



RESEARCH ARTICLE

SALIVARY ELECTROLYTES AND TOTAL PROTEINS IN DIABETIC PATIENTS WITH AND WITHOUT CHRONIC PERIODONTITIS AND HEALTHY ADULTS: A COMPARATIVE STUDY

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ABSTRACT

Aims and objectives: This study was conducted to evaluate and compare salivary electrolytes and total protein in diabetic patients with and without periodontitis and in healthy adults, to correlate clinical parameters with electrolytes in all the groups. Furthermore, the correlation between electrolytes of all groups was carried out.

Materials and Methods: 45 subjects of age group 25-60 years were selected and divided into 3 groups, Group 1: Healthy individuals, Group 2: Diabetic patients without chronic periodontitis, Group 3: Diabetic patients with chronic periodontitis. Clinical parameters (plaque index, bleeding index, probing pocket depth and clinical attachment loss) were recorded and non stimulated whole saliva was taken from all the subjects to perform biochemical analysis of salivary total proteins and electrolytes. Continuous variable with a normal distribution were expressed as means with Standard deviation. The intergroup difference was tested using one way ANOVA test. The categorical variables were expressed as numbers with percentage. The strength and direction of a linear relationship between two variables were measured using Pearson's Correlation Coefficient. All statistical analysis was performed by using SPSS (10.5) package. A value of $P < 0.05$ was considered as statistically significant.

Results: A statistically significant effect was demonstrated for plaque index and gingival index. The correlation of electrolytes between the groups was found but did not show statistical significance.

Conclusion: Though the estimated values of salivary electrolytes are not significant in the present study, it can add to the data already recorded in Indian population.

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INTRODUCTION

Periodontal disease is a chronic inflammatory disease of the oral cavity comprising a group of inflammatory conditions affecting the supporting structures of the dentition (Kolte *et al.*, 2012). Certain systemic conditions can also cause inflammatory changes in periodontal tissues.

Among these systemic diseases, diabetes mellitus is an established risk factor for periodontitis and most studies have reported that prevalence and severity of periodontitis are significantly higher in diabetics than non-diabetics (Lasisi and Fasanmade, 2012). Saliva is regarded as a promising medium for detection of markers of periodontal disease activity. The collection protocols are straight-forward, non invasive and can be performed easily. Thus the role of saliva in oral health has been a subject of continued research, as it contains a large number of proteins, peptides and electrolytes, which may serve as a potential source of biomarkers.

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Various studies have aimed to analyze the salivary levels of calcium (Ca^{+2}), magnesium (Mg^{+2}), potassium (K^{+}), sodium (Na^{+}) in CP but have shown conflicting results. Furthermore, there is very scarce literature available regarding the relationship between diabetes and salivary biochemical parameters and the effect of these changes on oral health. In addition, despite the fact that periodontitis is highly prevalent among diabetics, most studies do not relate the salivary composition in diabetic patients to periodontitis (Lasisi and Fasanmade, 2012). Therefore, this short-term clinical study of quantitative estimation of salivary electrolytes and total protein concentrations in saliva of healthy and periodontitis patients with and without diabetes was carried out. This will help to find the reliability of these electrolytes as diagnostic marker in periodontitis and to analyze the relation of these ions to one another, which could help in prevention and treatment of periodontal disease and to indicate disease activity in periodontitis.

Aims and Objectives

- To evaluate and compare the salivary electrolytes and total protein in diabetic patients with and without periodontitis and in healthy adults.
- To correlate clinical parameters with electrolytes in all the groups.
- Correlation between electrolytes of all groups.

MATERIALS AND METHODS

Eligible subjects of either sex aged 25-60 years visiting Department of Periodontics Dayananda Sagar College of Dental Sciences, Bangalore were considered for inclusion in the study. This study was divided into three different groups, each group consisting of 15 subjects: healthy individuals (Group-1), diabetic patients with CP (Group-2), diabetic patients without periodontitis (Group-3).

Inclusion criteria

Subjects in the age group of 25-60 years, who have a history of controlled Type-2 Diabetes mellitus (DM) (but not other systemic diseases, which could influence periodontal conditions), were selected for the study.

Exclusion criteria

Patients who have undergone periodontal therapy in the past three months, antibiotic therapy in the past six months were excluded from the study. Smokers and pregnant and/or lactating women were also excluded.

Method of data collection

All subjects were asked to sign a written informed consent form prior to commencement of the study.

Clinical Examination

Following clinical parameters were recorded, Plaque index (PI), (Silness *et al.*, 1964) Gingival index (GI) (Loe, 1967),

probing pocket depth (PPD) and clinical attachment loss (CAL) were recorded and non stimulated whole saliva was taken from all the subjects and biochemical analysis of salivary total proteins and electrolytes was done.

Sample collection

All participants were instructed not to eat or drink (except water) at least one hour prior to collection of saliva. They were asked to sit in a relaxed position and rinse his/her mouth several times with sterilized water. 30 minutes later, five millilitres (ml) of whole unstimulated saliva was collected into a sterile container.

Electrolytes detection

The collected saliva samples were centrifuged at 3000 revolutions per minute (rpm) for 30 minutes to obtain a clear supernatant fluid. The clear supernatant was analyzed using auto-analyzer for electrolytes and total protein detection for all the groups.

Statistical analysis

Continuous variable with a normal distribution were expressed as means with Standard deviation. The intergroup difference was tested using one way ANOVA test. The categorical variables were expressed as numbers with percentage. The strength and direction of a linear relationship between two variables were measured using Pearson's Correlation Coefficient. All statistical analysis was performed by using SPSS (version: 10.5) package. A value of $p < 0.05$ was considered as statistically significant.

RESULTS

The mean \pm SD of plaque index in Healthy, Diabetic with CP group and Diabetic without CP group were 0.30 ± 0.196 , 0.79 ± 0.263 and 0.43 ± 0.291 respectively. The comparison of mean PI between the groups shows statistically significant difference ($p < 0.05$) between the groups. Further analysis revealed that there is significant difference between Healthy and Diabetic with CP groups ($p < 0.001$). There is no statistically significant difference between Healthy and Diabetic without CP groups ($p = 0.583$). There is statistically significant difference between Diabetic with CP and diabetic without CP groups ($p = 0.001$).

The mean \pm SD of Gingival index in Healthy, Diabetic with CP group and Diabetic without CP group were 0.1713 ± 0.1214 , 0.68 ± 0.2007 and 0.4060 ± 0.2548 respectively. The comparison of mean Gingival Index between the groups shows statistically significant difference ($p < 0.05$) between the groups. Further analysis revealed that there is statistically significant difference between Healthy and Diabetic with CP, diabetic without CP group ($p < 0.001$ and $p = 0.008$ respectively). There is also statistically significant

Correlation between PI versus Electrolytes

There is Positive Correlation between Plaque Index and Electrolytes but no statistically significant and weak correlation was observed with sodium, potassium and Magnesium.

Table 1. Comparison of Mean Plaque Index between the Study Groups

Group	N	Mean	SD	Median	Min.	Max.	'F' value	'p' value
Healthy	15	0.30	0.196	0.20	0.00	0.70	15.120	<0.001*
Diabetic with CP	15	0.79	0.263	0.80	0.30	1.20		
Diabetic without CP	15	0.43	0.291	0.40	0.10	0.80		

*statistically significant

Table 2. Comparison of Mean Gingival Index between the Study Groups

Group	N	Mean	SD	Median	Min.	Max.	'F' value	'p' value
Healthy	15	0.1713	0.1214	0.1300	0.00	0.50	24.321	<0.001*
Diabetic with CP	15	0.6800	0.2007	0.7000	0.40	1.10		
Diabetic without CP	15	0.4060	0.2548	0.4000	0.10	0.87		

*statistically significant

Table 3. PPD

Group	N	Mean	SD	Median	Min.	Max.
Diabetic with CP	15	1.2027	0.23119	1.1600	.83	1.80

Table 4. CAL

Group	N	Mean	SD	Median	Min.	Max.
Diabetic with CP	15	1.0700	0.28496	1.0000	.80	1.80

Table 5. Correlation between PI and GI versus Electrolytes healthy group

Group	Versus	Total Protein (gm/dl)	Albumin (gm/dl)	Calcium (mg/dl)	Phosphorus (mg/dl)	Sodium (mmol/L)	Potassium (mmol/L)	Chloride (mmol/L)	Magnesium (mg/dl)	
Healthy	PI	Corr.	0.202	0.016	0.136	0.011	-0.019	-0.362	0.07	-0.014
		'p' valve	0.471	0.955	0.629	0.969	0.946	0.185	0.804	0.962
	GI	Corr.	0.423	0.113	0.499	0.46	-0.162	-0.249	0.164	0.211
		'p' valve	0.116	0.69	0.059	0.084	0.564	0.372	0.558	0.45

Table 6. Correlation between PI, GI, PPD and CAL versus Electrolytes in diabetic patients with CP

Group	Versus	Total Protein (gm/dl)	Albumin (gm/dl)	Calcium (mg/dl)	Phosphorus (mg/dl)	Sodium (mmol/L)	Potassium (mmol/L)	Chloride (mmol/L)	Magnesium (mg/dl)	
Diabetic with CP	PI	Corr.	0.044	0.022	0.211	0.255	0.121	0	-0.121	-0.245
		'p' valve	0.877	0.938	0.451	0.36	0.668	1	0.666	0.379
	GI	Corr.	-0.199	-0.218	0.048	0.133	-0.13	0.106	-0.382	-0.224
		'p' valve	0.477	0.434	0.866	0.636	0.644	0.706	0.16	0.421
	PPD	Corr.	0.714	0.733	0.758	0.569	0.765	-0.253	0.679	-0.167
		'p' valve	0.003	0.002	0.001	0.027	0.001	0.363	0.005	0.551
	CAL	Corr.	0.711	0.72	0.765	0.611	0.713	-0.144	0.622	-0.083
		'p' valve	0.003	0.002	0.001	0.015	0.003	0.608	0.013	0.767

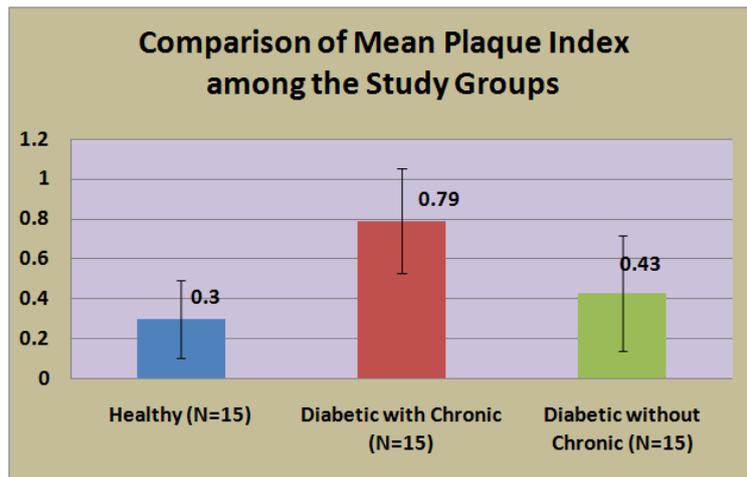
Table 7. Correlation between PI and GI versus Electrolytes in diabetic patients without CP

Group	Versus	Total Protein (gm/dl)	Albumin (gm/dl)	Calcium (mg/dl)	Phosphorus (mg/dl)	Sodium (mmol/L)	Potassium (mmol/L)	Chloride (mmol/L)	Magnesium (mg/dl)	
Diabetic without CP	PI	Corr.	0.110	0.175	-0.075	0.404	0.123	-0.414	-0.477	0.113
		'p' valve	0.697	0.533	0.792	0.136	0.663	0.125	0.072	0.689
	GI	Corr.	0.221	0.037	0.078	0.537	0.163	-0.161	-0.460	0.192
		'p' valve	0.428	0.895	0.783	0.039	0.562	0.567	0.085	0.493

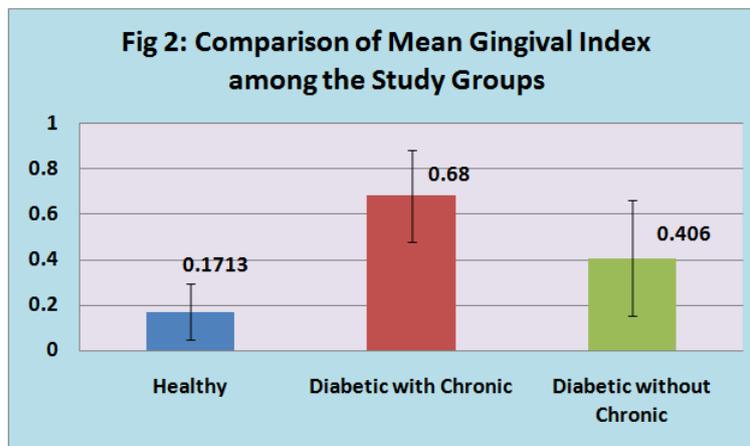
There is a weak negative correlation observed between PI versus Sodium, potassium and magnesium ($r=-0.019$, $r=-0.362$, $r=-0.014$ respectively). There is weak positive correlation was observed between PI and Total Protein, Albumin, Calcium, Phosphorus and Chloride ($r=0.202$, $r=0.016$, $r=0.136$, $r=0.011$ and $r=0.07$ respectively).

Correlation between GI versus Electrolytes

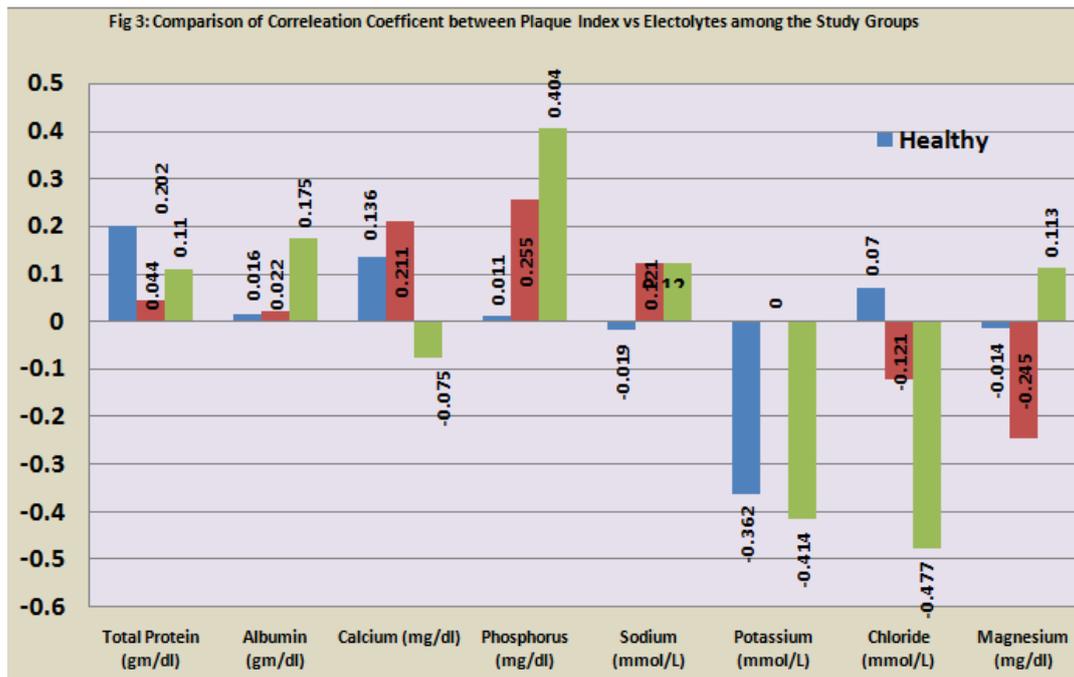
This study showed positive Correlation between Gingival Index and Electrolytes but there is no statistically significant and weak correlation expects sodium and potassium. There is a weak negative correlation observed between PI versus Sodium and potassium ($r=-0.162$, $r=-0.249$ respectively).



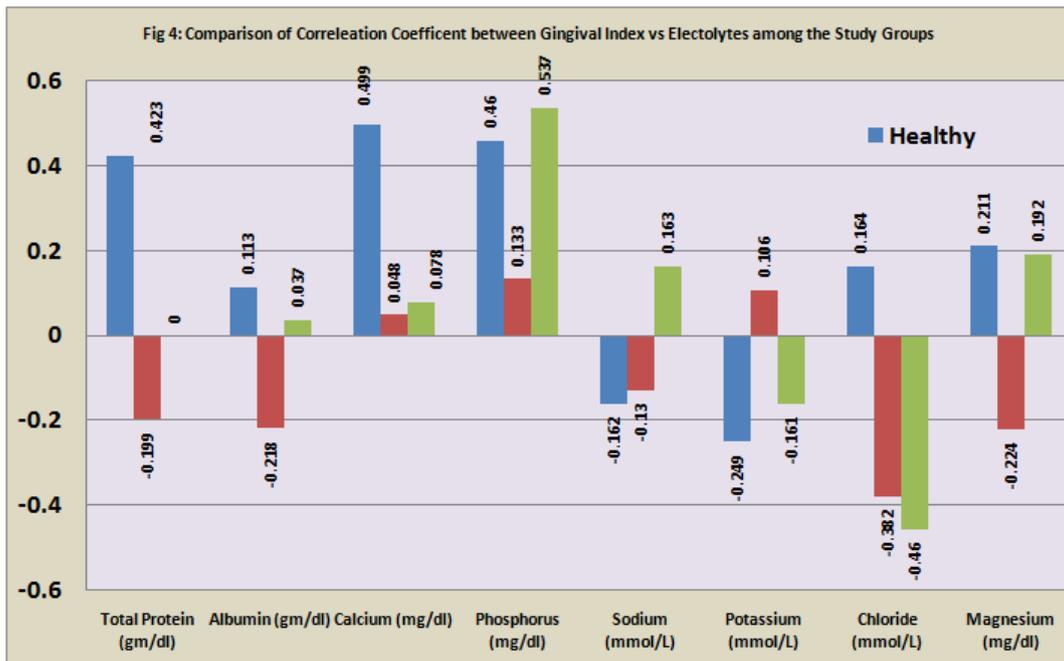
Graph-1: Comparison of mean plaque index among study groups



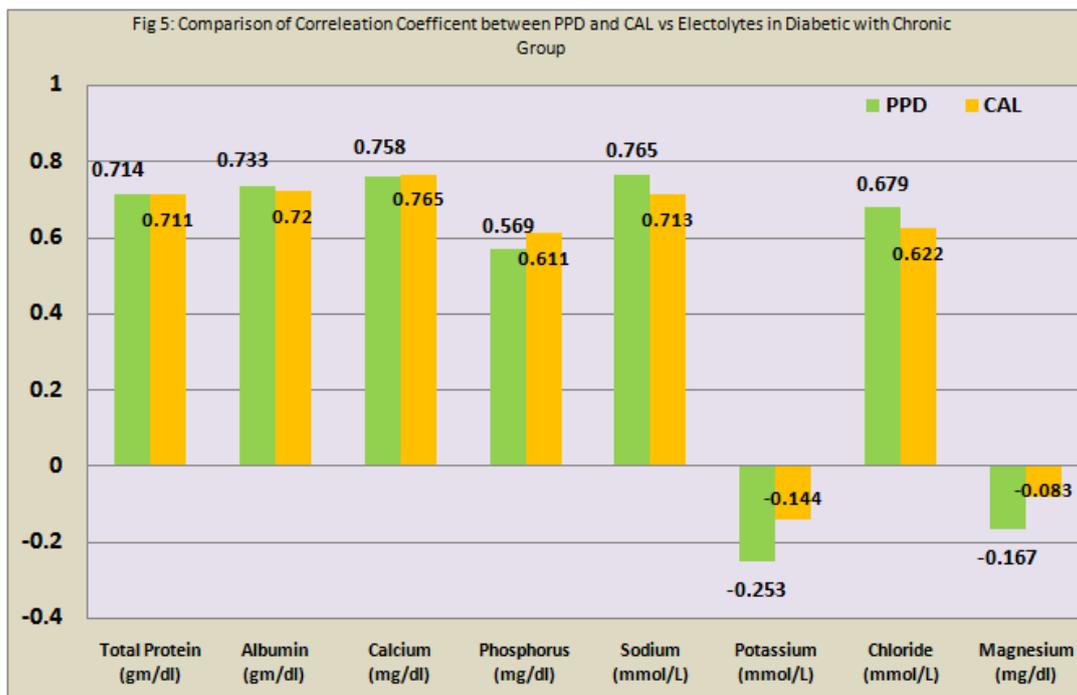
Graph-2: Comparison of mean gingival index among the study groups



Graph 3. Comparison of correlation coefficient between piaque index versus electrolytes among the study groups



Graph 4. Comparison of correlation coefficient between gingival index versus electrolytes among the study groups



Graph 5. Comparison of correlation coefficient between ppd and cal versus electrolytes in diabetic with chronic group

There is weak positive correlation was observed between PI and Total Protein, Albumin, Calcium, Phosphorus, Chloride and Magnesium ($r=0.423, r=0.113, r=0.499, r=0.460, r=0.164$ and $r=0.211$ respectively).

Correlation between PI versus Electrolytes

Correlation between Plaque Index and Electrolytes showed positive, weak correlation except Chloride and Magnesium.

There is no correlation observed between PI Versus Potassium ($r=0$). There is negative, weak correlation, observed between PI versus Chloride and Magnesium ($r=-0.121, r=-0.245$ respectively). But the above correlations were statistically not significant. There is weak positive correlation observed between PI and Total Protein, Albumin, Calcium, Phosphorus and Sodium ($r=0.044, r=0.022, r=0.211, r=0.255$ and $r=0.121$ respectively).

Correlation between GI versus Electrolytes

Correlation between Gingival Index versus calcium, phosphorus and Potassium showed positive weak correlation ($r=0.048$, $r=0.133$, $r=0.106$ respectively). There is negative weak correlation was observed between GI versus Total Protein, Albumin, Sodium, Chloride and Magnesium ($r=0.044$, $r=0.022$, $r=0.211$, $r=0.255$ and $r=0.121$ respectively). Again, all the above correlations were statistically not significant.

Correlation between PPD versus Electrolyte

Correlation between PPD versus Total Protein, Albumin, Sodium, Calcium, Sodium and Chloride showed positive statistically significant strong correlation ($r=0.714$, $r=0.733$, $r=0.758$, $r=0.765$, $r=0.679$ respectively). There is a positive statistically significant moderate correlation between PPD versus Phosphorus ($r=0.569$). There is negative statistically not significant weak correlation was observed between PPD versus Potassium and Magnesium ($r=-0.253$, $r=-0.167$ respectively).

Correlation between CAL versus Electrolyte

Correlation between CAL versus Total Protein, Albumin, Sodium, Calcium, Phosphorus, Sodium and Chloride showed positive statistically significant strong correlation ($r=0.711$, $r=0.720$, $r=0.765$, $r=0.611$, $r=0.713$, $r=0.622$ respectively). There is negative statistically not significant weak correlation was observed between PPD versus Potassium and Magnesium ($r=-0.144$, $r=-0.083$ respectively).

Correlation between PI versus Electrolytes

This study showed positive Correlation between Plaque Index and Electrolytes but statistically non significant value and weak correlation except Calcium, Potassium and Chloride. There is negative statistically not significant weak correlation observed between PI versus Calcium, Potassium, and Chloride ($r=-0.075$, $r=-0.414$, $r=-0.477$ respectively). There is weak positive correlation observed between PI versus Total Protein, Albumin, Phosphorus, Sodium and Magnesium ($r=0.110$, $r=0.175$, $r=0.404$, $r=0.123$ and $r=0.113$ respectively).

Correlation between GI versus Electrolytes

This study showed positive Correlation between Gingival Index versus Total Protein, Albumin, Calcium, Sodium and Magnesium but there is no statistically significant value and weak correlation ($r=0.221$, $r=0.037$, $r=0.078$, $r=0.163$ and $r=0.192$ respectively). There is negative statistically not significant weak correlation observed between GI versus Potassium, and Chloride ($r=-0.161$, $r=-0.460$ respectively). There is positive statistically significant moderate correlation observed between GI versus Phosphorus ($r=0.537$).

DISCUSSION

Salivary composition and functions are altered in diabetes. The use of saliva rather than blood for diagnosis has recently been promoted. Obtaining saliva is advantageous for patient as the procedure is non-invasive, 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 and thus altering the salivary constituents. In the present study, the comparison of mean plaque index and the gingival index between the groups shows statistically significant difference ($p<0.005$) between the groups. Further analysis revealed that there is significant difference between healthy and diabetic with CP groups ($p<0.001$).

There is no statistically significant difference between healthy and diabetic without CP groups ($p=0.583$). There is statistically significant difference between diabetic with CP and diabetic without CP groups ($p=0.001$). This is obvious since the accumulation of plaque is the primary cause of periodontal disease (Khamees *et al.*, 2012). With respect to potassium, in the present study there is no statistically significant increase in the potassium level, which is inconsistent with the results of Sharon *et al.* (1985) and Mata *et al.* (2004) who conducted a study on salivary electrolytes and found significantly increased potassium levels in diabetic groups compared to the non-diabetic groups. In this study there is no significant difference in the salivary concentrations of calcium, sodium, chloride levels in saliva of diabetic patients with periodontitis compared with controls, which is in accordance with the study conducted by Lasisi *et al.* 2012 and Ben-Ahrey *et al.* 1993.

It might be due to intact secretory capacity of the salivary glands in type 2 diabetes. In addition sodium and chloride ions are secreted in minute concentrations in the saliva (Sharon *et al.*, 1985) and this might account for the absence of significant difference. In this study there is no significant increase in the salivary concentrations of magnesium which agrees with the study conducted by the Sameeah *et al.* (2012) while disagreeing with Abid Aun *et al.* (2012) who found significant difference in salivary levels of magnesium ions between CP and control group. In the present study there is no significant increase in the salivary concentrations of phosphorus levels.

In this study, the salivary total protein and albumin levels are not significantly increased in any group. This is consistent with the findings of Dodds *et al.* (1997) and Panchbai *et al.* (2010) although, some studies have reported increased salivary protein concentration in diabetic patients, which was 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 (Tenovuo *et al.*, 1986; Laine *et al.*, 2002; Edgar, 1992). Conflicting results of the present study with observations of some of the earlier studies may be partly attributed to differences in the racial and ethnic background of the individuals studied, differences in methodologies employed to perform the evaluation of salivary electrolyte assays and in the interpretation of data obtained from these assays. The limitations of the present study may include unequal gender distribution, the effect of gender on salivary secretion and intern on electrolyte levels could not be assessed. Also smaller sample size is one of the limitation of the present study. One of the major strength of the present study was that the salivary electrolyte levels were assessed by using fully automatic analyzer (Autoanalyzer) which is economic, sensitive and reliable compared to other techniques. Electrolyte levels were assessed in saliva which is non invasive and less expensive as compared to serum.

Conclusion

Diabetes mellitus is known to alter the composition of saliva; few studies have shown alteration of salivary constituents in diabetes mellitus. Though the estimated values of salivary electrolytes are not significant in the present study, it can 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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