II – III	t=10.99 *P*<.001, HS		t=0.87 *P*=0.39, NS		t=8.22 *P*<.001, HS		t=10.8 *P*<.001, HS	
	I –II	t=0.69 *P*=0.49, NS		t=1.41 *P*=0.16, NS		t=4.69 *P*<.001, HS		t=4.72 *P*<.001, HS	

*: Unpaired't' test, Oneway ANOVA, HS : Highly significant, Unpaired t-test, S: Significant, *P*<.05 Sig. [S], NS: Not significant, *P*<.001 Highly Significant [HS], RBS: Random blood sugar, *P*>0.05 Not significant [NS]



Graph 1: Groupwise Comparison of Salivary sodium



Graph 2: Groupwise comparison of salivary potassium

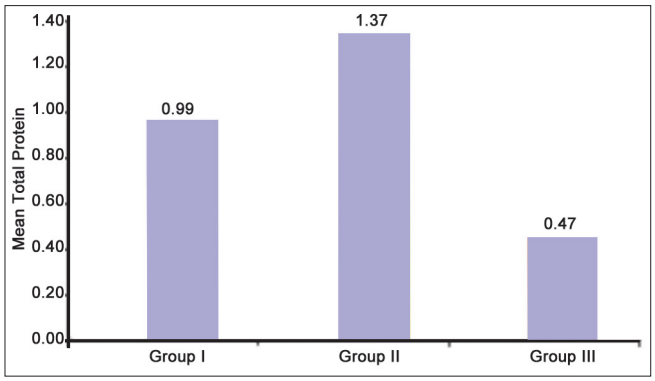
on comparison of Group I and III, Group II and III as well as Group I and II.

The statistical analysis by ANOVA factor showed a "*P*" value of <0.001 that was highly significant with "*F*" value of 55.8 for random blood sugar levels. For Salivary sodium, no significant difference of $P = 0.08$ with $F = 2.57$ was noted, but salivary potassium showed high significance $P \leq 0001$ with $F = 35.0$. Similarly total protein showed high significance $P \leq 0.001$ and $F = 64.7$.

DISCUSSION

Diabetes is known to influence the salivary composition and function.[11] The use of saliva rather than blood for diagnosis has recently been promoted.[12-14] Obtaining saliva is advantageous for patients, especially children and diabetic subjects, since the procedure is noninvasive, stress-free, and allows multiple samplings. The importance of optimal functioning of salivary glands in oral health is well known. Diabetes mellitus is one such disease affecting the salivary gland functioning[15] and thus altering the salivary constituents.

Sharon *et al.*,[16] Ben-Aryeh *et al.*, and Yavuzyilmaz *et al.* analyzed whole saliva, and found significantly higher potassium levels in diabetic groups compared to the nondiabetic group.[17-19] Sharon *et al.* citing studies in animals have hypothesized that in diabetes mellitus an autonomic neuropathy exists that causes sympathetic–parasympathetic imbalance.[16] This imbalance may perhaps exert a continuous stimulation on the salivary glands, bringing about increased potassium secretion into the saliva. Mander



Graph 3: Groupwise comparison of salivary total protein

et al. and Streckfus *et al.* analyzed individual gland saliva and they found no significant difference controls and diabetics.[20,21]

Marder *et al.* attempted to explain the higher concentration of potassium in whole saliva of diabetics by different factors such as peripheral vascular damage that had much higher potassium levels in diabetics than controls and elevated conductibility of the acinic cell membrane to potassium.[9] The findings of this study that whole saliva potassium levels are significantly higher in diabetic patients when compared to the control group. This result is in accordance with a previous study of Sharon *et al.*[16]

Laine *et al.* reported that smoking was associated with higher concentration of salivary potassium, sodium, and total protein. But this finding is entirely opposite of Khan *et al.* reported that potassium decreases with increase in the salivary flow rate of chronic tobacco users.[22] Our study showed significant increase in salivary potassium among diabetic smokers but the salivary sodium levels was not significant.

Total salivary protein levels in diabetic nonsmokers and diabetic smokers are increased on comparison with controls and our study is in general agreement with the findings of Yavuzyilmaz *et al.*,[19] Tenovero *et al.*,[23] Harrison *et al.*,[24] Pal *et al.*,[25] and López *et al.*[26] The increased salivary total protein in diabetics could be attributed to the increase in basement membrane permeability, allowing easy and increased passage of serum proteins into the whole saliva via salivary gland and gingival crevices.[23,27,28] Arati *et al.* and Streckfus *et al.* have demonstrated highly significant positive correlations in salivary total protein levels among uncontrolled and controlled diabetic groups.[11,19,21]

CONCLUSION

Diabetes mellitus is known to alter the composition of saliva. A few reported studies have shown alteration of salivary constituents in diabetes mellitus. Hence, the purpose of this study was to estimate and compare the levels of salivary potassium, sodium, and total protein in smoker diabetic patients and nondiabetic smokers and controls, and to explore potential of salivary electrolytes [Na^+, K^+] and total proteins as markers.

The estimated values of salivary constituents add to the data already recorded in Indian population. However, further studies using large samples are required to evaluate the findings in our study.

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