In Vivo Alteration of Phase II Transformation Enzyme (GST) Levels by Stainless Steel Orthodontic Appliances

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Abstract

Orthodontic appliances includes bands, brackets and wires which are made up of stainless steel with the composition of roughly 8-12% nickel and 17-22% chromium and other trace metals. Corrosion of orthodontic appliances can have a toxic effect on surrounding oral tissues. The main aim of the study is to analyze the Glutathione s transferase activity in saliva and blood samples of patients undergoing Orthodontic treatment. The study group included 40 participants and Samples were taken in different time intervals: Group 1- Collection of samples before the appliances fixed, Group 2- Collection of sample after one week of fixed appliances, Group 3- Collection of sample after three months of fixed appliances each from the Orthodontic Department of A.B Shetty Memorial institute of dental sciences. Glutathione S transferase was estimated using 1-chloro, 2, 4-dinitrobenzene (CDNB) method. In case of Saliva group 3 (211.17±1.84) significantly increased compare to group 1 (87.05±3.86) and group 2 (118.74±2.8). In case of RBC lysate group 2(532.33±3.2) and group 3(512.40±1.16) were increased when compare to group 1(107.12±2.54). The Glutathione s transferase levels of patients increased over a period of time after treatment with orthodontic appliances but other factors like age of the patient, diet rich in antioxidants, light and strenuous physical activity, medication, amalgam fillings and genetic constitution of an individual also influenced the results.

Key words: Stainless steel orthodontic appliances, Glutathione S transferase, in vivo study

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Introduction

Orthodontic appliances includes bands, brackets and wires which are made up of stainless steel with the composition of roughly 8-12% nickel and 17-22% chromium and other trace metals. Corrosion of orthodontic appliances can have a toxic effect on surrounding oral tissues [1]. It has been proved by many research studies that orthodontic appliances release metal ions through discharge of electrogalvanic currents with saliva acting as medium for continuous erosion over period of time [2]. Leached metal ions from orthodontic appliances can lead to metal mediated generation of free radicals which causes various chemical alterations in DNA bases, increased lipid peroxidation and altered homeostasis of calcium and sulfhydryls [3]. Lipid peroxides formed by assault of radicals on polyunsaturated fatty acids residues of phospholipids can further react with redox metals finally producing mutagenic and carcinogenic malondialdehyde, 4-hydroxynonenal and other exocyclic DNA adducts (ethanol or propane adducts) [4]. Nickel and chromium can yield free radical species straightaway from molecular oxygen in a two step process to yield superoxide anion and in a preserved manner to produce highly toxic hydroxyl radical. The pro-oxidative effects are intensified by the fact that the free radicals thus generated also inhibit anti-oxidant enzymes and exhaust the intracellular glutathione levels [5].

The placement of orthodontic bands, brackets and wires increases the risk of plaque accumulation, caries and decalcification and there is risk of corrosion from the components of stainless steel [6].

Endogenously synthesized antioxidants namely glutathione transferase and superoxide dismutase may be altered in saliva in response to the fixed orthodontic appliance therapy. Although there are ample reports involving the increased risk of dental caries and periodontal disease due to placement of orthodontic appliances [7].

Glutathione Transferase is a phase II enzyme which plays an important role in detoxification process and its level increases during various kinds of stress conditions. Glutathione s transferase protects the cellular biomolecules from reactive oxygen species and others Free Radical induced damage. Orthodontic appliances include bands, brackets and wires and are made up of stainless steel. It contains approximately 8–12% nickel and 17–22% chromium and other trace metals [8]. The corrosion of the orthodontic appliances can have a toxic effect on the surrounding oral tissues [9,10]. It has been proved by many research studies that orthodontic appliances release metal ions through discharge of electro-galvanic currents, with saliva acting as the medium for

continuous erosion over a period of time [6,11-14]. Persistence of a low metal ion concentration can induce biological effects inside cells; Leached metal ions from orthodontic appliances can lead to metal-mediated generation of free radicals which causes various chemical alterations to DNA bases, increased lipid peroxidation, and altered homeostasis of calcium and sulfhydryls. Lipid peroxides, formed by the assault of radicals on polyunsaturated fatty acid (PUFA) residues of phospholipids, can further react with redox metals finally producing mutagenic and carcinogenic malondialdehyde, 4-hydroxynonenal and other exocyclic DNA adducts (etheno or propano adducts) [15-17]. Nickel and chromium can yield free radical species (FRS) straightaway from molecular oxygen in a two step process to yield superoxide anion and in a preserved manner to produce highly toxic hydroxyl radical. The pro-oxidative effects are intensified by fact that the free radicals thus generated also inhibit cellular antioxidant enzymes and exhausts the intracellular glutathione levels [18].

The glutathione transferase enzyme family includes many cytosolic, mitochondrial, and microsomal forms and now better known as Membrane-Associated Proteins in Eicosanoid and Glutathione metabolism (MAPEG) proteins. GTs are universally present in both eukaryotes and in prokaryotes, where they catalyze a variety of reactions by accepting endogenous and xenobiotic substrates [19-21]. GTs constitutes up to 10% of cytosolic protein in certain mammalian organs, Glutathione s transferases catalyses the conjugation reaction of Reduced Glutathione through a sulfhydryl group (-SH) to electrophilic centers of various substrates and this activity results in detoxification of peroxidised lipids generated by free radical damage and breakdown of xenobiotic compounds. The mammalian GT super-family exists in cytosolic dimeric isoenzymes of molecular weights ranging from 45–55 kDa size that have been assigned to atleast six classes: Alpha(α), Mu(μ), Pi(π), Theta(Θ), Zeta(ζ) and Omega(ω). GT contributes in detoxification by conjugating toxic compounds with reduced glutathione to facilitate its dissolution in the aqueous cellular and extra cellular media, [22-25] and hence its excretes out of the body.

The main aim of the study is to analyze the Glutathione s transferase activity in saliva and blood samples of patients undergoing Orthodontic treatment.

Material and Method

The patients required for this study were selected from the department of orthodontics and Dentofacial orthopaedics, ABSMIDS, Deralakatte, Mangalore, Karnataka, India.

40 patients were selected for the study, aged between 17- 40 years which were treated with fixed orthodontic appliances.

They were given detailed information about the research work being carried out and were included in the research study only after obtaining written consent from them.

Ethical clearance certificate was obtained from the institute with the ethical clearance No: NU/CEC/PhD-30/2011.

Stainless steel orthodontic appliances were procured from Rocky Mountain Orthodontics, Mangalore, Karnataka, India.

Sample preparation for Glutathione s transferase assay - 1:4 diluted RBC lysate was prepared and analysed immediately. And 5ml of saliva was collected in small beaker and immediately Centrifuged at 10,000rpm for 15 minutes and the supernatant was used for the assay. If not used immediately the samples were stored in deep freezer (-20^{o} C).

Glutathione transferase levels estimation - The estimation of Glutathione s transferase levels was performed according to the method of Warholm et al [26]. Reagents used were of Molecular Biology grade and were purchased from Sigma and all tests were performed in duplicates. To the 1ml cuvette 890µl of Phosphate buffer, 50µl of 20mM reduced glutathione (GSH) , 50µl of 20mM 1-chloro, 2, 4-dinitrobenzene (CDNB) and 10µl of test Sample was added. OD is measured at 340nm. Calculation was done by using following formula.

The increase in absorbance is directly proportional to the Glutathione S transferase activity. The linearity of the reaction must be determined by plotting the absorbance values against time. Calculate the change in absorbance (ΔA340)/minute, in the linear range of the plot, for the sample and for the blank using the following equation:

$$(\Delta A340)/min = \frac{A340\ (final\ read) - A340\ (initial\ read)}{Reaction\ time\ (min.)}$$

GST activity was calculated using the following equation:

GST specific activity= **(ΔA340)/ min ×V (ml) ×dil / εmM×Venz(ml)**

Where:

dil = the dilution factor of the original sample

ε mM (mM-1cm-1)- the extinction coefficient for CDNB conjugate at 340 nm.

Statistical analysis: The GT levels of patients over a period of three months were statistically treated by the software Graph Pad prism 3.0 and one way ANOVA followed by Bonferroni test was performed on the data with 95 % confidence level.

Results

In our present study we monitored the level of Glutathione-S-Transferase enzyme levels in subjects treated with metal stainless steel Orthodontic appliances. The general characteristic of subjects is given in Table 1.

Table 1. General Characteristics of Subjects

Parameters	*Controls*	*Patients*
Number of subjects	40	40
Male	20	20
Female	20	20
Age (years)	17-40	17-40
Fixed appliances (months)	0-3	0-3

Glutathione-S-Transferase enzyme levels was found to be significantly ($P<0.05$) increased in RBC lysate compared to saliva and also found to be significantly increased after three months ($P<0.05$) after implantation. The results are shown in Fig 1.

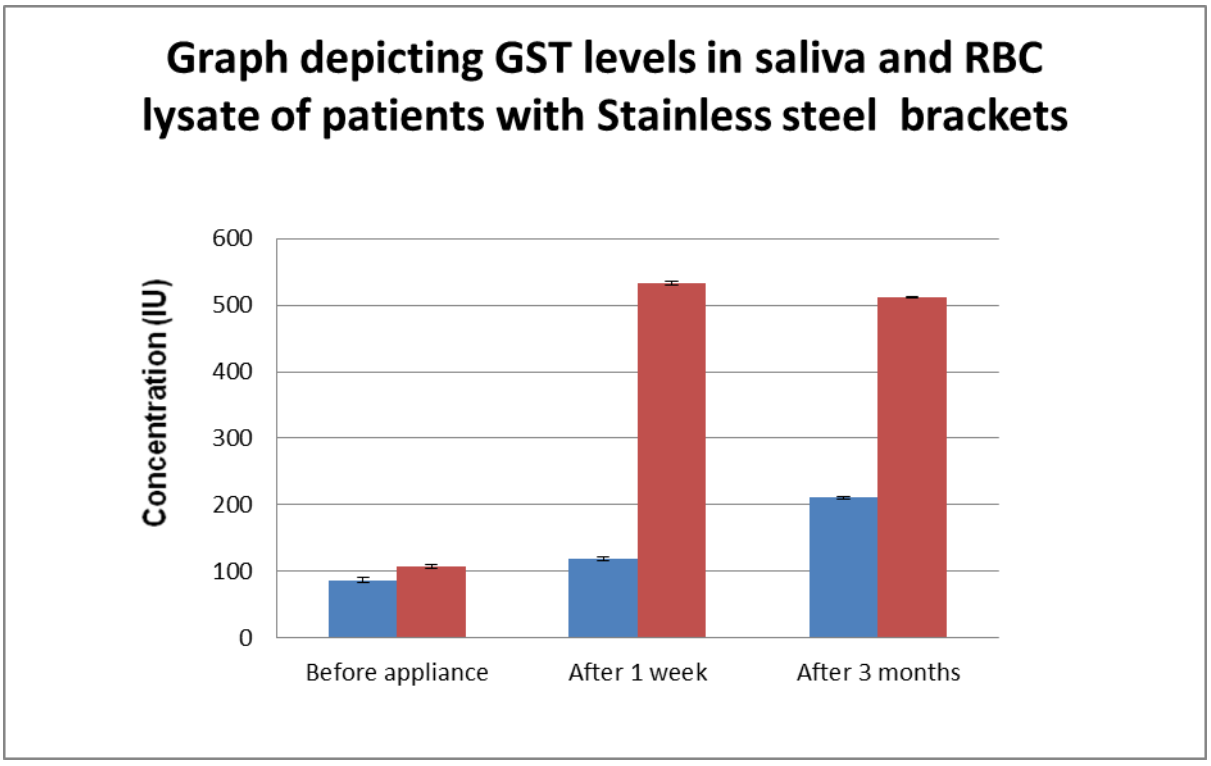

Figure 1. Mean levels of GT in Saliva and RBC lysate, before implanting appliance (B.A), after one week (1W), after 3 months (3M)

Table 2. Mean Levels of GST Activity in Saliva and RBC Lysate

Subjects	**SALIVA**	**RBC LYSATE**
Before appliance	87.05±3.86	107.12±2.54
After 1 week	118.74±2.8	532.33±3.2
After 3 months	211.17±1.84	512.40±1.16

The levels of GT were elevated in saliva (211.17±1.84 U/ml) and in RBC lysate (512.4±1.16 U/ml) significantly after three months post implantation of stainless steel appliances (P value = 0.02).

Glutathione S transferase activity of Saliva was significantly increased in case of group 3 compared to group 1 and 2. In case of RBC lysate group 2 was showed increased activity than group1.

Discussion

Studies previously conducted have verified low levels of metal ions released from orthodontic steel appliances and their acute toxicity [8-17], but till date no attempts have been made to

monitor stress enzyme levels in orthodontic subjects. Glutathione S-transferase (GST) plays an important role in detoxification of xenobiotics. [27]. The levels of Glutathione S Transferase increased probably because of the generation of free radicals and toxic intermediates released by the stainless steel appliance and their subsequent interaction with the biomolecules within the cell. The excess production or decreased clearance of ROS through the antioxidant mechanism may result in oxidative stress which is directly related to lipid peroxidation within the cells [28,29]. The levels of Glutathione s transferase increased probably because of the generation of free radicals and toxic intermediates released by the stainless steel appliance and their subsequent interaction with the biomolecules within the cell. During stress conditions the cell turns on the gene expression of certain anti stress enzymes and antioxidants molecules like Glutathione and Metallothionein as defense mechanisms to neutralize free radicals and prevent these highly reactive molecules from further damaging the cell and its component. In this study it was observed that Glutathione s transferase levels were more in RBC lysate than in saliva, the important factors which can influence the results are diet, presence of amalgam fillings, physical activity, occupational factor and genetic factors.

The Glutathione s transferase levels of patients increased over a period of time after treatment with orthodontic appliances but other factors like age of the patient, diet rich in antioxidants, light and strenuous physical activity, medication, amalgam fillings and genetic constitution of an individual also influenced the results.

Conclusion

The Glutathione s transferase levels of patients increased over a period of time after treatment with orthodontic appliances but other factors like age of the patient, diet rich in antioxidants, light and strenuous physical activity, medication, amalgam fillings and genetic constitution of an individual also influenced the results.

Consumption of antioxidants can decrease the damage caused by free radicals as it quenches the reactive species and neutralizes its harmful effect. Though the levels of metal ions released are within tolerable limits, Consumption of antioxidant rich diet is advisable in orthodontic patients undergoing treatment for longer durations.

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