

# Oxidative Stress Levels in Orthodontic Patients and Efficacy of Antioxidant Supplements in Combating Its Effects- A Randomized Clinical Study

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## ABSTRACT

**Introduction:** Orthodontic appliances are considered to be biocompatible although adverse effects attributed to release of nickel ion which are free radicals in oral cavity. These free radical produce damages both in cellular and extracellular components phospholipid membrane, proteins, mitochondrial and nuclear DNA leading to Oxidative stress which is normally counter balanced by the action antioxidant mechanisms. However in higher concentrations of free radicals resulting in cellular death and apoptosis Orthodontic appliances such as brackets , wire, resins has considered as potential allergen leading to release of free radicals. The study was conducted to determine and compare the role of oxidative stress and role of antioxidants in saliva of patients undergoing fixed orthodontic appliances therapy at different time intervals.

**Materials & Method:** A double-blinded, parallel, randomized clinical study was designed consisting of 40 healthy participants, aged 15-30yrs. The samples were divided into two groups Group A without antioxidants supplements and Group B with antioxidants supplements. Salivary MDA levels and gingival health index was recorded at different time intervals from each group. Salivary Lipid peroxidation (Malondialdehyde) level was estimated using Thiobarbituric acid (TBA) method and gingival status was investigated using Silness & Loe gingival index. Intragroup and intergroup comparison was statistically analyzed using student's paired T test.

**Result:** Increased salivary MDA levels and mild to moderate amount of gingivitis is seen in both groups. This is more pronounced after 24 hours of appliance placement. Improvement in salivary MDA levels and gingival health status is observed during the course of treatment, In Group B the salivary MDA levels and gingival health index score reached below their pretreatment values. However even after a span of 3 months the levels remained higher to their base values in Group A.

**Conclusion:** Improvement in salivary MDA levels and gingival health status is observed following antioxidant therapy during course of treatment, indicating combating nature of antioxidant supplements in orthodontic patients.

**Keywords:** Antioxidants, Free radicals, Lipid peroxidase, MDA, Oxidative stress.

## INTRODUCTION

Recent decades have seen great interest in the biocompatibility aspects of biomaterials.<sup>1,2</sup> In normal physiology there is a dynamic equilibrium between ROS activity and antioxidant defence capacity. Normal cellular defence mechanisms destroy most of these ROS and free radicals.<sup>3</sup> Likewise, any damage to cells is constantly repaired.<sup>4</sup>

However appliances and additional devices used during orthodontic treatment are exposed to different factors, such as temperature, pH, mechanical stress (corrosion), and micro flora (bio-corrosion) in oral cavity.<sup>2</sup> All these factors lead to release of toxic metal ions from alloy. Degradation products released from brackets and/or arch wires undergoes redox cycles and thus indirectly generates free radicals leading to oxidative stress.<sup>5</sup>

Free radicals are highly reactive and have very short span of life. Considering this, the damage produced by a free radical ROS damages all components of the cell, including proteins, lipids, and DNA.<sup>6</sup> However, the most important cellular targets of oxidative stress are the phospholipid membranes.<sup>7</sup> When it reacts with lipids the by-product of the reaction is lipid peroxidation.<sup>8</sup> It is the process in which free radicals "steal" electrons from the lipids present in cell membranes, resulting in cell damage.<sup>9</sup> Lipid peroxides attack on polyunsaturated fatty acids one of the by-product of this reaction is MDA.<sup>10</sup>

Saliva, an important physiologic fluid, containing a highly complex mixture of substances, is rapidly gaining popularity as a diagnostic tool.<sup>11</sup> It carries important relevant information in the form of biomarkers and act as a mirror of oral and systemic health.<sup>12</sup> Saliva can be used as a medium to analyse the oxidative stress levels in terms of MDA.<sup>13</sup>

Antioxidants neutralize free radicals. They may be classified as enzymatic and non enzymatic. They act as scavengers, helping to prevent cell and tissue damage.<sup>14</sup> The most well research non enzymatic antioxidant includes lipid soluble vitamin E and water soluble vitamin C and vitamin A.<sup>15</sup>

Recently, it has been claimed that the imbalances in the levels of free radicals and antioxidants in saliva may play an important role in the onset of periodontal diseases.<sup>15</sup>

However literature lack studies whether dietary supplements of antioxidants can combat with the oxidative stress induced by orthodontic appliances and improve the gingival health status of orthodontic patients. Hence this study was undertaken to study the effect of dietary supplements on salivary MDA level and gingival health status of orthodontic patients.

## MATERIALS AND METHOD

### Study Design

The study was designed to be double blinded and randomized consisting of forty healthy patients aged 18-30yrs who were supposed to undergo fixed orthodontic treatment. The patients were randomly selected from the outpatient department of orthodontics using simple random sampling techniques. The CONSORT statement was used as a guide for this study.<sup>16</sup>

### Ethical Approval and Informed Consent

Ethical approval of this prospective clinical study was obtained from the local ethics committee of our institute and University vide letter no MUHS /PG/E-2/1190/14. Written consent was retrieved before commencement of the study.

### Sample Size Calculation

The sample size was determined from a previous study.<sup>17</sup> Our standard difference=0.6 and power= 80; 20 was the number of volunteers needed per group.

### Clinical Study Protocol

Inclusion criteria: No history of systemic disease, not undergoing any prior medication, not suffering from xerostomia.

Exclusion criteria; Patients suffering from systemic diseases &/or xerostomia and those reporting the use of medications.

### Randomization

Patients were allocated to the control group or an experimental group with an allocation ratio of 1:1 using a simple randomized controlled trial method with coin toss method.

### Clinical Procedure

Group 1- Controlled group [without antioxidants] consisted of twenty patients. Group 2- Experimental group [with antioxidants] consisted of twenty patients.

Subjects belonging to both the groups were treated with fixed orthodontic mechanotherapy. Appliance of choice was SS MBT 0.022" prescription with 0.014" NiTi wire for initial levelling and aligning. Subjects belonging to Group 2 were prescribed daily dose of one tablet OXITARD [Himalaya Drug Company] (Vitamin A, vitamin E and vitamin C), whereas subjects from Group 1 were given daily dose of 1 tablet placebo for three months.

Oxitard is a non-enzymatic, secondary, dietary supplement drug having high ORAC (Oxygen Radical Absorbance Capacity) value. Higher the ORAC value of the drug, higher is its antioxidant potential.

Saliva collection method: Whole saliva was collected from each subject. Saliva collection was collected from each participant at four different occasions, i.e one day prior to appliance placement, 24hrs, 7 days and 3months after complete appliance placement. The coding of the saliva samples was not disclosed to the laboratory personnel to minimize experimental bias.

### Outcomes

The saliva was centrifuged at 3000 rpm for 15 min in centrifuging machine. Salivary MDA levels using Satoh TBA method<sup>18</sup> (1978) and gingival health status using Loe and Silness gingival index<sup>18</sup> (1963) (chart no 1) was assessed for all the patients at four different time intervals.

SCORE	CRITERIA
0	NORMAL GINGIVA
1	MILD INFLAMMATION, SLIGHT CHANGE IN COLOUR, SLIGHT EDEMA, NO BLEEDING ON PROBING
2	MODERATE INFLAMMATION: MODERATE GLAZING, REDNESS, EDEMA, HYPERTROPHY AND BLEEDING ON PROBING
3	SEVERE INFLAMMATION: MARKED REDNESS, EDEMA, ULCERATION, TENDENCY TO SPONTANEOUS BLEEDING

Chart No.1. Gingival Health Index<sup>17</sup>

**Error of the Method**

All the measurements were done by single examiner. The intra examiner reliability for data readings was assessed using Kappa statistics which was found to be 94%.

**RESULT**

Intragroup and intergroup comparison of salivary MDA levels and gingival health index was statistically analysed using standard paired t test.

**Comparison of Mean Level**

In Group A, MDA levels were found to be (0.348, 0.089), (1.166, 0.293), (0.908, 0.206), (0.759, 0.118) at time intervals. The values were found to be statistically significant (0.000)(Table no. 1)

In Group B, MDA levels were found to be (0.348, 0.087), (1.067, 0.31), (0.773, 0.292), (0.289, 0.081) at time intervals. The values were found to be statistically significant (0.000) at all the time intervals after appliance placement. (Table no. 2)

**Intergroup comparison of salivary MDA level:**

MDA levels when compared among the groups using Student T test showed statistically insignificant difference at time intervals (P = 0.203, 0.304, 0.098) respectively, however after 3 months there was significant difference MDA levels among two groups. (P=0.000) (Table no. 3.)

**Gingival health status**

In Group A, the mean and standard deviation of gingival health index score were found to be (1.11, 0.297), (2.44, 0.471), (2.56, 0.153), (2.15, 0.146) at time intervals. When compared using student T test, the values were found to be statistically significant (0.000) at all the time intervals after appliance placement. (Table no. 4)

In Group B, the mean and standard deviation of gingival health index score were found to be (1.15, 0.375), (2.16, 0.264), (1.14, 0.356), (0.54, 0.335) at time intervals. The values were found to be statistically significant (0.000) (Table no. 5).

**Table 1: Comparison of Mean MDA Level Controlled Group (Group A)**

TIME	MEAN	SD	T VALUE	P VALUE	RESULT
PRE TREATMENT	0.348	0.089			
AFTER 24HRS	1.166	0.293	12.598	0.000	SIGNIFICANT
AFTER SEVEN DAYS	0.908	0.206	12.049	0.000	SIGNIFICANT
AT 3 MONTHS	0.759	0.118	13.738	0.000	SIGNIFICANT

**Table 2: Comparison of Mean MDA Level Experimental Group (Group B)**

TIME	MEAN	SD	T VALUE	P VALUE	RESULT
PRE TREATMENT	0.384	0.087			
AFTER 24HRS	1.067	0.31	9.48	0.000	SIGNIFICANT
AFTER SEVEN DAYS	0.773	0.292	5.69	0.000	SIGNIFICANT
AT 3 MONTHS	0.289	0.808	3.57	0.001	SIGNIFICANT

**Table 3: Inter Group comparison of MDA Level at different time interval**

TIME	INTER GROUPS	SD	T value	P value	RESULTS
PRETREATMENT	GROUP A	0.089		0.203	NOT SIGNIF-ICANT
	GROUP B	0.089	1.294		
AFTER 24 HRS	GROUP A	0.293		0.304	NOT SIGNIF-ICANT
	GROUP B	0.31	1.043		
AFTER 7 Days	GROUP A	0.206	1.698	0.098	NOT SIGNIF-ICANT
	GROUP B	0.292			
AFTER MONTHS	GROUP A	0.118	14.631	0.000	NOT SIGNIF-ICANT
	GROUP B	0.808			

**Table 4: Comparison of Gingival Health Status - without Antioxidant Group (Group A )**

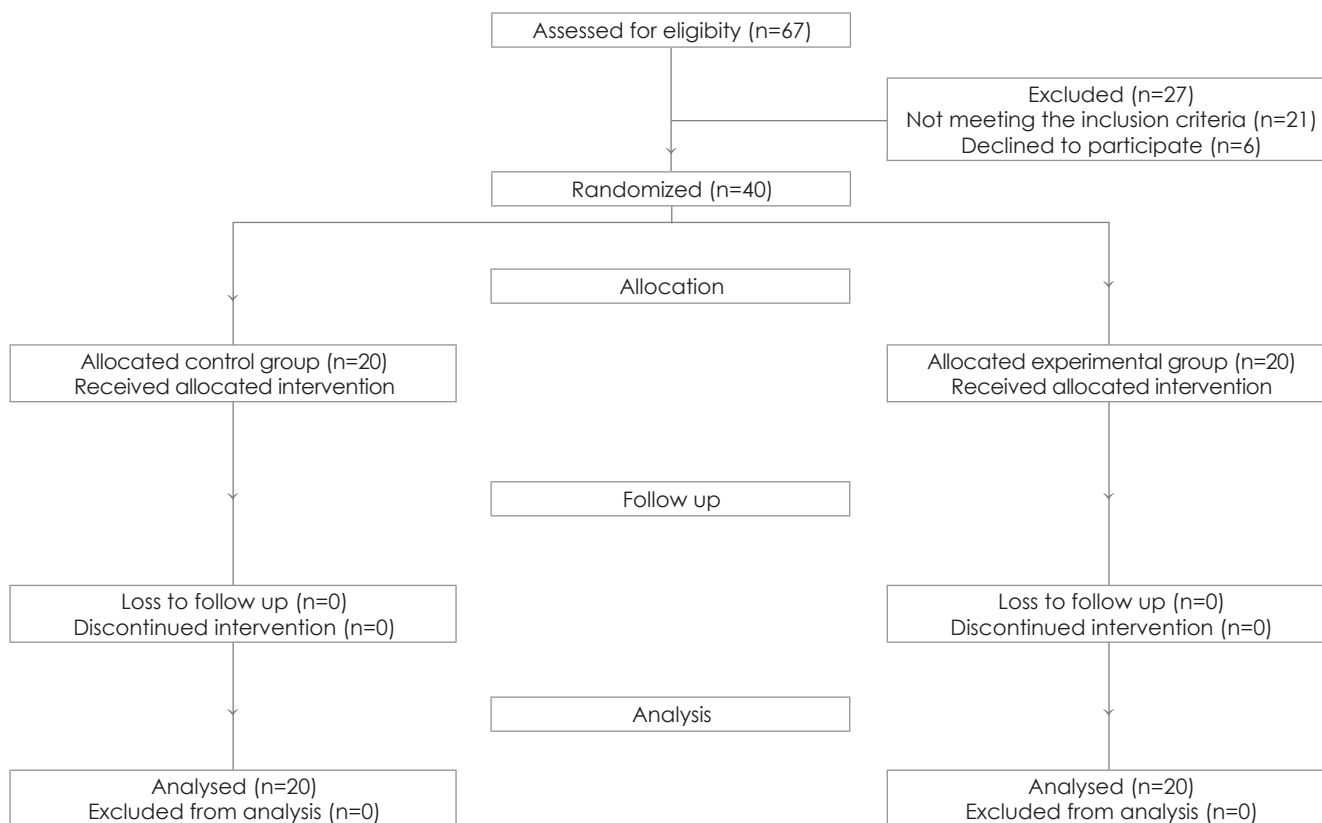
TIME	MEAN	SD	T VALUE	P VALUE	RESULT
PRE TREATMENT	1.11	0.297			
AFTER 24HRS	2.44	0.471	10.46	0.000	SIGNIFICANT
AFTER SEVEN DAYS	2.56	0.153	18.89	0.000	SIGNIFICANT
AT 3 MONTHS	2.15	0.146	13.67	0.000	SIGNIFICANT

**Table 5: Comparison of Gingival Health Status - with Antioxidant Group (Group B )**

TIME	MEAN	SD	T VALUE	P VALUE	RESULT
PRE TREATMENT	1.15	0.375			
AFTER 24HRS	2.16	0.264	9.9	0.000	SIGNIFICANT
AFTER SEVEN DAYS	1.14	0.356	2.23	0.034	SIGNIFICANT
AT 3 MONTHS	0.54	0.335	5.38	0.000	SIGNIFICANT

**Table 3: Inter Group Comparison of Gingival Health Status at different time interval**

TIME	INTER GROUPS	SD	T value	P value	RESULTS
PRETREATMENT	GROUP A	0.297		0.763	NOT SIGNIF-ICANT
	GROUP B	0.375	0.317		
AFTER 24 HRS	GROUP A	0.471		0.215	NOT SIGNIF-ICANT
	GROUP B	0.264	2.11		
AFTER 7 Days	GROUP A	0.293	13.09	0.508	NOT SIGNIF-ICANT
	GROUP B	0.356			
AFTER MONTHS	GROUP A	0.145	19.31	0.000	SIGNIF-ICANT
	GROUP B	0.334			



**Chart No. 2. CONSORT flow diagram**

**Intergroup comparison of gingival health index score**

Gingival health index score when compared among the groups showed statistically insignificant difference at time intervals. (P = 0.753, 0.215, 0.508) respectively, however after 3 months there was significant difference in MDA levels among two groups. (P=0.000)(Table no. 6).

**DISCUSSION**

Over the past few years, strong evidence has emerged to implicate oxidative stress in pathogenesis of dental

disease. At low concentration, they stimulate the growth of fibroblasts and epithelial cells in culture, but at higher concentrations it may result in tissue injury.<sup>19</sup>

An Orthodontic appliance remain in mouth for 2 years or more where saliva is the connecting media, may produce electro galvanic currents that produce a discharge of ions.<sup>20</sup>

The harmful effects of free radicals are counterbalanced by an antioxidant mechanism.<sup>21</sup> A change in this

balance in favour of free radicals deteriorates the oxidative balance and produces oxidative tissue damage associated with oxidative stress.<sup>21,22</sup>

Saliva reflects general health status of the human organism and is easy to collect, it can be used as a non-invasive diagnostic tool.<sup>23</sup>

This study was undertaken to determine whether oxidative stress are induced by orthodontic appliance, role of antioxidants on oxidative stress and also its effect on gingival health.

In present study MDA levels were significantly increased after 24hrs in comparison of pre-treatment values in both groups. There was constant decline in the MDA levels after seven days to three months but yet not reaching to the base values. This can be attributed to the fact that metal ions release from appliances are higher in first 24hrs. The results were same with findings reported by Nilfroushan et al<sup>24</sup> and Maja khuta et al.<sup>25</sup> The study was also in accordance to D'Attillio et al<sup>26</sup> and Olteanu et al<sup>27</sup> who stated that radical ions were higher in early stages of treatment.

In antioxidant group the results were also seen to exceed from base line to seven days and were statistically significant, however after three months, MDA levels were lower than the baseline values and were significant. The antioxidants prescribed to the patients combated with the free radicals formed due to metal ions. These findings were in correlation of Rai et al<sup>28</sup> and Das et al<sup>29</sup> who studied the effects of antioxidants in diseases.

The MDA level among the two groups showed insignificant difference. However after 3 months there was significant difference in MDA levels among the two groups. (P=0.000). These findings indicate that oxidative stress are built up at 3 months was combated by antioxidants.

The second part of study was to determine the effects of oxidative stress on gingival health and role of antioxidants during the treatment. Oral hygiene was maintained yet gingival inflammation was seen in both the groups. As explained earlier releases of metal ions were higher at initial stages of appliance placement. This increase in oxidative stress levels in the initial period may be the reason of gingival tissues inflammation as suggested by Grimsdottir et al<sup>30</sup> and Buljan et al.<sup>31</sup> The results of our study were in accordance to the findings of Ajith Pillai<sup>32</sup> and Faccioni et al<sup>33</sup> who reported in their study that metal release of minimum concentration of 1.18 g could damage the gingival fibroblast at

72hrs exposure. At three months in antioxidant group, the patient's GI score dropped from base values. The results of our study are seen in collaboration to the results of study conducted by Benjamin.<sup>17</sup> They reported reduction in BOP, GI in the patients treated with local applicant of antioxidant –essential oil gel. The highly significant difference between the two groups can be explained by the fact that antioxidant supplements prescribed had its effect on combating with oxidative stresses generated by the orthodontic appliance.

The results of our study are in contrast with the results of Elcin Esenlick<sup>34</sup> studied role of (vitamin E) on lipid peroxidation levels in GCF of 50 orthodontic patients may be due to the difference in medication and protocol followed. In our study antioxidants supplements prescribed for 3 months included vitamin A, vitamin C and vitamin E, whereas in Esenlick study only vitamin E was prescribed for a 1 month. Micronutrients such as beta-carotene and vitamins A, C and E can be depleted during inflammation.<sup>6</sup> As mitochondria, release ROS in the cell. In a study in Schectman<sup>35</sup> et al suggested that vitamin C enters the mitochondria and protects against oxidative injury. These vitamins maintains structural and functional integrity of epithelial tissues and physiological or metabolic parameters of periodontal health.<sup>35</sup> Gingival health status is observed during the course of treatment, but do not reach its pre-treatment values. This may be due to oxidative stress induced by the leached metal ions from the appliance. Prescription of antioxidant has shown to deal with this problem in a time span of 3 months. However in this study, no changes in the archwire or ligatures were done throughout the study.

## CONCLUSION

Our study concludes that even after thorough oral hygiene maintenance in orthodontic patients, increased salivary MDA levels and mild to moderate amount of gingivitis is seen in both groups. Improvement in salivary MDA levels and gingival health status is observed during the course of treatment, however even after a span of 3 months the levels remained higher to their base values due leaching of ions. where as in Antioxidant group the salivary MDA levels after 3 months were below the pretreatment values. Prescription of antioxidant tab has shown to deal in a time span of 3 months Hence further studies are recommended to study long term effect of the fixed appliance on lipid peroxidation and whether antioxidant supplements should be continued throughout the treatment to combat with oxidative stress.



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