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Original Article

Comparison of serum nitric oxide, sialic acid and alkaline phosphatase in type 2 DM patients with and without chronic periodontitis

Ankur Tailor [1], Nina Shenoy [1]*, Amitha Ramesh [1], Smitha Shetty [1]

[1] Department of Periodontics, NITTE (Deemed to be University), AB Shetty Memorial Institute of Dental Sciences (ABSMIDS), Mangalore, Karnataka, India

* Correspondence to: Nina Shenoy, Department of Periodontics, NITTE (Deemed to be University), AB Shetty Memorial Institute of Dental Sciences, Medical Sciences Complex, Deralakatte, Mangaluru – 575018, Karnataka, India. Phone: 0091-9844344488; E-mail: drninavijaykumar@nitte.edu.in

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Abstract

Periodontitis and diabetes cause a hyperinflammatory immune response with elevated reactive oxygen species and cytokines. A few mediators of the ensuing inflammation are nitric oxide (NO), sialic acid (SA) and ALP (alkaline phosphatase). Hence, this study aimed to compare and correlate the level of serum NO, SA and ALP in diabetes mellitus with and without chronic periodontitis patients prior to and after non-surgical periodontal treatment. A total of 100 patients were included: healthy controls (Group A), DM (Group C, D) and chronic periodontitis (Group B, C). Serum samples of NO, sialic acid and ALP were analyzed before and after the treatment. Post-hoc Tukey HSD test was used for intergroup comparison levels, and the Analysis of Variance Test (ANOVA) was used to compare values before and after treatment in all groups. P<0.05 was considered statistically significant. Baseline means NO, SA and ALP levels were highest in DM with periodontitis (65.37, 156.09, 272 µM/L), respectively. Inter-group comparison of NO, SA and ALP values before and after treatment was statistically significant (P<0.001). Diabetes significantly impacts the exacerbation of inflammation and elevation of serum NO, SA and ALT in periodontitis. Additionally, periodontal therapy produced a beneficial result.

Keywords: reactive oxygen species, biomarkers, nonsurgical periodontal therapy.

Introduction

Periodontal disease is a chronic inflammatory condition of the tissues that are brought on by gram-negative bacterial infections and is, for the most part, asymptomatic. However, the inflammatory host response is largely responsible for the clinically observed detrimental tissue changes [1]. The metabolic disorder called diabetes mellitus is defined by a partial or total deficiency in insulin production and/or a resistance to insulin's metabolic effects on target tissues. Type 2 diabetes mellitus (DM), the more prevalent variety, is brought on by the pancreatic beta cells' inability to produce enough insulin, which causes insulin resistance [2]. One defining trait of diabetes mellitus is hyperglycemia, linked to consequences including coma. Poor glycemic control and the ensuing hyperglycemia result in various molecular and cellular processes, including oxidative stress, an increase in the generation of proinflammatory mediators, and vascular alterations that put people at risk for diabetic complications [2].

Studies have reported that patients with periodontitis have elevated circulating levels of inflammatory markers and Reactive Oxygen Species [2–4]. These host response biomarkers can be found in gingival crevicular fluid (GCF), saliva and serum samples and can potentially be used as diagnostic markers. Infection and the ensuing inflammatory reactions are mediated by nitric oxide (NO), which is produced from L-arginine by the enzyme NO synthase (NOS). Nitric oxide also serves a dual function in the pathophysiology of host damage [2]. Sialic acid is crucial to glycoproteins and glycolipids in both cells and body fluids. Sialic acid levels in serum and plasma should be between 1.58 and 2.22 mmol/L. Sialic acid levels have been found to rise during inflammatory processes, most likely as a result of an increase in the amount of acute-phase glycoprotein that has been richly sialylated [5]. Alkaline phosphatase (ALP), also known as orthophosphoric-monoester phosphohydrolase, was one of the first host enzymes in GCF to be discovered. It is a membrane glycoprotein that is made by a large number of cells in the periodontium and gingival crevice region. ALP Level has been used as a diagnostic marker for periodontal disease since it may distinguish between healthy and inflammatory areas [6].

Therefore, this study was designed to compare and correlate the level of serum nitric oxide, sialic acid and alkaline phosphatase in type II diabetes mellitus with and without chronic periodontitis patients prior to and after non-surgical periodontal treatment and to ascertain whether these markers contribute to the severity of periodontitis in patients with diabetes.

Material and methods

This study was conducted over a 2-year period from 2012 to 2014 and comprised a total of 100 patients aged between 25 and 65 years, reporting to a dental college in Karnataka. Institutional research and ethical committee clearance was obtained, and prior informed consent was taken from the participants.

Systemically and periodontally healthy patients (Group A). Patients diagnosed with Type II DM on oral hypoglycaemics (Group C and D), and those diagnosed with chronic periodontitis (Group B and C) with a minimum complement of 20 teeth were included.

The patients were divided into four groups of 25 subjects, each as follows:

- Group A – Systemically healthy without chronic periodontitis (control group);
- Group B – Systemically healthy with chronic periodontitis;
- Group C – Type II diabetes mellitus and chronic periodontitis;
- Group D – Type II diabetes mellitus without chronic periodontitis.

Pregnant/lactating women, smokers, systemic diseases other than type 2 diabetes mellitus, antibiotic/anti-inflammatory/micronutrient supplemental therapy three months prior to the study and periodontal treatment six months prior to the study were excluded.

Laboratory and clinical data collection

Before starting any treatment, clinical parameters such as probing depth, clinical attachment levels, gingival index (Loe & Silness, 1963) and plaque index (Silness & Loe, 1967) were recorded (baseline value) and blood samples were collected to assess the serum nitric oxide, sialic acid, and alkaline phosphatase level. Scaling and root planing were done for Group B and C, and oral hygiene instructions were given. On the 21st day, the gingival and plaque index for Group B and Group C was recorded, and subjects with a gingival and plaque index of more than 1 were excluded. Blood samples were collected again to assess the nitric oxide, sialic acid, and alkaline phosphatase levels. 5 ml of venous blood for serum was collected in sterile tubes and kept at 4˚C for 30 minutes before centrifugation for 10 minutes at room temperature.

Statistical analysis

Quantitative evaluation of nitric oxide, sialic acid and alkaline phosphatase levels in serum was tabulated using mean and standard deviation. Intergroup comparison before non-surgical periodontal treatment of the serum levels of nitric oxide, sialic acid and alkaline phosphatase was assessed using post-hoc Tukey HSD test Analysis of Variance Test (ANOVA) was used for comparison of mean and standard deviation before and post-treatment in all groups for the levels of nitric oxide, sialic acid and alkaline phosphatase. $P<0.05$ was considered to be statistically significant.

Results

Table 1 depicts the mean and SD significant difference in all groups prior to SRP (underlined values indicate statistically significant).

Table 2 shows that in the nitric oxide levels, there was a significant difference when Group A was compared with Groups B, C and D. There was no significant difference when Group B was compared with Groups C and D. Also, there was no significant difference between Groups C and D. The sialic acid levels all the groups showed significant difference. The alkaline phosphatase levels in all the groups showed a significant difference, except between Groups A and B and Groups C and D.

Table 3 compares mean and standard deviation values using a one-way ANOVA test following nonsurgical periodontal treatment in all groups of nitric oxide, sialic acid and alkaline phosphatase. There was a significant difference in all groups, except serum values of nitric oxide levels.

The table also depicts the comparison of the difference in mean and standard deviation in all groups between before and after non-surgical therapy for the levels of nitric oxide, sialic acid and alkaline phosphate. There was a significant difference between all groups of nitric oxide, sialic acid and alkaline phosphatase levels.

Discussion

In periodontitis, a number of bacterial virulence factors either cause the host tissues to deteriorate or trigger the release of biological mediators from host tissue cells, which in turn causes host tissue destruction [7]. Reactive oxygen species and oxidative stress are produced in excess as a result of numerous molecular and cellular reactions that are sparked by hyperglycemia. Proinflammatory cytokines are also produced in excess, which upregulates proinflammatory responses and causes AGEs and vascular abnormalities. This may worsen insulin resistance and make it more challeng-

Table 1: Mean and SD of serum levels of nitric oxide, sialic acid and alkaline phosphatase in all 4 groups levels before scaling and root planning using one-way ANOVA test.

	N	Mean	Std. Deviation	Statistics/ mean squares	df2(welch)/ F(Anova)	P-value
Before NO in µm/L						
Group A: Control	25	50.76	6.74	15.954	52.322	<0.001**
Group B: Systemically healthy with periodontitis	25	58.5	11.7			=
Group C: Type 2 DM with periodontitis	25	65.37	8.8			=
Group D: Type 2 DM without periodontitis	25	61.40	9.7			=
Total	100	59.02	10.7			=
Before SA in Mg/Dl						
Group A: Control	25	58.70	6.6	46149.66	739.077	<0.001**
Group B: Systemically healthy with periodontitis	25	74.62	6.5			=
Group C: Type 2 DM with periodontitis	25	156.09	10.3			=
Group D: Type 2 DM without periodontitis	25	106.75	7.48			=
Total	100	99.04	38.1			=
Before ALP in U/L						
Group A: Control	25	180.6	55.9	52766.89	23.019	<0.001**
Group B: Systemically healthy with periodontitis	25	179.32	52.7			=
Group C: Type 2 DM with periodontitis	25	272.0	37.72			=
Group D: Type 2 DM without periodontitis	25	240.8	42.72			=
Total	100	218.2	61.82			=

Note: NO – Nitric Oxide; SA – Sialic Acid; ALP – Alkaline Phosphatase; ** – P<0.05 High statistical significance.

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Table 2: Comparison of nitric oxide, sialic acid and alkaline phosphatase levels in all 4 groups levels before non-surgical treatment using post-hoc Tukey HSD test.

Tukey HSD Dependent Variable	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	P-value
Before NO in µM/L	Group A: Control	Group B: Healthy with periodontitis	-7.7904	2.671987	0.023**
		Group C: Type 2 DM with periodontitis	-14.6132	2.671987	<0.001**
		Group D: Type 2 DM without periodontitis	-10.6412	2.671987	0.001**
	Group B: Systemically healthy with periodontitis	Group C: Type 2 DM with periodontitis	-6.8228	2.671987	0.058
		Group D: Type 2 DM without periodontitis	-2.8508	2.671987	0.71
	Group C: Type 2 DM with periodontitis	Group D: Type 2 DM without periodontitis	3.972	2.671987	0.45
Before SA in mg/dL	Group A: Control	Group B: Healthy with periodontitis	-15.9252	2.235035	<0.001**
		Group C: Type 2 DM with periodontitis	-97.3932	2.235035	<0.001**
		Group D: Type 2 DM without periodontitis	-48.0532	2.235035	<0.001**
	Group B: Systemically healthy with periodontitis	Group C: Type 2 DM with periodontitis	-81.468	2.235035	<0.001**
		Group D: Type 2 DM without periodontitis	-32.128	2.235035	<0.001**
	Group C: Type 2 DM with periodontitis	Group D: Type 2 DM without periodontitis	49.34	2.235035	<0.001**
Before ALP in U/L	Group A: Control	Group B: Healthy with periodontitis	1.3368	13.54208	1
		Group C: Type 2 DM with periodontitis	-91.362	13.54208	<0.001**
		Group D: Type 2 DM without periodontitis	-60.224	13.54208	<0.001**
	Group B: Systemically healthy with periodontitis	Group C: Type 2 DM with periodontitis	-92.6988	13.54208	<0.001**
		Group D: Type 2 DM without periodontitis	-61.5608	13.54208	<0.001**
	Group C: Type 2 DM with periodontitis	Group D: Type 2 DM without periodontitis	31.138	13.54208	0.105

Note: NO – Nitric oxide; SA – Sialic Acid; ALP – Alkaline Phosphatase; ** – P<0.05 High statistical significance.

ing for patients to manage their diabetes. Reducing periodontal inflammation may aid in lowering blood levels of inflammatory mediators that cause insulin resistance, improving glycemic regulation and systemic inflammation [4, 8].

The serum study can contribute to the diagnosis and prognosis of the disease even if periodontal disease is diagnosed predominantly by clinical examination and recording periodontal clinical parameters [7]. According to available evidence, the elevated serum levels of various inflammatory markers present in periodontitis patients compared to those in unaffected control populations indicates that periodontitis contributes to systemic inflammation [9]. A number of studies have shown raised levels of nitric oxide, sialic acid and alkaline phosphatase in various inflammatory conditions such as periodontitis [2, 10–13]. This study aimed to evaluate and compare serum nitric oxide, sialic acid and alkaline phosphatase levels in chronic periodontitis patients with and without type II diabetes mellitus prior to and following non-surgical periodontal treatment (SRP).

Periodontal diseases are chronic inflammatory infections associated with gram-negative bacteria, which in turn stimulate macrophages to generate nitric oxide [14]. The enzyme nitric oxide synthase generates a highly reactive radical (NOS) from the amino acid arginine. Depending on the source and amount of NO generated, NO has a variety of functions in the immunological and inflammatory responses [15]. The higher difference in mean values at baseline of NO was recorded in Group C (subjects with DM and CP) and D (subjects with DM without periodontitis), as compared to Group B (systemically healthy subjects with CP), the probable reason being in diabetic patients with CP increased oxidative stress could contribute to high-level nitric

Table 3: Comparison of serum NO, SA and ALP levels after non-surgical treatment and difference in all three groups using one-way ANOVA test.

Groups	N	Mean	Std. Deviation	Statistics/ mean squares	df2(welch)/ F(Anova)	P-value
After NO in μM/L						
Group B: Systemically healthy with periodontitis	25	51.93	11.30	104.328	0.864	0.426
Group C: Type 2 DM with periodontitis	25	48.18	12.61			=
Group D: Type 2 DM without periodontitis	25	48.66	8.67			=
Total	75	49.59	10.96			=
After SA in mg/dL						
Group B: Systemically healthy with periodontitis	25	65.74	7.07	23905.85	439.374	<0.001**
Group C: Type 2 DM with periodontitis	25	127.3	8.46			=
Group D: Type 2 DM without periodontitis	25	91.97	6.43			=
Total	75	95.02	26.43			=
After ALP in U/L						
Group B: Systemically healthy with periodontitis	25	139.63	53.60	15908.99	7.888	0.001**
Group C: Type 2 DM with periodontitis	25	183.34	42.03			=
Group D: Type 2 DM without periodontitis	25	183.31	37.56			=
Total	75	168.76	48.91			=
NO DIFF						
GROUP B: Systemically healthy with periodontitis	25	6.61	4.26	13.318	43.251	<0.001**
Group C: Type 2 DM with periodontitis	25	17.19	14.9			=
Group D: Type 2 DM without periodontitis	25	12.74	5.4			=
Total	75	12.18	10.3			=

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Table 3: Continued.

Groups	N	Mean	Std. Deviation	Statistics/ mean squares	df2(welch)/ F(Anova)	P-value
SA DIFF						
GROUP B: Systemically healthy with periodontitis	25	8.88	2.64	144.386	37.248	<0.001**
Group C: Type 2 DM with periodontitis	25	28.73	5.3			=
Group D: Type 2 DM without periodontitis	25	14.78	1.04			=
Total	75	17.46	9.05			=
ALP DIFF						
GROUP B: Systemically healthy with periodontitis	25	39.6	57.38	15365	6.284	0.003**
Group C: Type 2 DM with periodontitis	25	88.6	46.69			=
Group D: Type 2 DM without periodontitis	25	57.58	43.15			=
Total	75	61.98	52.86			=

Note: NO – Nitric Oxide; SA – Sialic Acid; ALP – Alkaline Phosphatase; ** – P<0.05 High statistical significance. The levels of serum nitric oxide, sialic acid and alkaline phosphatase before and following scaling and root planning in all the groups were evaluated.

oxide [1]. Prior to non-surgical treatment, a statistically significant difference was observed in the levels of serum nitric oxide in all the groups as compared to the control (P<0.05) (Table 1). These results are consistent with numerous other studies showing greater nitric oxide levels in inflammatory sites [10, 14–17]. Gram-negative bacteria, such as porphyromonas gingivalis and prevotella intermedia in chronic periodontitis, promote nitric oxide production (NO) [18]. However, there was no significant difference in the nitric oxide level when group C (subjects with DM and CP) was compared with Group D (subjects with DM without CP) (Table 2). There was a significant decrease in mean nitric oxide levels in all the groups before and after SRP (Table 3). The probable explanation could be the improvement in the inflammatory status resulting in decreased nitric oxide levels. These results were in agreement with the results obtained by Latha N *et al.* in 2018, which reported a statistically significant reduction in nitric oxide levels three months following non-surgical therapy in chronic periodontitis patients with and without type 2 diabetes [14]. In 2019, Gayathri *et al.* also reported a significant reduction in nitric oxide levels in CP with and without type 2 DM four weeks after initial periodontal therapy [19]. A bidirectional relationship exists between diabetes and periodontitis. The role of NO is similar in both diseases due to the hyperinflammatory state. This suggests that nitric oxide plays an important role in diabetic patients with periodontitis.

This study showed a higher serum concentration SA in all the groups compared to the control prior to scaling and root planing (Table 1). These results suggest increased serum SA in chronic periodontitis, which is in accordance with similar studies [11, 20, 21].

In an inflammatory state, SA concentration is elevated as a result of increased levels of richly sialylated acute-phase glycoproteins. Markedly increased SA levels in inflammatory diseases, including periodontitis, confirm the evidence that saccharides play a crucial role in the immune system. High levels of SA were attributed to the synthesis of sialoproteins and cleavage of globulins from damaged tissue [22].

The intergroup comparison was statistically highly significant (p<0.001) (Table 2). Inter-group comparison of mean values (Table 1) indicates a higher SA level in Group C (Subjects with DM and CP). SA could play an additional inflammatory response in diabetic patients with periodontitis. There was a significant decrease in serum SA levels in all groups before and after SRP due to an improved inflammatory state (Table 3). The findings were similar to the results obtained by Suresh R *et al.* in 2019, in which there was an elevation of total serum SA level in chronic periodontitis and non-insulin-dependent diabetes mellitus subjects when compared to healthy subjects [21]. After non-surgical treatment, as well as a comparison of all the groups before and after non-surgical therapy indicated a significant reduction in the total serum sialic acid levels (Tables 2 and 3). Oktay S *et al*. also reported that non-surgical treatment of periodontal disease significantly decreased salivary and serum SA levels [11]. In contrast, Ide *et al*. found no appreciable changes in the levels of sialic acid and other inflammatory markers, concluding that an improvement in periodontal health did not affect the levels of vascular markers [23].

Alkaline phosphatase, a membrane-bound glycoprotein, is present on the membranes of the majority of human cells. It is primarily produced by polymorphonuclear leukocytes (PMNs) in the periodontal environment. Its additional sources are the bacteria present in supra- and subgingival plaque, along with fibroblast and osteoblast activity. ALP, SGPT (serum glutamic pyruvic transaminase), and SGOT (serum glutamic oxaloacetic transaminase) are a few of the enzymes whose activity is changed in diabetes mellitus (DM) [24].

Group C (subjects with DM and CP) had the highest mean value of alkaline phosphatase (Table 1). Other investigations have reported similar outcomes [13, 25]. In the inter-group comparison, the alkaline phosphatase levels in all the groups showed a significant difference, except between Groups A and B and Groups C and D (Table 2). The mechanical plaque control may have caused a considerable drop in serum ALP levels in this analysis (Table 3). Similar outcomes were seen by Jeyasree *et al*. in 2018 [9], who found that in phase I periodontal treatment, there was a significant drop in the serum and saliva levels of ALP following phase I periodontal treatment [12]. Similar findings have been observed in studies with GCF and saliva [6, 26, 27].

The literature on the complications of diabetes and periodontal disease is replete with information suggesting that altered immune responses may be a precursor to both conditions, resulting in a synergistic effect when they coexist in the host. Numerous studies have also shown that people with diabetes and periodontitis had higher levels of inflammatory markers [28–30]. In 2018, Naiff *et al*. agreed that periodontal treatment had been linked to a subsequent decline in serum levels of IL-6, TNF-, CRP, and ROS and promoting local clinical inflammation reduction [28]. This data emphasizes the crucial link of inflammatory mediators in the relationship between periodontitis and diabetes. Patients with severe periodontitis and diabetes, compared to those with milder forms, have decreased PMN chemotaxis and faulty PMN apoptosis, which prolongs the release of MMPs and reactive oxygen species (ROS) due to PMN retention in the periodontal tissue, leading to greater tissue degradation. The main triggers of acute-phase proteins in type 2 diabetes are elevated levels of TNF- and IL-6, both of which also exacerbate insulin resistance by impairing intracellular insulin signaling [31].

This study showed a statistically significant difference in serum nitric oxide, sialic acid and alkaline phosphatase in DM patients with chronic periodontitis compared to other groups. It was also noted that there was a marked reduction in levels of serum nitric oxide, sialic acid and alkaline phosphatase before and after non-surgical treatment (Table 3). Nitric oxide, sialic acid, and alkaline phosphatase measurements in the serum are a quick and easy way to check for periodontal disease and do not require a skilled examination. Nitric oxide, sialic acid, and alkaline phosphatase serum levels reflect periodontal tissue inflammation and degeneration. They may therefore serve as significant biomarkers for the diagnosis of active periodontal disease as well as a secondary assessment of the effectiveness of periodontal therapy. Hence, due to the reduction of inflammatory markers such as NO, SA and ALP following periodontal treatment, it may be concluded that periodontal treatment can improve glycemic control, thereby reducing the molecules responsible for periodontal destruction. This is in accordance with a comprehensive systematic review and meta-analyses by WJ Teeuw *et al*. in 2010 and a systematic review by Hasuike A *et al*. in 2017 [32, 33]. Also, a meta-analysis by Ata-Ali F in 2020 concluded that periodontal therapy is linked to better glycemic control in individuals with type 2 DM [34].

Longer follow-up of patients for 3 months rather than 21 days, parameters such as probing depth and CAL were not evaluated following treatment, glycemic control status was not evaluated, the sample population selected in each group was small and the study was a cross-sectional design; hence no causal relationship could be established, which were few of the limitations of this study.

Conclusions

Diabetes plays an important role in inflammation and aggravating inflammatory conditions in periodontitis. According to the findings of this study, patients with type 2 diabetes mellitus and chronic periodontitis

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had considerably greater serum levels of nitric oxide, sialic acid and alkaline phosphatase than systemically healthy patients. Hence, it can be concluded that diabetes plays a significant role in aggravating inflammatory responses in periodontitis. There was a significant difference in the levels of NO, SA and ALP prior to and after non-surgical treatment. Furthermore, periodontal treatment proved beneficial in reducing their levels; therefore, they can be utilized as diagnostic biomarkers. Oral and periodontal health maintenance should be introduced as fundamental elements in managing diabetes. Additional longitudinal studies comparing periodontal parameters with glycemic control can be carried out to further comprehend and characterize these biomarkers' potential.

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Conflict of interest

The authors declare no conflict of interest.

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