Estimation and Correlation of Salivary Alkaline Phosphatase and Total Protein Levels in Chronic Periodontitis Subjects with or without Uncontrolled Diabetes mellitus

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ABSTRACT

Aim: The aim of the present study is to estimate and compare the salivary levels of alkaline phosphatase and total protein in generalized chronic periodontitis subjects with or without uncontrolled type II diabetes.

Materials and Methods: 60 subjects were included in this study. After the consideration of inclusion and exclusion criteria, subjects were divided into 2 groups, group I subjects had uncontrolled diabetes mellitus with generalized chronic periodontitis (DM+CP) and group II generalized chronic periodontitis subjects (CP). Clinical parameters i.e. plaque index, gingival index, and clinical attachment level & salivary ALP and total protein levels were also estimated using a fully automated analyser.

Results: The present study showed a higher mean salivary ALP and total protein levels among the patients with type II diabetes mellitus (Group I) than non-diabetic individuals (group II).

Conclusion: Salivary ALP and total protein levels reflects the inflammation and destruction of periodontal tissues, suggesting a useful marker in diagnosis of ongoing periodontal destruction.

Keywords: **chronic periodontitis; salivary biomarkers; Diabetes mellitus**

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Introduction: Periodontal disease is common chronic inflammatory infection that have multifactorial etiology and numerous risk factors.[1] Severity and pattern of disease progression are influenced by many factors e.g. Like smoking, stress, any systemic disease or condition and genetic.[2] Epidemiological studies confirm that diabetes is risk factor for periodontal disease and uncontrolled blood sugar levels have a direct relationship with disease severity and tissue destruction. The process under this mechanism mainly has effect of immune function, inflammation, neutrophil response and cytokine activity.[3]

salivary enzyme, protein and other component of saliva are observed as marker for periodontitis and their production increases with periodontal tissue destruction and bone loss. Alkaline phosphatase is a salivary glycoprotein produced by PMNs and microorganism, directly in relation with fibroblastic and osteoblastic activity in sub gingival pocket.[4] Level of total proteins in saliva is also significant component during inflammation.[5] Studies revealed a link between systemic inflammation and periodontal disease. Thus, many studies are done to evaluate the role to systemic biomarker in periodontal inflammation

because periodontal tissue destruction also have negative effects on systemic health, which alter immune function, neutrophil function and cytokine production.[6] Biomarkers, solely are not fulfil the diagnostic requirement in any disease but however they are very reliable and significant tool to diagnose the disease and its severity and progression. Hence, the present study was undertaken to Estimate and compare the salivary levels of alkaline phosphatase and total protein in generalized chronic periodontitis subjects with or without uncontrolled type II diabetes.

Materials and methods: A total number of 60 subjects were comprised in this study. After the consideration of inclusion and exclusion criteria, subjects were divided into two groups, group I subjects had uncontrolled diabetes mellitus with generalized chronic periodontitis (DM+CP) and group II generalized chronic periodontitis subjects (CP). Institutional ethical committee has been approved the study and informed consent has been taken from every subjects. Clinical parameters i.e. plaque index, gingival index, and clinical attachment level were measured. The salivary ALP and albumin levels were also estimated using a fully automated analyser.

The inclusion criteria include Type II diabetes mellitus patients whom diagnosis made with diabetes at least a year before the study began according to ADA[7] (American Diabetes Association standards), they were aged 30-65 years. They received insulin or oral hypo-glycemic medications as prescribed by their doctor. Patient should have at least 30% or more of the teeth with 3-5 mm of Clinical Attachment Loss. The exclusion criteria include patients with a history of systemic disease other than diabetes, and those who were under anti-inflammatory/antibiotic therapy for the previous six months, tobacco consumer subjects because the pathophysiology for periodontitis was altered in these diseases. Individuals who had undergone periodontal treatment within the preceding 6 month were also not included. Patients who had undergone antibiotic medication within the three months prior or who had any other systemic disorders were excluded.

Estimation Of Clinical Parameters: For clinical examination, each subject was examined. Plaque index (PI), gingival index (GI), and CAL were recorded at four sites of each tooth of all teeth using William's periodontal probe. One single examiner recorded all the clinical data.

Estimation of ALP and Total protein:

Requested the patient sit up straight and saliva was collected by spitting. We gather around 5 millilitres of saliva. The lab has separated the saliva supernatant by centrifuging a sample of saliva at 3000 rpm for 15 minutes. Twenty microliters of the leftover sample are combined with the ALP reagent in the Erba Mannheim kit, and the fully automated analyser measures the ALP level. It is noted what the readings on the analyser's screen indicate. The total protein kit (biuret technique, end point) from Erba was used to estimate the total protein content in saliva samples. The biuret technique is used to determine the total protein content of saliva after it has been estimated using the UV absorption method. In an alkaline media, protein and cupric ions combine to generate a coloured complex. Based on this idea, the total protein content of saliva is measured by combining unadulterated saliva with the biuret reagent and observing the colour changes using a biowave spectrophotometer set at a wavelength of 546 nm.

Statistical Analysis: The results were analyzed using descriptive statistics. Categorical data were summarized as in proportions and percentages (%) and discrete as Mean ± SD (standard deviation). Data was analysed using the software MS Office Excel software & SPSS 23 for Windows. $p<0.05$ was considered statistically significant.

Results: The mean age of participants in Group 1 (DM+CP) is 45.67 with a standard deviation of 6.05, while in Group 2 (CP), the mean age was 46.30 with a standard deviation of 5.46 suggesting that the difference in age was not statistically significant.

The mean plaque index in Group 1 is 2.27 with a standard deviation of 0.64, while in Group 2, it is 2.13 with a standard deviation of 0.57 and for the gingival index, Group 1 has a mean of

1.90 with a standard deviation of 0.61, while Group 2 has a mean of 1.73 with a standard deviation of 0.45. the p-value is greater than 0.05 for both PI and GI, suggesting that there was no significant difference in plaque index and gingival index between the two groups.

Group 1 has a significantly higher mean CAL (4.60) compared to Group 2 (3.90), It suggest that there was a substantial difference in clinical attachment levels between the two groups, and this difference was statistically significant.

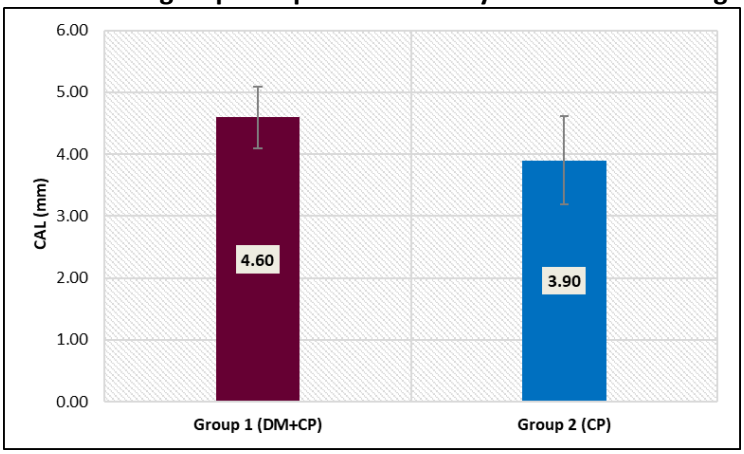
ALP levels were significantly higher in Group 1 (52.80) compared to Group 2 (39.01), as shown by the t-test with a high t-value of 7.28 and a p-value less than 0.001. This indicates a substantial and statistically significant difference in ALP levels between the two groups. Total protein levels were higher in Group 1 (4.18) compared to Group 2 (3.70). there was a statistically significant difference in total protein levels between the two groups, with Group 1 having higher levels. In summary, it was found that while age, plaque index, and gingival index do not significantly differ between the groups, clinical attachment level (Graph 1), ALP levels (Graph 2), and total protein levels (Graph 3) has been shown significant differences (Table1).

The results of a multivariate analysis (Table 2 & Graph 4) that assess the effect of diabetes (DM) on various study parameters, while also considering age as a potential confounding factor. The table includes different dependent variables (PI, GI, CAL (mm), ALP and total protein levels) and their relationship with independent variables (intercept, age, CP+DM, and a reference group CP only).

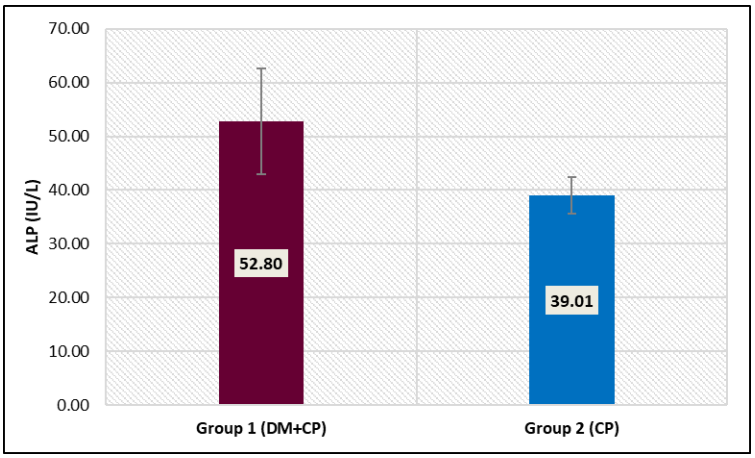
Variable	Group 1 (DM+CP)		Group 2 (CP)		Significance
	Mean	SD	Mean	SD	
AGE	45.67	6.05	46.30	5.46	t=0.43, p=0.672
PLAQUE INDEX	2.27	0.64	2.13	0.57	z=0.92, p=0.359
GINGIVAL INDEX	1.90	0.61	1.73	0.45	z=1.07, p=0.285
CAL (mm)	4.60	0.50	3.90	0.71	z=3.77, **p<0.001**
ALP (IU/L)	52.80	9.81	39.01	3.37	t=7.28, **p<0.001**
TOTAL PROTEIN (g/dl)	4.18	0.81	3.70	0.54	t=2.74, **p=0.008**

*Mann Whitney test was applied for Plaque index, Gingival index and CAL

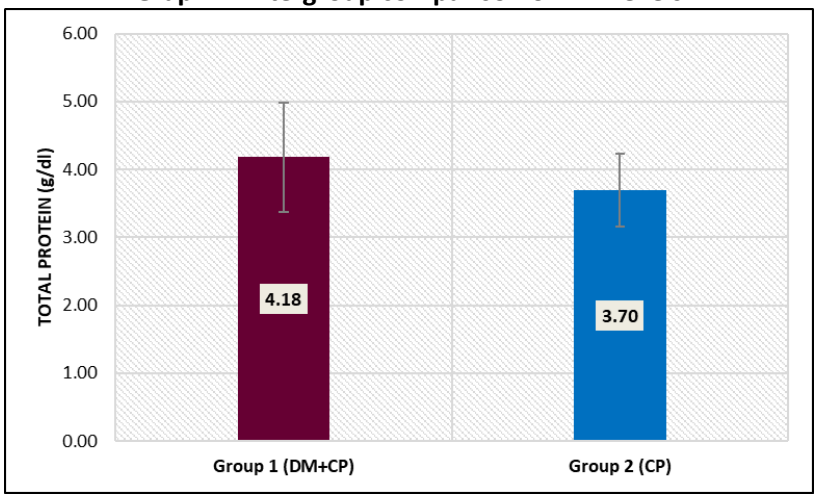
Table 1: Intergroup Comparison of Study Parameters and Age



Graph1: Intergroup comparison of CAL



Graph 2: Intergroup comparison of ALP levels

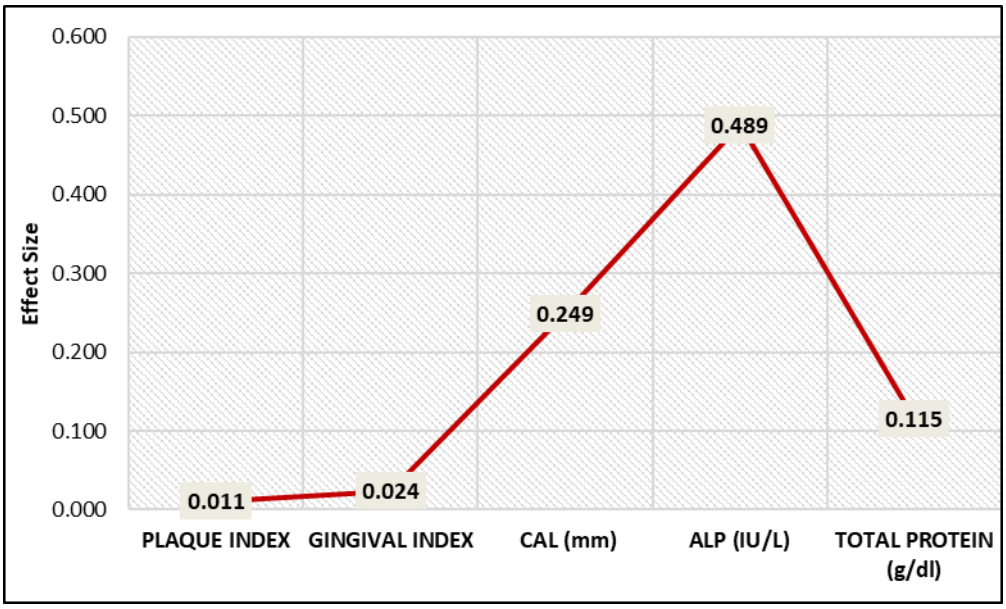


Graph 3: Intergroup comparison of Total Protein levels

Dependent Variable	Independent	B	SE	t-value	p-value	Effect size
PLAQUE INDEX	Intercept	2.89	0.65	4.47	**<0.001**	0.259
	AGE	-0.02	0.01	-1.19	0.239	0.024
	CP+DM	0.12	0.16	0.79	0.435	0.011
	CP only	Ref.				
GINGIVAL INDEX	Intercept	1.84	0.58	3.19	**0.002**	0.152
	AGE	0.00	0.01	-0.19	0.846	0.001
	CP+DM	0.17	0.14	1.19	0.241	0.024
	CP only	Ref.				
CAL (mm)	Intercept	4.31	0.66	6.52	**<0.001**	0.427
	AGE	-0.01	0.01	-0.63	0.531	0.007
	CP+DM	0.69	0.16	4.35	**<0.001**	0.249
	CP only	Ref.				
ALP (IU/L)	Intercept	29.08	7.81	3.72	**<0.001**	0.195
	AGE	0.21	0.17	1.29	0.202	0.028
	CP+DM	13.93	1.89	7.38	**<0.001**	0.489
	CP only	Ref.				
TOTAL PROTEIN (g/dl)	Intercept	3.58	0.74	4.83	**<0.001**	0.290
	AGE	0.00	0.02	0.16	0.875	0.000

	CP+DM	0.49	0.18	2.72	**0.009**	0.115
	CP only	Ref.				

Table 2: Multivariate Analysis Showing Effect of DM Over Study Parameters considering Age as Confounding Factor



Graph 4: Effect of DM Over Study Parameters considering Age as Confounding Factor

ALP levels were 29.08. Age has a small, non-significant positive effect (B = 0.21, p = 0.202) on ALP levels. In contrast, the presence of CP+DM has a highly significant positive effect (B = 13.93, p < 0.001). Total protein levels were 3.58, there was also age has a non-significant effect (B = 0.00, p = 0.875) on total protein levels. However, the presence of CP+DM has a significant positive effect (B = 0.49, p = 0.009), suggesting that individuals with both chronic periodontitis and diabetes have higher ALP and total protein levels.

In summary, this multivariate analysis indicates that the presence of both chronic periodontitis and diabetes (CP+DM) were associated with significant differences in CAL, ALP levels, and total protein levels. Age does not appear to have a significant effect on most of these variables, except for ALP levels where it has a small, non-significant positive effect. These findings provide insights into the relationships between these variables and the impact of diabetes in individuals with chronic periodontitis, while controlling for age as a potential confounder.

Discussion: The inflammation of periodontal tissue triggers an immunoinflammatory response, releasing a variety of anabolic and catabolic products at the interface between the diseased periodontal tissue and the tooth, including salivary enzymes, chemokines, and pro- and inflammatory cytokines.[8] These products have been assessed as not only the diagnostic biomarker but also evaluated the severity of disease. Periodontal disease is highly prevalent chronic inflammatory condition with multiple risk factor and because of its complex etiopathogenesis, there is persistent need of new biomarkers in the field of periodontology.[9] diabetes has been established as an obvious risk factor for periodontal disease and increased the frequency and severeness of disease. [10]

In individuals with diabetes, less salivation and changes in the subgingival microflora cause plaque to accumulate more and the inflammatory response to increase. Advanced glycation end products (AGEs) are overflowing into periodontal structures, hastening the inflammation of the periodontal tissue. Reactive oxygen species are created when an AGE binds to its receptor for an advanced glycation end product. This process raises

oxidative stress, damages endothelial cells, and damages vessels.[11,12]

In the current investigation, we examined the levels of total protein, salivary ALP, and clinical parameter values between patients with chronic periodontitis who were non-diabetics and those who had diabetes. At baseline, diabetics had significantly higher levels of GI, PD, and CAL than non-diabetics. CAL is higher in group 1, but there was no statistically significant difference in the gingival and plaque indices between the two groups. The patients with type II diabetes mellitus had a significantly higher mean salivary ALP level than the non-diabetic subjects in the current investigation. Shaheen et al.[13] and Kulbergi V et al.[4] reported similar findings, indicating that individuals with type II diabetes mellitus had a considerably higher level of salivary ALP than non-diabetics. Todorovic et al[14] also reported a positive corelation between gingival inflammation and ALP levels.

The present study also determined salivary total protein levels in chronic periodontitis subjects with or without diabetes mellitus, using simple biochemical methods. The elevated protein levels of periodontal diseases are most likely due to enhanced synthesis and secretion by the individual glandular saliva. It has known protective actions against infection through immunoglobulins. Besides, certain salivary enzymes such as β-glucuronidase, aspartate aminotransferase, and alkaline phosphatase have been reported in increased concentrations in periodontal disease and can be regarded as contributors to initiation and progression of periodontal disease.[15] in favor to our study Karthiga et al.[16] described that the mean salivary total protein values in the control and periodontitis groups are 0.87 g/ml (SD = 0.21) (87 g/dl [SD = 21]) and 1.67 g/ml (SD = 0.48) (167 g/dl [SD = 48]). The rise in these values was statistically significant (P =0.001). Pratibha KM et al[17] found significantly increased levels of salivary total proteins in diabetics. More periodontal tissue-derived proteins or increased microbial activity are likely responsible for the rise in salivary protein levels. Mandel et al[18] postulated that one explanation for the increased transfer of proteins from the exocrine glands into their secretions in some individuals could be greater basement membrane permeability, which is frequently linked to diabetes. In contrast to our finding Dodds et al.[19] reported that the amounts of albumin and total protein in saliva are not significantly elevated in type II diabetes mellitus subjects.

Diabetes mellitus is a metabolic disease caused by insufficient insulin, which is linked to changes in the activity of several different enzymes, including SGOT, SGPT, and ALP. Diabetes mellitus not only causes macrovascular and microvascular problems but also makes people more vulnerable to infections, especially from opportunistic bacteria that dominate the oral microbiota. There is a connection between diabetes mellitus and damaged periodontal tissues. ALP is secreted by the wounded tissues from PMNs, which results in the degree of ALP activity and the degradation of connective tissue. directly correspond to the degree of periodontal tissue inflammation.[13] According to the current investigation, salivary ALP levels declined in step with the clinical values (GI, PD, and CAL). It has been demonstrated that in inflamed gingival tissues, the amount of cellular damage and metabolic alterations are reflected in the enzyme activity.

Conclusions: Based on the study's findings, it can be said that persons with diabetes mellitus who had chronic periodontitis had considerably higher levels of salivary alkaline phosphatase and total protein enzyme than people with systemic health. Salivary ALP and total protein level screening for periodontal disease may be a practical, easy, and convenient method that doesn't require specialised examiners. Salivary ALP is a good diagnostic marker for ongoing periodontal damage because it reflects the inflammation and degradation of periodontal tissues.

1. Armitage GC: Periodontal diagnoses and classification of periodontal diseases. Periodontol 2000. 2004, 34:9-21.
2. Hajishengallis G, Chavakis T, Lambris JD: Current understanding of periodontal

disease pathogenesis and targets for host-modulation therapy. Periodontol 2000. 2020, 84:14-34.
3. Preshaw PM, Alba AL, Herrera D, Jepsen S, Konstantinidis A, Makrilakis K, Taylor R. Periodontitis and diabetes: a two-way relationship. Diabetologia. 2012 Jan;55(1):21-31.
4. Kalburgi V et al. Comparison of salivary alkaline phosphatase levels among diabetics and non-diabetics with chronic periodontitis. Journal of Oral Health Research, 2010;1(4):146-149.
5. Panchbhai AS, Degwekar SS, Bhowte RR. Estimation of salivary glucose, salivary amylase, salivary total protein and salivary flow rate in diabetics in India. J Oral Sci. 2010 Sep;52(3):359-68.
6. Lalkota H, Alluri LSC, Da Silva APB, Gummalla S, Kondareddy KMR. Estimation and Correlation of Serum Albumin and Serum Alkaline Phosphatase Levels Between Smokers and Non-smokers With Generalized Chronic Periodontitis. Cureus. 2021;13(8):e17474.
7. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2009 Jan;32 Suppl 1(Suppl 1):S62-7.
8. Ferreira MC, Dias-Pereira AC, Branco-de-Almeida LS, Martins CC, Paiva SM: Impact of periodontal disease on quality of life: a systematic review. J Periodontal Res. 2017;52:651-65.
9. Giannobile WV: Salivary diagnostics for periodontal diseases. J Am Dent Assoc. 2012;143:6S-11S.
10. Newman, T., Klokkevold, C. (2009) Classification of diseases and conditions affecting periodontium. Carranza's Clinical Periodontology. Ed 10. Philadelphia: Saunders: 103–104.
11. Soskolne WA, Klinger A. The relationship between periodontal diseases and diabetes: an overview. Ann Periodontol. 2001 Dec;6(1):91-8.
12. Ohlrich EJ, Cullinan MP, Leichter JW. Diabetes, periodontitis, and the subgingival microbiota. J Oral Microbiol. 2010;21(2):1-8.
13. Shaheen A, Khattak S, Khattak AM, Kamal A, Jaffari SA, Sher A. Serum alkaline phosphatase level in patients with type 2 diabetes mellitus and its relation with periodontitis. KUST Med J. 2009; 1:51-4.
14. Todorovic T, Dozic I, Barrero MV, Ljuskovic B, Pejovic J, Marjanovic M et al. Salivary enzymes and periodontal disease.Med Oral Patol Oral Cir Bucal. 2006;11:E115-9.
15. Zin, Thaw, et al. "Salivary total protein levels among healthy controls, chronic gingivitis patients and chronic periodontitis patients." Journal of Oral Research and Review, 2021;13(1):18-24.
16. Karthiga DG, Geetha RV, Vishnu Priya V, Gayathri R. Comparative analysis of salivary protein in individuals with and without periodontitis. Int J Pharm Sci Rev Res 2017;43:23
17. . Karthiga DG, Geetha RV, Vishnu Priya V, Gayathri R. Comparative analysis of salivary protein in individuals with and without periodontitis. Int J Pharm Sci Rev Res 2017;43:23-4.
18. K M P, Johnson P, Ganesh M, Subhashini AS. Evaluation of Salivary Profile among Adult Type 2 Diabetes Mellitus Patients in South India. J Clin Diagn Res. 2013 Aug;7(8):1592-5.
19. Mandel ID. The diagnostic uses of saliva. Journal of Oral Pathology and Medicine. 1990;19(3):119-25.
20. Dodds, M.W. and Dodds, A.P. 1997. Effects of glycemic control on saliva flow rates and protein composition in non-insulin dependent diabetes mellitus. Oral Surg Oral Med Oral Patho Oral Radio Endo, 83:465-470.