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A COMPARATIVE ASSESSMENT OF SERUM VITAMIN C AND SERUM LEAD AMONG PERIODONTITIS AND DIABETIC PATIENT

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Abstract

Background and Aims: *Pre and post-operative nutritional status are not often tested on a consistent basis when patients are analyzed and diagnosed with Type 2 Diabetes Mellitus (T2DM) or have chronic periodontitis. Thus, this study pursues to evaluate the predictive value of serum vitamin C and lead levels in this population at baseline and after periodontal treatment.* ***Materials and Methods:*** *In an interventional study setting we enrolled 120 subjects which were later categorized into four sub-groups: set 1 – individuals with chronic periodontitis, set 2 – individuals with T2DM, set 3 – individuals with T2DM and chronic periodontitis respectively set 4 - healthy individuals as control group. Scaling and root planing was performed only to individuals with periodontitis and T2DM. At baseline and 21 days after periodontitis treatment blood was obtained from the subjects and analysis of serum vitamin C and serum lead was done and data obtained was statisticaly analysed.* ***Results:*** *ANOVA test showed baseline values of both the parameters to be statistically significant different between groups and within groups ($p<0.001$). Alteration was seen in the parameters postoperatively regarding serum vitamin C was increased and lead level decreased; paired t-test showed statistically significant difference ($p<0.001$).* ***Conclusion:*** *Serum Vitamin C can be considered a key biomarker. Increased lead level can be a risk factor for commencement and progression of T2DM and chronic periodontitis.*

key words: *Biomarker, Nutritional Status, Infection, Subclinical.*

Background and Aims

Pre- and post-operative nutritional status is a predictive biomarker during delayed wound healing and undesirable short term complications development [1,2]. Increased risk of infection and tissue damage is seen in patients suffering from longstanding diabetes mellitus [3]. Gingival tissues manifest periodontitis in Type 2 Diabetes Mellitus patients (T2DM) [3]. An thought-provoking fact is that chronic, long-lasting diseases like diabetes and periodontitis are systemically linked for the reason that both are initiated mainly due to oxidative stress [3].

Chronic lead exposure in humans leads to tissue damage [4]. Tissue damage and infection alter the plasma nutrients of quite a lot of important nutritional biomarkers, including

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vitamin C [5]. Any tissue damage or infection is diagnosed by clinical effect present in one and subclinical infection will be predictable by measuring biomarkers of inflammation [5]. In spite of knowing that nutrition plays an important role in improving the health and overall wellbeing in patients who are diabetic or a common oral condition like chronic periodontitis [1,3] it is hardly ever measured in clinical situations. In this study we have made an effort to associate the pre and post treatment values of serum lead and vitamin C in patients having long lasting periodontitis and T2DM.

Material and Methods

An interventional study was conducted at an Institute located in Mangalore, India. Institutional ethical committee approved to give clearance to conduct the study. Sample size assessment was done based on the minimum expected difference in the vitamin C and lead levels in the previous studies done. The subjects were categorized into 4 set of thirty participants in all as test groups were a) thirty subjects (set 1) detected and identified with chronic periodontitis b) thirty subjects (set 2) who were analyzed and diagnosed to have T2DM c) thirty subjects (set 3) detected and analyzed with both chronic periodontitis and T2DM d) thirty subjects (set 4) were taken as a control group without any oral and medical history fulfilling the inclusion and exclusion criteria.

The diagnosis criteria for chronic periodontitis was done according to 1999 American Academy of Periodontology classification [6] and T2DM patients according to the American Diabetes Association Classification [7]. The purpose of the study was described and enligthened to the patients and written informed consent was obtained.

Inclusion and exclusion criteria was similar to other studies conducted [8] earlier and it is as below: subjects aged between twenty-five to sixty years with ≥ twenty teeth present in the oral cavity were counted within the study. Patients with a history of any antibiotic/anti-inflammatory therapy or having a history of any systemic disease or condition, individuals who had undertaken any periodontal treatment three months previous to study, pregnant/breast feeding ladies, tobacco consumption and ghutka chewers, subjects who consumed health supplements for more than 3 months and T2DM who had history high sugar level were barred from the study.

Figure 1. *Collection of Venous Blood.*

Before begining any treatment, blood samples were obtained from the subjects (Figure 1) to assess the serum vitamin C by means of visible spectrophotometer and lead levels using atomic absorption spectro-photometry. Scaling and root planing, oral hygiene instruction was delivered to the test group and they were reminded to be recalled after 21 days after the treatment. Blood samples were obtained again from the subjects on the day of follow up from the test group and sent for analysis .

Biochemical Analysis: 5mL of venous blood was obtained from subjects and was centrifuged (Figure 2).

Figure 2. *Centrifuge Machine.*

Estimation of serum lead was done after digesting serum by making use of concentrated nitric acid. The digested serum sample was then read in the atomic absorption spectrophotometer (Figure 3).

Figure 3. *Atomic Absorption Spectrophotometer.*

Figure 4. *Visible Spectrophotometer.*

Estimation of serum vitamin C was done using visible spectrophotometer (Figure 4).

Serum sample was collected in a clean test tube and 5% TCA (Tri chloro acetic acid) added to it and allowed to precipitate proteins for about ten minutes and centrifuged. The supernatant solution was transferred into another test tube and DTC (2,4-dinitrophenyl hydrazine, Cupric sulphate, Thiorea) reagent is added, the tube was plugged and the mixture was incubated at 60c for 60 minutes in a water bath, simultaneously a blank with TCA and DTC reagents is also incubated under similar condition. Following 60 minutes of incubation, the reaction mixture was cooled in an ice bath and 1ml of 4.5m sulphuric acid was added to it and when it reached room temperature the optical density was read at 540nm against blank. Spectrophotometer was used to read the concentration of serum vitamin C.

Statistical analysis

The collected information was summarized by using the descriptive statistics such as frequency and percentage of qualitative data, mean and standard deviation for quantitative data. To compare the outcome measures inferential statistics such as one way ANOVA (> two groups), Independent sample "t" test (two groups) and paired "t" test (pre and post) were computed. The ANOVA results were further analyzed by using the post hoc Tukey test. The p value < 0.05 was considered as significant. Data management and analysis were performed by using Microsoft Excel and SPSS 21 (SPSS Inc, Chicago, IL, USA).

Results

All the 120 subjects completed the study uneventfully. The vitamin C values measured at baseline is presented in Table 1. The ANOVA test demonstrates a significant variance between groups with a p value lower than 0.001

Table 1. Descriptive statistics regarding serum vitamin C in mg/dL at baseline.

Group	n	Mean	S.D	F value	p value
Controls	30	0.99	0.33	49.65	< 0.001
Periodontitis without T2DM	30	0.46	0.11		
T2DM with Periodontitis	30	0.38	0.126		
T2DM without Periodontitis	30	0.46	0.13		

Post hoc test between the groups showed a statistically significant difference in the values of serum vitamin C among healthy v/s T2DM with periodontitis group, healthy v/s T2DM without periodontitis and healthy v/s healthy with periodontitis since ($p<0.001$; Table 2).

Table 2. Comparison of Serum Vitamin C level between the Groups.

Groups	Mean difference	Std error	P value
Controls vs. periodontits without T2DM	0.52	0.56	<0.001
Controls vs. T2DM with periodontitis	0.61	0.05	<0.001
Controls vs. T2DM without periodontitis	0.52	0.05	<0.001
Periodontitis without T2DM vs. T2DM with periodontitis	0.08	0.05	0.390
Periodontitis without T2DM vs. T2DM without periodontitis	0.00064	0.056	1.00
Patients with periodontitis, with vs. without T2DM	0.088	0.056	0.397

Student paired t test showed a significant difference in mean serum vitamin C levels post operatively when compared to the baseline values in all the 3 sets (Table 3).

Table 3. Pre and Post Treatment comparison of serum vitamin C level.

Group	Visit	Mean	Std dev	T value	P value
Without T2DM, with periodontitis	Pre-op Post-op	0.469 0.788	0.118 0.160	11.13	<0.001
T2DM with periodontitis	Pre-op Post-op	0.380 0.578	0.126 0.131	7.12	<0.001
T2DM without periodontitis	Pre-op Post-op	0.469 0.643	0.134 0.156	5.31	<0.001

A significant variation between studied group was found also regarding serum lead concentrations (p<0.001; Table 4)

Table 4. Mean and Standard deviation of serum Lead in ug/dl at baseline.

Group	n	Mean	S.D	F value	p value
Controls	30	5.020	1.86	14.63	< 0.001
Periodontitis without T2DM	30	8.18	2.60		
T2DM with Periodontitis	30	9.15	2.84		
T2DM without Periodontitis	30	6.79	1.91		

Serum lead value between healthy v/s healthy with periodontitis and healthy v/s T2DM with periodontitis showed significant difference which has been statically significant since p< 0.001 as shown in (Table 5).

Table 5. Comparison of serum lead level between the groups.

Groups	Mean difference	Std error	P value
Controls vs. periodontits without T2DM	3.16	0.664	<0.001
Controls vs. T2DM with periodontitis	4.13	0.664	<0.001
Controls vs. T2DM without periodontitis	1.77	0.664	0.043
Periodontitis without T2DM vs. T2DM with periodontitis	0.968	0.664	0.467
Periodontitis without T2DM vs. T2DM without periodontitis	1.39	0.66	0.162
Patients with periodontitis, with vs. without T2DM	2.36	0.66	0.003

It was observed that the mean serum lead levels were significantly different post-interventions vs. baseline in all three studied sub-groups (p<0.001; paired t-student test; Table 6).

Table 6. Pre- and post-treatment comparison of serum lead level.

Group	Visit	Mean	Std dev	T value	P value
Healthy with periodontitis	Pre-op Post-op	8.188 6.344	2.60 2.15	6.51	<0.001 significant
Type 2 diabetes with periodontitis	Pre-op Post-op	9.15 6.18	2.84 2.67	10.13	<0.001 significant
Type 2 diabetes without periodontitis	Pre-op Post-op	6.79 5.03	1.91 1.94	5.117	<0.001 significant

Discussions

The importance and impact of nutrition and toxic metals in treating a range of chronic inflammatory diseases is proved in earlier literature, still in present years we have seen a major growth and contribution in research probing this phenomenon. However, of late the impact of nutrition and toxic metals in treating periodontal disease has engrossed slight consideration [9].

Micronutrients like vitamin C are essential in small quantities in one's diet to uphold range of biological process and for supporting optimal health [10]. Literature review done on patients with T2DM explain that due to reduced renal

absorption of vitamin C in conditions where they have increased insulin resistance, blood glucose may strive with vitamin C for uptake into certain cells and tissues, and hence cellular regulation of vitamin C may be impaired [11]. Studies have shown decreased vitamin C in subjects as a consequence of inflammation. When your system has low plasma vitamin C there is elevated threat and possibility of subclinical inflammation [12]. It is proved that for the commencement and advancement of periodontal breakdown number of risk factors play a major impact and role, nutritional status being one among them [13]. Inappropriate production of oxidants is associated to inflammatory diseases. Reduced antioxidant defenses may add to disease progression after the infection in certain type of inflammatory tissue injury [14].

The primary outcome of our present study was that serum vitamin C level was low in test groups which was significant statistically (Table 1 and Table 2) and this is consistent with previous study results [10,11].

When human system is in good condition, a well sense of balance exists among oxidants and antioxidants, and this is seen in all tissues of the body. If this equilibrium gets upset by extra production of oxidants and/ or exhaustion of local antioxidants the resulting oxidant production causes oxidative stress which leads to the consequence of local tissue damage .Thus the above literature [15] justify for the increased commencement and progression of periodontitis in patients having increased oxidative stress [15]. Nutrients and antioxidants are supposed to reduce lead absorption. Increase in vitamin C, magnesium level, increased glutathione levels will decrease the effect of lead poisoning [16]. Previous survey done in U.S. population showed a statistically significant correlation between lead blood levels in periodontitis patients and they also stressed for maximum such studies to confirm this association [17]. In the present study the cause for increased serum lead level as seen in (Table 4 and Table 5) is explained by previous studies [15-17].

Earlier studies have shown that Interventional study that employed scaling and, root planing to reduce periodontal inflammation resulted in restoration of gingival crevicular fluid, and total antioxidant concentration to those of healthy controls [18]. After periodontal therapy, in the present study, we saw an improvement of serum vitamin C (Table 3) and a decrease in the serum lead level (Table 6) and the reason for this can be supported and explained by previous study [18].

Frequent research work have revealed variations in the micronutrient status in patients with T2DM and in some research work scarcity of certain minerals or vitamins have been interrelated with the manifestation of T2DM complications, but the scope and magnitude of the pathogenic role of malnutrition in T2DM has been disputed [19].

No single mechanism can explain the reason for the changes seen among the parameters at baseline and after 20 days of periodontal therapy. Nutritional influence can be due to increased intake of healthy diet, improvement in lifestyle. In the present study studied sample dimension was too low, which makes external validity of the study questionable. This was a limitation of the study. Similar to previous studies [19] the extent of role of malnutrition and toxic metals in the pathogenesis of T2DM or chronic periodontitis is disputed in the present study since it was conducted for a short duration of time. This is a further limitation of the study.

We, as dentists, treat cases with people suffering from diabetes and chronic periodontitis on a large scale. Nutritional status of the patients maintains optimal health. Thus, clinically it can be a biomarker to check one's well-being and

also to check prognosis post operatively especially with a non-healing or failing cases like refractive periodontitis.

Vitamin C is an important nutritional supplement. Such studies will focus light ,especially on the nutritional benefits of population exposed to high lead toxicity, medically compromised and also for maintaining good oral health.

Since T2DM and chronic periodontitis are two diseases which are worldwide present our main aim should be to completely eradicate these diseases. In developing countries like India, where research in the health sector is still in the emerging phase, and dental sector is highly neglected such studies are essential.

Future perspectives regarding this study are to conduct longitudinal studies on a larger group of population for higher reliability and better outcome.

Conclusions

The present study obtained serum limits of vitamin C and lead at baseline and post operative. The baseline value of serum vitamin C was low and lead level high in patients with T2DM and periodontitis when compared with healthy controls. Post operative we observed improvements in serum vitamin C and decreases in the serum lead level when compared to baseline. Nutritional status (especially serum vitamin C) and toxic metals like serum lead level should be screened in patients with T2DM and chronic periodontitis.

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