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"Comparative Assessment of the Effects of Vitamin C and Oxitard™ On the Plasma Total Anti-Oxidant Capacity (TAOC) In Patients with Chronic Periodontitis (Chp) When Used As Adjuncts to Non-Surgical Periodontal Therapy"

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Abstract:
Background: *Antioxidants exist in all body fluids and tissues and protect against free radicals and protect the cells from harmful oxidants (ROS). The human body incorporates a plethora of complex antioxidant systems. Hence, the total antioxidant capacity (TAOC) assessment method has been developed to reduce the costly and time-consuming task of measuring individual species. Periodontitis could be associated with reduced local antioxidant defense.*
Aim: *To investigate the Total Antioxidant Capacity of (TAOC) of plasma in subjects with chronic periodontitis compared to that of periodontally healthy subjects.*
Methodology: *60 subjects were selected.*
Group A:*15 periodontally healthy subjects (SRP)*
Group B: *SRP + Vitamin C (9 males, 6 females)*
Group C:*{SRP + OXITARD™ (11 males and 4 females)*
The clinical parameters that were recorded included Probing pocket depth (PD), Clinical attachment level (CAL), Gingival index (GI), Plaque index (PI) and Bleeding on probing (BOP) at baseline, 1 month and 3 months.
Result: *This study showed evidently that subjects with ChP are associated with significantly lower levels of plasma TAOC.*
Keywords: *Chronic periodontitis, TAOC, Reactive oxygen species, vitamin C*

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I. Introduction

Chronic periodontitis is an infectious disease associated with a variety of microbes and characterized by the inflammation of the supporting tissues of the teeth and the progressive destruction of its attachment apparatus.[1]The inflammatory and the immune responses to this microorganism that colonize the periodontal and associated tissues involve the systemic and peripheral systems of the body. This creates a complex bi-directional, series of host- microbial interactions involving cellular and humoral factors and networks of cytokines, chemokines and growth factors.

More specifically, a loss of homeostatic balance between proteolytic enzymes (eg: neutrophil elastase) and their inhibitors and reactive oxygen species (ROS) and the antioxidant defense systems that protect and repair vital tissue, cell and molecular components that is believed to be responsible for periodontal tissue destruction.[2]

ROS is a collective term, which includes oxygen derived free radicals. A free radical may be defined as any species capable of independent existence that contains one or more unpaired electrons Reactive oxygen species cause tissue damage by a variety of mechanisms, which include the following[3]: 1. DNA damage 2. Lipid peroxidation (through activation of cyclooxygenases and lipoxygenases) 3. Protein damage including gingival hyaluronic acid and proteoglycans (Bartold et al 1984)4. Oxidation of important enzymes (e.g. Anti-proteases, α Antitrypsin, stimulation of pro inflammatory cytokine release by monocytes and macrophages by depleting intracellular thiol compounds and activating nuclear factor). Over the past few years, strong evidence has emerged to implicate oxidative stress in the pathology of periodontitis.[2] Low levels of certain free radicals

and ROS can stimulate the growth of fibroblast and epithelial cells in culture , whereas higher levels may result in tissue injury.[4] Control and modulation of ROS activity can normally achieved by its interaction with antioxidants. The antioxidant has been described as a substance that when present at low concentrations compared to that of an oxidizing substrate, significantly delays or prevents oxidation of that substrate.[5] Antioxidants exist in all body fluids and tissues and protect against free radicals.[1] Antioxidants in the body protect the cells from harmful oxidants (ROS) by removing the oxidants or repairing the damage caused by ROS in vivo.[5] The human body incorporates a plethora of complex antioxidant systems. Therefore, the total antioxidant capacity (TAOC) assessment method has been developed to reduce the costly and time-consuming task of measuring individual species.

Ascorbic acid protects against endogenous oxidative DNA damage on human sperm.[6]Vitamin C is a powerful water-soluble antioxidant, which is present in the organism at high concentrations. It is able to scavenge superoxide anion radical, hydrogenperoxide, hydroxyl radical, singlet oxygen, and nitrogen oxide species (Stahl and Sies 1997).

OXITARD ™

A number of plants and plant isolates have been reported to protect free radical induced damage in various experimental models. The essential oil of Syzygiumaromaticum is a very good antioxidant in vivo and in vitro (Deans et al., 1995; Lemberkovies et al., 1992). The antioxidant properties of Vitisvinifera (Gabreilskee et al., 1997), Mangiferaindica (Ghosal et al., 1996), Daucuscarota (Kim and Lee, 1994) and Glycyrrhizaglabra(Hatano et al., 1991) have been reported. Emblicaofficinalis is rich in vitamin C and is also a well-known antioxidant.[7]

Hence, the present study was conducted to investigate the Total Antioxidant Capacity of (TAOC) of plasma in subjects with chronic periodontitis treated with Vit C supplements and OXITARD™ compared to that of periodontally healthy subjects.

II. Materials And Methods

Sixty subjects were selected for the study from the Outpatient Department of Periodontics.

15 periodontally healthy subjects, aged 23-65(7 males and 8 females) with Clinical Attachment Level (CAL) ≤1mm, Probing Pocket Depth (PD) <3mm at any sites were included as control group for the estimation of Total Antioxidant Capacity of Plasma (TAOC).

45 Systemically healthy subjectsof both sexes, willing to undergo treatment with ≥ 20 teeth present with age group 23-65 years and presence of ≥ 2 non-adjacent sites per quadrant that are not first molar or incisor, with pocket depth ≥ 5mm,showing radiographic bone loss ≥ 30% of root length were included as test group.

Pregnant and lactating females, subjects using mouthwashes and/or Vitamin supplements within the previous 3 months before the study, smokers and tobacco users were excluded from study. Subjects taking antimicrobial drugs or NSAIDS within 3 months period before the study were excluded.

All subjects were motivated and educated regarding oral hygiene to be practiced during the study period (Modified Bass Technique of tooth brushing). They were randomly distributed into 3 groups based upon a lottery draw, and each group consisted of 15 subjects.

Group A (11 males and 3 females) – Scaling and root planning (SRP) alone.

Group B{SRP + Vitamin C **(**9 males, 6 females)}– SRP with an adjunctive dose of vitamin C in the form of chewable tablets, LIMCEE chewable tablets manufactured by Abbott healthcare, India twice a day for 30 days.

Group C {SRP + OXITARD™(11 males and 4 females)}-SRP was done and an adjunctive dose of a commercially available antioxidant capsule , Oxitard manufactured by Himalaya Herbals® ,India was prescribed to the subjects to be taken twice daily for 30 days.

Scaling and root planning was performed using Ultrasonic scalers and universal curettes.University of North Carolina probe (UNC 15) was used for periodontal probing measurements. All Baseline clinical measurements were done one week before the plasma sampling was done. The clinical parameters that were recorded included Probing pocket depth (PD), Clinical attachment level (CAL), Gingival index (GILoe and Silness, 1963), Plaque index (PISilness and Loe, 1964) and Bleeding on probing (BOP).

Fasting Venous blood samples were collected for plasma sampling.

Assessment of Plasma TAOC

TAOCwas evaluated using the FRAP assay (Ferric Reducing Ability of Plasma) according to the method by Benzie and Strain, 1996.The method is based on the reduction of ferric (Fe3+) to ferrous (Fe2+) ion at low pH. This causes a formation of blue colored ferrous-tripyridyltriazine (Fe2± TPTZ) complex which absorbs at 593 nm.Results were expressed as mmol/l (mM)

III. Data Analysis

The analysis was performed with a statistical software package **(SPSS, Version 21)**

A one way Analysis of Variance (ANOVA) was applied for the clinical measurement. A post –Hoc test was done for intergroup comparison. The independent T-test was used to analyze the differences between the test groups and the control group for the TAOC measurements.

IV. Results

There was significant reduction in the PD, CAL, PI, GI and BOPin all group at 1 month and 3 month and it was statistically significant as shown in **Table 1.(**P< 0.05)

The mean TAOC levels for subjects with Chronic Periodontitis were less than that of healthy subjects which increased to a level about that of healthy subjects after treatment.

Table 2shows the Mean plasma TAOC levels of three groups at baseline, 1month and 3 month. A statistically non significant difference can be seen at 1 month and 3 month post treatment for the mean plasma TAOC for the test group when compared to the control group with p value > 0.05. This showed that the TAOC levels reached the level of Control group after treatment.

Table no.1 comparison of PD, CAL, PI, BOP at baseline, 1 month and 3 month

Parameter	Group	Baseline SD	1month SD	3months SD	p value
Probing depth(mm)	**A**	6.01 + 0.51	4.94 + 0.42	4.27 + 0.47	0.001*
	B	6.14 + 0.35	5.16 + 0.31	4.52 + 0.40	0.001*
	C	6.65 + 0.45	5.56 + 0.51	4.96 + 0.45	0.001*
	Total	6.27 + 0.51	5.22 + 0.48	4.58 + 0.52	0.001*
CAL(mm)	**A**	5.80 + 0.67	4.80 + 0.56	4.00 + 0.53	0.001*
	B	6.33 + 0.61	5.20 + 0.56	4.53 + 0.51	0.001*
	C	6.60 + 0.73	5.60 + 0.73	4.93 + 0.45	0.001*
	Total	6.24 + 0.74	5.20 + 0.69	4.48 + 0.6	0.001
GI	**A**	2.50 + 0.36	1.25 + 0.37	1.08 + 0.35	0.001*
	B	2.47 + 0.39	1.20 + 0.33	1.10 + 0 .34	0.001*
	C	2.56 + 0.38	1.20 + 0.31	1.10 + 0.26	0.001*
	Total	2.51 + 0.37	1.23 + 0.33	1.09 + 0.3	0.001*
PI	**A**	1 .85 + 0.83	0.93 + 0.26	0.78 + 0.23	0.001*
	B	2.17 + 0.62	1.16 + 0.29	0.99 + 0.27	0.001*
	C	2.57 + 0.36	1.13 + 0.21	1 .03 + 0.21	0.001*
	Total	2.20 + 0.69	1 .07 + 0.27	0.93 + 0.26	0.001*
BOP %	**A**	59.26 + 14.26	34.04 + 8.72	29.63 + 8.83	0.001*
	B	58.39 + 13.95	32.03 + 6.95	29.90 + 6.57	0.001*
	C	54.77 + 11.53	28.07 + 6.83	26.69 + 7.03	0.001*
	Total	57.47 + 13.14	31 .38 +7.79	28.74 + 7.51	0.001*

Table no. 2 Mean plasma TAOC of all group before and after treatment

Group	Baseline	1 month	3 month	P value
Group A	598.00 ± 32.93	704.20 ± 34.82	696.26 ± 35.76	**0.001***
Group B	601.20 + 25.93	720.93 + 41.84	712.06 + 44.12	0.428
Group C	595.93 + 34.16	728.93 + 41.01	718.60 + 40.61	0.143

V. Discussion

The periodontal tissues represent a perfect area to study the mechanisms of ROS mediated injury and the defenses of antioxidants occurring as a response to microbial infections and oxidative stress has been considered a strong feature of periodontitis.

The results of the present study showed that baseline TAOC in plasma was significantly lower in subjects with ChP than controls. This reflects the systematically increased ROS and decreased antioxidants defenses in patients with periodontal disease.

The data from the present study are in agreement with the results of Pavlica et al2004,[8] who found positive relations between plasma TAOC and periodontitis in dogs. Also, results are consistent with Brock et al 2004,[9] who found that the mean total antioxidant concentrations of serum and plasma from patients with periodontal disease were lower than healthy control samples, and the results of Chapple et al 2002,[10] who reported that GCF glutathione values and TAOC in plasma and GCF are reduced in ChP.

On the other hand, our baseline plasma levels are in contrast to that of Chapple et al 2007[11] who investigated the TAOC of plasma and GCF in European patients with periodontal disease. This may be because of the reagents used in the study which differed and the analysis which was done also differed. We used a calorimeter to check the results whereas a spectrophotometer was used by Chapple et al (2007).[11] All the European standard of living and the dietary intake differs from that of us Indians a diet which consists mainly of vegetables and lentils.

Our baseline line plasma level TAOC also differs from the result of study done by Ali E. Sulaiman et al 2010.[1] This may be due to the assay which they did to measure the TAOC , in our study we did a simpler FRAP assay whereas they made use of an ABTS assay which uses a spectrophotometer to measure the TAOC.

The present study shows that plasma TAOC levels after 1 month post-treatment and 3 months post treatment were significantly higher than baseline plasma TAOC for the two therapy groups (P <0.05). Also, it is noticeable that post-treatment TAOC levels were restored among both therapy groups to equal baseline levels of healthy subjects (P >0.05). This is in line with the results of Grant et al 2010,[12] who found that non-surgical therapy restores the redox balance in patients with ChP when glutathione was measured in GCF samples before and 3 months after therapy.

On the other hand, this is inconsistent with the results of Chapple et al. 2007, who reported that successful periodontal therapy did not alter plasma TAOC at 3 months post-treatment in patients with ChP. This disagreement might be caused by the different intervals used for measuring clinical parameters between the two studies. Moreover, our data shows that the adjunctive use of vitamin C and OXITARD™,herbal antioxidant did not improve the clinical measures and plasma TAOC levels after 1 month post-treatment compared to non-surgical treatment alone.

This is similar to the results ofLeggott et al 1991,[13] who did not find a relationship between PD and BOP measures and the concentration of ascorbic acid after using a dose of vitamin C (250 mg per day for 32 days) as an adjunct to non-surgical periodontal treatment. Also, this is in agreement with Vogel et al.1986,[14] who reported no effects of vitamin C supplementation on the development of experimental gingivitis.

VI. Conclusion

This study showed evidently that subjects with ChP are associated with significantly lower levels of plasma TAOC. Successful non-surgical periodontal therapy seems to reduce oxidative stress along with periodontal inflammation and can restore plasma TAOC at 1 month post-therapy and 3 months post therapy to the level of periodontally healthy subjects. In addition, the adjunctive dose of vitamin C or the herbal antioxidant did not offer added improvements compared to non-surgical periodontal treatment alone.

The study however shows scope for use of antioxidants as adjuncts to nonsurgical periodontal therapy, such as TAOC of GCF which may offer a better avenue for further research.

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