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A CORRELATION OF CHRONIC PERIODONTITIS WITH SERUM ALKALINE PHOSPHATASE LEVEL IN TYPE 2 DIABETES MELLITUS INDIVIDUALS – A CLINICO-BIOCHEMICAL STUDY

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ABSTRACT

Introduction: Various enzymes are released from host cells during the initiation and progression of periodontal disease. Alkaline Phosphatase (ALP) is an enzyme found in cells of the periodontium, including osteoblasts, fibroblasts and neutrophils. Studies show that concentrations of this enzyme in gingival crevicular fluid (GCF) from diseased sites are significantly higher than healthy sites. The injured tissues secrete alkaline phosphatase from the neutrophil that causes destruction of connective tissues.

Aim: Present clinical study was undertaken to investigate a possible correlation between serum ALP levels in periodontitis with type 2 diabetics and non-diabetics subjects.

Materials and Methods: In the present study 160 subjects were taken in the range of 30-70 years. Among these Group A comprised of 40 healthy subjects. Group B comprises 40 type II Diabetes Mellitus subjects with Periodontitis. Group C comprises of 40 type II Diabetes Mellitus subjects without Periodontits and Group D comprises of 40 periodontitis subjects without type II Diabetes Mellitus. The baseline of clinical examinations included measurement of clinical attachment loss, oral hygiene index-Simplified and Russel's periodontal index. Laboratory measurements include serum alkaline phosphatase.

Results: Serum alkaline phosphatase level was significantly raised in type 2 diabetes mellitus subjects with or without periodontitis and non diabetic subjects with periodontitis when compared to controls.

Conclusion: From this study it was concluded that there is a positive correlation between chronic periodontitis and serum alkaline phosphates level in type 2 diabetes mellitus individuals which has been revealed by statistical analysis.

Keywords: Serum Alkaline Phosphatase, Chronic Periodontitis, Type 2 Diabetes Mellitus, Russel's Periodontal Index

INTRODUCTION:

Periodontal diseases consist of a group of inflammatory diseases initiated by bacteria that colonize the teeth and infect their surrounding soft tissues. The end result of this infection is the clinical manifestation of disease, which results in several distinct signs and symptoms. Majority of these clinical signs and symptoms include radiographic evidence of bone loss and increasing pocket depth.

At present periodontitis is diagnosed almost entirely on the basis of an array of clinical measurements including probing depth (PD), clinical attachment level (CAL), bleeding on probing (BOP), plaque index recordings and radiographic findings, however PD and CAL measurements by periodontal probes and radiographic bone levels provide information about the past periodontal destruction and neither elucidate the current state of the disease activity nor predict the future. Therefore one of the major challenges in the field of Periodontology is to discover a method of predicting the future of periodontal disease or at least to declare the current state of disease activity. The increased prevalence and severity of periodontitis, the sixth complication of diabetes; in the sequence of retinopathy, nephropathy, neuropathy, macrovascular disease and altered wound healing is commonly seen in diabetics **[1]**.

Long term periodontitis can lead to more serious problems like heart attack and stroke. Oral manifestation of diabetes mellitus(DM) like xerostomia,

glossopyrosis and oral candidiasis and impaired wound healing are important factors considered in the treatment of oral diseases in patients with DM **[2]**. Nuetrophils are the first cells that migrate into tissues, phagocytocise microbes and their complexes and injured tissues. Periodontal disease is frequently diagnosed in diabetic patients.

Various enzymes are released from host cells during the initiation and progression of periodontal disease **[3]**. Alkaline Phosphatase (ALP) is an enzyme found in cells of the periodontium, including osteoblasts, fibroblasts and neutrophils. Studies show that concentrations of this enzyme in gingival crevicular fluid (GCF) from diseased sites are significantly higher than healthy sites **[4]**.

There is relation between injured periodontal tissues and DM. The injured tissue secrete alkaline phosphatase from the neutrophil that causes destruction of connective tissues and level of ALP activities directly correlate with the intensity of inflammatory process of periodontal tissue **[4]**.

Phosphatases are enzymes that catalyze the splitting of phosphoric acid from monophosphoric esters. Two types are commonly estimated in serum, a mixture of *alkaline phosphatase* with maximum activity at about pH 10 and *acid phosphatase* at pH 5 and 6. It is present in liver, kidney, bone, intestine and placenta. It is also found in many cells of periodontium including neutrophils, osteoblasts and fibroblasts. ALP is released from polymorphonuclear neutrophils during inflammation, osteoblasts during bone formation and periodontal ligament fibroblasts during periodontal regeneration **[5]**. Thus it has dual involvement in the process of periodontal inflammation and healing/regeneration.

Alkaline phosphatase (ALP) (EC 3.1.3.1; Orthophosphoric monoester phosphohydrolase; ALP) comprise a group of enzymes that catalyze the alkaline hydrolysis of phosphoric acid from monophosphoric esters, generating an organic radical and inorganic phosphate **[6]**. For the most part, ALP is present in all tissues and especially found to be secreted in the cells of periodontium, including osteoblasts, fibroblasts, and neutrophils. ALP requires divalent ions like Mg2+, Co2+, and Mn2+ for the activation and has Zn2+ as its constituent metal ion. ALP physiologically increases during normal bone growth, while pathological increase is related to hepatobiliary disease and bone disease associated with increased osteoblastic activity **[7]**. The enzyme ALP plays a key role in bone metabolism. It is a membrane-bound glycoprotein produced by

many cells such as polymorpho nuclear leukocytes, osteoblasts, macrophages and fibroblasts within the area of the periodontium and gingival crevice. The response of an organism to the periodontal infection includes production of several enzymes and inflammation markers which can be analyzed both in serum as well as saliva. Recent studies have shown a correlation between high ALP levels and periodontitis, by proving the effect in periodontal diseases **[8]**.

The ideal periodontal diagnostic method should be able to screen susceptible subjects in the general population, secondly it should differentiate active or inactive site, thirdly it should predict future tissue destruction in particular individuals and sites, and finally to monitor the response to periodontal therapy.

There are enough studies available in the literature, correlating the levels of these enzymes in GCF with the severity of periodontal disease **[9]**. However, there are inherent problems in collecting GCF in a routine dental practice. The sampling technique is not easy as a long time is required for sample collection and it only reflects gingival inflammation at each specific site sampled. Thus, GCF is not suitable for community practice or in public health practices **[10]**. Saliva, even though it is faster and more convenient to collect, the sensitivity of the ALP in saliva is found to be inadequate when compared to its presence in GCF. The ratio of GCF ALP levels to those of saliva within individuals was 530:1 **[11]**. Hence serum as sample source was considered based on its convenience, high reliability and on the finding that alveolar bone loss could be reflected at the serum level **[12]**. Hence the present clinical study was undertaken to investigate a possible correlation between serum ALP levels in periodontitis with type 2 diabetics and non-diabetics subjects.

MATERIAL AND METHODS:

Study Design and Study Population:

In this observational case control study, 160 subjects were selected on purposive selection criteria from the Outpatient Department of General Medicine and Department of Periodontology. The duration of this study was from January 2015 to September 2016. The ethical clearance was obtained from institutional ethical committee. The written consent was taken from all the subjects included in this study. The subjects selected were in the age range 30–70 years, of whom 40 subjects assigned to group A (Control group) and 40 subjects assigned to group B, C, D each (Test group). Group A comprised of 40 healthy subjects. Group B comprises 40 type II Diabetes Mellitus subjects with Chronic Periodontitis. Group C comprises

of 40 type II Diabetes Mellitus subjects without Chronic Periodontits and group D comprises of 40 Chronic Periodontitis subjects without type II Diabetes Mellitus.

Group A: Inclusion Criteria:

- Forty healthy subjects as controls
- Dentate patients
- Age group 30–70 years

Group B: Inclusion Criteria:

- Forty patients with type II DM as diagnosed by physician having chronic periodontitis
- Dentate patients
- Age group 30-70 years

Group C: Inclusion Criteria:

- Forty patients with type II DM as diagnosed by physician with healthy periodontium
- Dentate patients
- Age group 30-70 years

Group D: Inclusion Criteria:

- Forty patients diagnosed as having chronic periodontitis
- Dentate patients
- Age group 30–70 years

Group A, B, C and D: Exclusion Criteria:

- If patients had undergone periodontal therapy for last 3 months
- Patients on medications (antibiotics) known to influence the periodontal tissues for last 6–8 weeks
- Patients with any systemic diseases
- Patients with other diagnosed diseases affecting ALP levels
- Patients having habit of smoking and alcoholics

Following parameter were measured in all the patients.

Oral Hygiene Index – Simplified, Russel's Periodontal Index, Clinical Attachment Level, Serum Alkaline Phosphatase

Biochemical Analysis:

Two milliliters of fasting venous whole blood sample was collected and centrifuged at 1500 rpm for 10 minutes **(Figure 1)**.

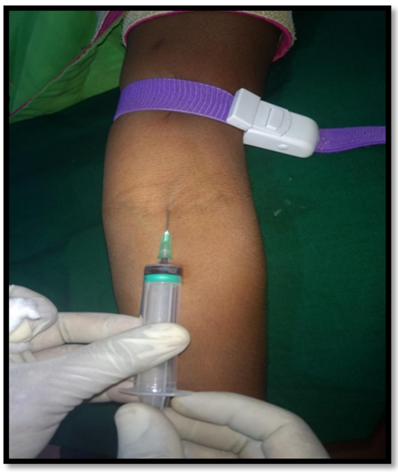


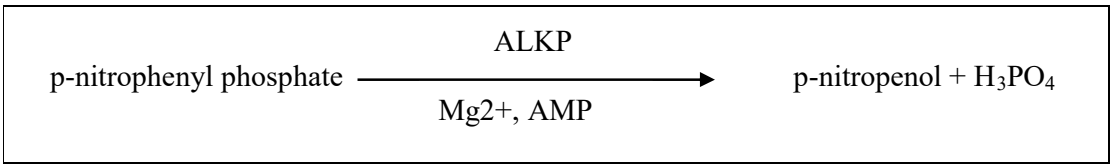
Figure 1: Blood Sample Collection

The serum obtained was used for the estimation of Alkaline Phosphatase. The investigation was carried out in the Centralized clinical laboratory of Pravara Institute of Medical Sciences (Loni) by automated machine, System Vitros Company 5.1 FS (Chemistry Analyzer) from Ortho clinical diagnostics (Johnson & Johnson Co.) using dry chemistry. Reagents used in automated machines were p-

nitrophenyl phosphate, 2-amino-2-methyl-1-propanol and magnesium sulfate **(Figure 2)**.

Figure 2: System Vitros Company 5.1 FS (Chemistry Analyzer) [Ortho clinical diagnostics]

Reaction Scheme:



Statistical analysis:

Descriptive statistics were expressed as mean ± standard deviation (SD) for each group. Age wise comparison, serum alkaline phosphatase level, blood glucose level, OHI-S, PI and Avg. CAL comparison among groups were analyzed using Anova test and post hoc Bonferrroni test. Gender wise comparison among groups was analyzed using chi square test. In the above tests, p value less than or equal to 0.05 ($p<0.05$) was taken to be stattstically significant

All analyses were performed using SPSS software version 2.0.

RESULTS:

The average age of Group A subjects was 47.6, group B was 51.2, group C was 49.7 and group D was 51.5. The age was not significant among all the groups

Table 1 shows the serum alkaline phosphatase level comparison among groups. It shows that the serum alkaline phosphatase level is statistically significant in group A, B, C and D as p value is <0.05. When group C and group D were compared, they were statistically nonsignificant.

Table 2 shows the blood glucose level comparison among groups. It shows that the serum blood glucose level is statistically significant in group A, B, C and D as p value is <0.05. When group A and group D were compared, they were statistically nonsignificant.

Graph 1 shows the average OHI-S scores of all the groups and the graph shows the average score of Group B and D is higher than Group A and C

Graph 2 shows the average CAL scores of all the groups and the graph shows the

average score of Group B and D is higher than Group A and C

Table 3 shows the intra group comparison of study subjects for serum alkaline phosphatase level. Group A shows statistically significant results when compared to group B, C and D. Similarly Group B shows statistically significant results when compared to group A, C and D. Group C shows statistically significant results when compared to group A and B but group C and D are statistically nonsignificant.

Table 4 shows the intra group comparison of study subjects for blood glucose level. Group A shows statistically significant results when compared to group B and C but group A and D are statistically nonsignificant. Similarly Group B shows statistically significant results when compared to group A, C and D. Group C shows statistically significant results when compared to group A,B and D.

Total 64 females and 96 males were included in study but there was no statistical difference gender wise.

Table 1: Serum Alkaline Phosphatase level comparison among groups

	Mean	Std. Deviation	Minimum	Maximum	F/p value
A	92.02*	14.93	43.00	127.00	67.63 / 0.001(S)
B	234.22	84.14	110.00	433.00	
C	192.95	67.74	111.00	393.00	
D	94.85*	12.903	72.00	119.00	

Test applied: ANOVA with post hoc Bonferrroni test; S=Significant
*** = when compared its gives non-significant results**

Table 2: Blood glucose level comparison among groups

		Mean differences	P value
A	B	-96.5	0.000 (S)
	C	-73.62	0.000 (S)
	D	-67.77	0.000 (S)
B	A	96.5	0.000 (S)
	C	22.87	0.000 (S)
	D	28.72	0.000 (S)
C	A	73.62	0.000 (S)
	B	-22.87	0.000 (S)
	D	5.85	1.000

Test applied: Unpaired t test; S=Significant

Table 3: Intra-group comparison of study subjects (Serum Alkaline Phosphatase)

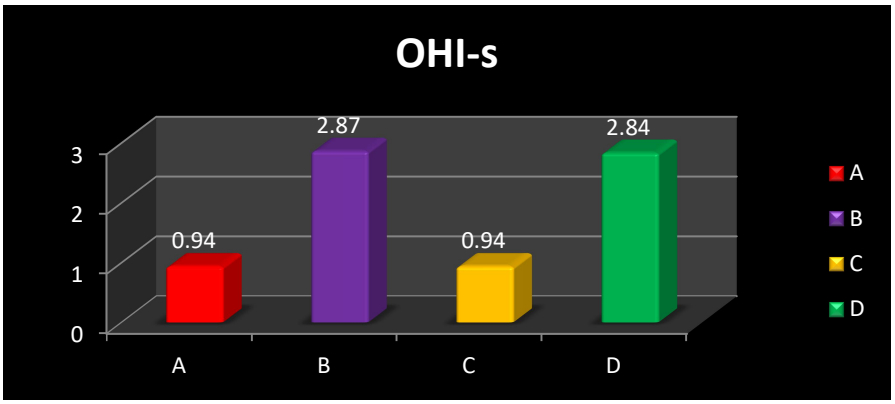
		Mean differences	P value
A	B	-142.2	0.000 (S)
	C	-100.92	0.000 (S)
	D	-2.82	1.000
B	A	142.2	0.000 (S)
	C	41.27	0.006 (S)
	D	139.37	0.000 (S)
C	A	100.92	0.000 (S)
	B	-41.27	0.006 (S)
	D	98.1	0.000 (S)

Test applied: Unpaired t test; S=Significant

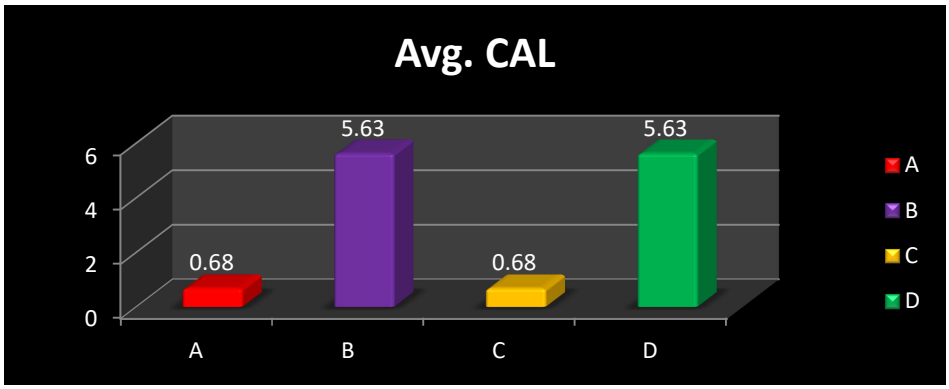
Table 4: Intra-group comparison of study subjects (Blood Glucose Level)

	Mean	Std. Deviation	Minimum	Maximum	F/p value
A	80.8	19.92	40.00	122.00	143.29 / 0.001(S)
B	177.3	26.13	106.00	253.00	
C	154.42*	20.28	121.00	197.00	
D	148.57*	20.86	126.00	236.00	

Test applied: ANOVA with post hoc Bonferrroni test; S=Significant
*** = when compared its gives non-significant results**



Graph 1: OHI-S comparison among groups



Graph 2: Average CAL comparison among groups

DISCUSSION:

The present study was undertaken to evaluate the gingival and periodontal health status of patients with and without Type II DM. Oral hygiene status was assessed by Oral Hygiene Index- Simplified (OHI-S). Periodontal health status was assessed by Russell's Index (Pl) and clinical attachment level (CAL). Serum alkaline phosphatase and random blood sugar level were assessed in all the subjects of all the groups including controls.

Periodontal disease is defined as when 2 or more teeth sites with pocket depth greater than 4 mm or clinical loss of more than 4 mm and bleeding on probing. (Task Force Report on the update to the 1999 Classification of Periodontal Diseases and Conditions)

Oral hygiene index- Simplified and periodontal index both are highly

significant in group B and D compared to group A and C. Significant difference in mean values of the indices was noted among subjects with non periodontitis, non diabetics and non periodontitis, diabetics when compared those having a periodontitis with or without diabetics.

New diagnostic tests are required to detect disease activity. Alkaline phosphatase is actually a heterogenous group of enzymes. They are widely distributed in the body with significant activities in liver, bone, gastrointestinal tract and placenta.

A number of studies have shown raised serum ALP levels in various physiological and pathological conditions e.g. during bone growth physiologically while pathological increases are associated with hepatobilliary and bone diseases **[7]**. Diabetes mellitus is a metabolic disorder arising from insulin insufficiency which is associated with altered activity of various enzymes e.g. ALP, SGPT and SGOT etc. **[13]**. Besides the microvascular and macro vascular complications in diabetes mellitus a compromised immune state is also a condition that increase the susceptibility of diabetes to different infection, particularly including opportunist micro-organisms such as constituting oral micro flora **[14]**.

The aim of our study is to compare the serum ALP levels among the type 2 diabetes patients with and without periodontitis. The duration of diabetes is known to be related to the development of complications **[15]**. ALP showed a significant rise in both diabetic and non-diabetic patients with periodontitis (Group B and Group C, 177.3 ± 26.13 and 154.42 ± 20.28 resp.) as compared to control (Group A, 80.2 ± 19.92). Diabetic patients even in the absence of periodontitis showed an increase in mean values of serum ALP. Comparing diabetics (Group B) and nondiabetics (Group D, 154 ± 20.86) both having periodontitis, raised blood glucose level in diabetic patients and change in medium make the individual susceptible to infection due to depressed immunity **[16]**. It can be seen that significant increase of ALP and clinical attachment loss in diabetics with periodontitis (Group B) and non-diabetics with periodontitis (Group D) **[17]**. The broad spectrum of pathogen is defeated by the individuals when immunity is intact **[18]**. Raised value of serum ALP and diabetic patients has been reported by Grossi, 1998, **[19]** Iwamoto *et al*. 2001 **[20]**, Siddiqui *et al*. 2005 **[13]**. Raised value of serum ALP in our population with periodontitis (Group D), particularly when they are diabetics (Group B) and also in diabetics without periodontitis (Group C) is in agreement with a studies of Armitage and Pushpa Rani *et al* **[21, 22]**.

Bone forming cells have been shown to have alkaline phosphatase activity and changes in this enzyme in serum and bone have been used as markers for bone metabolism in several diseases (Christenson, 1997). Alkaline phosphatase is commonly associated with bone metabolism with osteoblasts showing high levels of alkaline phosphatase **[23]**.

The response of an organism to the periodontal infection includes production of several enzymes and inflammation markers which can be analyzed both in serum and saliva **[24]**. In peridontitis, one of the mechanism of collagen loss is fibroblast phagocytize collagen fibers which contributes to the total ALP level **[25]**.

In a study of serum ALP as a potential marker in progression of periodontal disease in cirrhosis patient by Jaiswal *et al* in 2011 **[26]**, there existed an increased ALP levels with increase in CAL and PD values. Similar results were obtained in our study which revealed an increase in serum ALP level as with increased CAL and PD values in Group D when compared to Group A. This was in agreement with studies done by Ishikawa and Cimasoni **[27]** Gibert P *et al*, **[28]** Totan A **[29]** and Todorovic T **[9]** who compared the enzyme activities in subjects with periodontitis and in healthy controls.

It is well established that inadequate management and or control of hyperglycemia predisposes diabetic patients to a number of complications. Although periodontitis is a potential complication of diabetes, emerging evidence suggests that treatment of periodontal infections in diabetics could improve glycemic control. Patients with controlled diabetes had no more periodontal destruction than healthy controls and increased periodontal destruction was found in diabetic patients **[30, 31]**. Simultaneously, with the worsening of the diabetes with periodontitis, serum ALP and frequency of pockets seem to increase. On the contrary, some investigators have found increased periodontal destruction in diabetic patients. In some cases, drastic deterioration of periodontal conditions has even led to the diagnosis of diabetes mellitus **[32]**. The results of this study show that increased serum ALP in type 2 diabetes mellitus with periodontitis may be linked to alveolar bone loss. We observed a clear progressive increase in serum ALP level among type 2 diabetes mellitus with periodontitis (group B) compared to type 2 diabetes mellitus without periodontitis (group C) and nondiabetes with periodontitis patients (group D) which is in agreement with study done Shaheen *et al*.

[33] However, serum ALP level was found to be nonsignificant among non-diabetic patients without periodontitis (group A). This implies to the fact that type 2 diabetes mellitus with periodontitis (group B) are severely affected with alveolar bone loss and tooth loss when compared to patients having type 2 diabetes without periodontitis (group C) and nondiabetes mellitus with periodontitis (group D). When serum ALP level was compared between diabetics without periodontitis (group C) and non diabetics with periodontitis (group D), the result was not significant. Raised serum ALP in chronic periodontitis patients is suggestive of its role in bone apposition. As the severity of chronic periodontitis increases, the rate of bone loss also increases which may lead to decrease in serum ALP as shown in a study done by Gopu Chandran *et al*. **[34]**.

The finding that human serum has more significant ALP activity among the type 2 diabetes mellitus with periodontitis indicates the probable origin of this enzyme from alveolar bone **[35]**. The increased ALP enzyme activity causes the release of phosphate ions, which in turn leads to more calculus formation and may worsen the condition of periodontitis in type 2 diabetes mellitus.

Thus present study showed that the serum alkaline phosphatase level was significantly high in subjects having type 2 diabetes mellitus with or without periodontitis and subjects having periodontitis without type 2 diabetes mellitus compared to controls. Serum ALP level was significantly high in subjects having type 2 diabetes mellitus with periodontitis when compared to subjects having type 2 diabetes mellitus without periodontitis and subjects having periodontitis without diabetes mellitus.

Many theories have been advanced to explain the pathogenesis of diabetic complications but as until now, no one has provided a complete and satisfactory answer to this problem. The observation that a significant increased level of serum ALP in subjects having type 2 diabetes mellitus with periodontitis could possibly explain more credible mechanistic justification on the role of diabetes in the pathogenesis of periodontal disease. The suggestion of the relationship between ALP in patients with type 2 diabetes mellitus and periodontitis provides a testable hypothesis that may offer novel insights into the pathogenesis of diabetic complications. The significantly elevated level of serum ALP can serve as a biomarker to the rapid development of periodontitis in type 2 diabetes patients.

CONCLUSION:

Considering the fact about the lack of many researches in serum alkaline phosphatase of

chronic periodontitis in type 2 DM patients, this stands as an initial attempt to suggest the role of serum alkaline phosphatase as a potential biochemical assay in type 2 diabetic patients with chronic periodontitis on a longitudinal basis. To conclude, this study describes the interesting research area of oral health in relation to systemic diseases. Increasing awareness of both physicians and Periodontists/dentists for possible relationships and interaction between oral and general health is of major importance and should been encouraged. This is the simple test to diagnose periodontitis in diabetic and non diabetic subjects. This test is quick, with a very good sensibility, giving the clinician details about status of periodontitis in diabetics and non diabetics. Serum ALP is not very technique sensitive when compared to gingival crevicular fluid and saliva. In this study, this test is unable to distinguish severity of periodontitis. Serum alkaline phoaphatase level in other forms of periodontitis like aggressive periodontitis is not studied.

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Conflicts of interest: None

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