EFFECTS OF CURCUMIN ON FREE RADICALS RELEASED FROM FIXED ORTHODONTIC APPLIANCES: A RANDOMIZED CONTROLLED TRIAL

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ABSTRACT:
Objectives: To evaluate the effects of curcumin mouthwash on oxidative stress levels in patients undergoing fixed orthodontic treatment by assessing the levels of 8-hydroxydeoxyguanosine in GCF.
Design and Setting: 50 patients (27 females and 23 males) participated in the study and were randomly allocated between two groups. Group 1 - subjects who were given curcumin mouthwash and toothpaste; group 2 – subjects who acted as control group (only toothpaste).
Methods: The levels of 8-hydroxydeoxyguanosine from GCF samples of patients were used to assess the oxidative stress levels of the periodontiumat three time periods: pretreatment (T0),3 months of treatment (T1) and 6 months of treatment (T2). Kruskal Wallis test was used to test the null hypothesis that there was no difference in biomarker levels across the three time periods for both experimental and control group followed by Dunn's post hoc analysis.The non-parametric Mann Whitney U test was used to perform the comparisons between the experimental and control group during the three time periods (P <0.05).
Results: The median value for 8 hydroxydeoxyguanosine level was lowest when taken at the 6 months interval (8.24 ng/L; IQR=6.94-9.84) in experimental group (Curcurmin mouthwash + toothpaste group). The results of Mann-Whitney U test showed that there was statistically significant difference between experimental (Curcurmin mouthwash + toothpaste) and control groups (Toothpaste group) during the 6th month.
Conclusion: Curcumin mouthwash can serve as an effective way of reducing the oxidative stress levels in patients undergoing fixed orthodontic treatment.

Keywords: Biomarker, curcumin, oxidative stress, antioxidants; Fixed orthodontic appliances, 8–hydroxydeoxyguanosine

INTRODUCTION

A wide range of materials are introduced in orthodontics from time to time with improved properties. Although newer dental materials with modified surface chemistry have been introduced, still the conundrum of bio-compatibility and cytotoxicity need to be addressed. As orthodontic appliances are subjected to constant loading and fluctuating oral conditions, the metal ions tend to leach from these materials with time.Free radicals released from fixed orthodontic appliances induce oxidative stress by peroxidation of membrane phospholipids

which results in concomitant increase in ROS induced periodontitis [8,12]. Study by Ortiz et al.onbracket materials concluded that stainless steel brackets induces greater toxicity in human fibroblast culture [14].

Owing to the short half-life of free radicals they generally go undetected in biological fluids; hence we take the biomarkers of oxidative stress such as 8-OHdG which are modified nucleosides to check the level of oxidative stress in the localised tissues [9,19].

Curcumin is a yellow pigment and a phytocolloid present in curcuma longa (Turmeric). It prevents free radical mediated peroxidation of phospholipids present in cell membrane and also oxidative damage of genetic materials and proteins.

Although oxidative stress after fixed orthodontic treatment is well documented in literature, so far there are no studies which evaluate the role of natural supplements in reducing the free radical levels. Therefore, this study evaluates the effects of curcumin in reducing the free radical levels associated with fixed orthodontic appliances.

Objectives
- To evaluate the effects of curcumin mouthwash on oxidative stress levels in patients undergoing fixed orthodontic treatment by assessing the levels of 8-hydroxydeoxyguanosine in GCF.

Hypothesis of the study
- The null hypothesis of the study is "The curcumin mouthwash does not alter the freeradical release in orthodontic patients with fixed appliance".
- The alternate hypothesis of the study is "Curcumin mouthwash may reduce the freeradical release as compared to the conventional group".

MATERIALS AND METHODS

The study was conducted over a period of 6 months. Sampling software G power version 3.1.9.2 (IBM CORP, CHICAGO, IL, USA) was used to compute the sample size and was evaluated to be 50 with 25 in each group (with an alpha error of 5% and power of 80%).
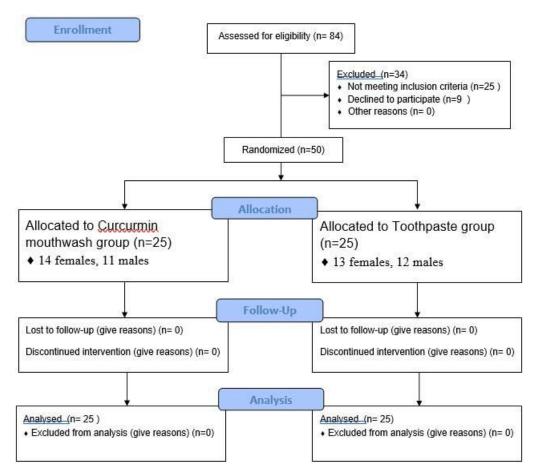
A total of 84 patients (44 females and 40 males) were initially enrolled in the study (Figure 1). The patients were selected based on the following inclusion and exclusion criteria:

Inclusion Criteria
- Subjects of both genders.
- Age group(18 to 25 years)
- Non-extraction cases.
- First premolars extraction cases.

Exclusion Criteria
- History of previous orthodontic, orthopaedic or surgical treatment.
- Patients with past history of trauma, injury to facial structures.
- Patients under any anti-inflammatory and antioxidant medications.
- Patients with any tooth coloured restorations
- Patients undergoing second premolar extraction.

CONSORT 2010 Flow Diagram

Figure 1: CONSORT flow-diagram

34 patients didn't meet the eligibility criteria and 9 patients were not willing to participate in the study.A total of 50 randomly selected patients(27 females and 23 males)were finally allocated to the two intervention groups after randomization. Computer based random number list was prepared prior to the commencement of the study which were kept in sealed envelopes for random allocation into two groups.

The subjects were divided into two groups of 25 each. Group 1 was the control group using the regular toothpaste (Figure 2a). Group 2 was the experimental group using the regular toothpaste along with the curcumin mouthwash (Figure 2b). All the patients were bonded with 0.022"x0.028" slot MBT prescription (ORMCO, mini 2000, Orange CA, USA) stainless steel brackets.All the patients included in the study had healthy periodontium with no signs of any periodontal inflammation or disease. All the patients followed rigorous oral hygiene measures and oral prophylaxis was done for all patients during their periodic monthly appointments.

Figure 2(a) & (b). Interventions used in the study

The study was conducted with the approval of Institutional Ethical committee. The nature of the study was explained to the participants and informed consent was obtained from them prior to the GCF sample collection.

Curcumin mouthwash is not available commercially, the mouthwash was prepared using standard formulations (Figure 2b). 10 ml of mouthwash contains 0.1% curcumin and rest comprised of distilled water. The curcumin mouthwash had a shelf-life of 5 days (50 ml) after which the same mouthwash cannot be used because of the absence of preservative. So, each patient was given 7 packs of mouthwash because the study was carried out in orthodontic patients, the regular monthly check-up will be held at an average duration of 25 days.

The GCF samples were collected at three different time-interval from the same patient. T0 (Baseline) will be before orthodontic bonding. The study duration was 6 months from the day of bonding. The archwire sequence used during the course of the study was either 0.014" NiTi/0.016" NiTi as the initial aligning archwire followed by 0.016×0.022 NiTiarchwire and 0.019×0.025 NiTiarchwire as the subsequent aligning archwires. For standardisation of collection of GCF samples across the patients, GCF sample was collected during 3rd month of treatment (T1) (patients were uniformly in 0.016×0.022 NiTiarchwire) and during 6th month of treatment (T2) (patients were uniformly in 0.019×0.025 NiTiarchwire). Since nickel-titanium archwires produce greater oxidative stress, this duration of orthodontic treatment is selected to assess oxidative stress.

The GCF samples were collected in the embrasure region between second premolar and molar using the deep intracrevicular technique using 10µl micropipettes. Minimum 5µl of GCF sample is collected from each patient. The collected GCF samples are transported through 400 µl of phosphate buffer solution and stored in -80ºC. A two-site sandwich ELISA kit which is marker specific (8 hydroxydeoxyguanosine) was used for analysis.

Statistical Analysis

The distribution of the data was analysed by Shapiro-Wilk test that revealed that data was not normally distributed. Therefore, Kruskal Wallis test was used to test the null hypothesis that there was no difference in biomarker levels across the three time periods for both experimental and control group followed by Dunn's post hoc analysis.The non-parametric Mann Whitney U test was used to perform the comparisons between the experimental and control group during the three time periods. P <0.05 was considered to be statistically significant.

RESULTS:

Table 1 shows the descriptive statistics for the two intervention groups. Median and interquartile range (IQR) were computed for experimental and control group during threetime periods (baseline, 3rd month and 6th month).

Table 1: Descriptive table for the intervention groups

Parameter	Experimental group (Curcurmin + toothpaste group)	Control group (Toothpaste group)	P value
Age(years)	23.5±2.45	22.3±2.74	NS
Sex	14 females, 11 males	13 females, 12 males	NS
Pre-treatment 8 - OHdG levels (Mean±SD)	8.6±0.86 ng/L	8.56± .78 ng/L	NS

Intra group comparison of the biomarker within the intervention groups was done using Kruskal Wallis test, which reveals that among all the time periods, that is baseline, 3rd month and 6th month, there is a statistically very highly significant difference (0.001) with relation to the biomarker concentration/ levels. Since Kruskal Wallis test was statistically significant, Dunn's post hoc test was done for pairwise comparison between the three different time periods for both the experimental and control group.

Dunn's post hoc analysis for the Intra group comparison of the biomarker within the groups reveals that among all the time periods, that is baseline vs 3rd month, baseline vs 6thmonth

and 3rd month vs 6th month, there is a statistically significant difference with relation to the biomarker concentration/ levels **(Table 2)**.

Table 2: Intra group comparison of the biomarker within the groups using Dunn's Post hoc analysis

Groups	Experimental group (Curcurmin + toothpaste group)	Control group (Toothpaste group)
	P value^A	P value^A
Baseline vs 3rd month	0.006**	0.054*
Baseline vs 6th month	<0.001**	0.048*
3rd month vs 6th month	<0.001***	0.025*

P Value^{A}Dunn's Post Hocanalysis
*P<0.05 is statistically significant
**P<0.01 is statistically highly significant
***P<0.001 is statistically very highly significant

The median value for 8 hydroxydeoxyguanosine level was lowest when taken at the 6 months interval (8.24 ng/L; IQR=6.94-9.84) in experimental group (Cucurmin mouthwash + toothpaste group). The marker level invariably increased for all time periods in control group (toothpaste group) with the highest value at 6 months interval (18.4 ng/L; IQR=14.86-20.2)**(Table 3, Figure 3).** The results of Mann-Whitney U test showed that there was statistically significant difference between experimental (Curcurminmouthwash+toothpaste group) and control groups (Toothpaste group) during the 6th month.

Table 3:Inter group comparison of the biomarker levels using Mann Whitney U test

Time periods	Experimental group (Curcurmin + toothpaste group)				Control group (Toothpaste group)				P value^A
	Median	Minimum	Maximum	IQR	Median	Minimum	Maximum	IQR	
Baseline	8.45	7.42	11.4	8.36-8.56	8.42	7.39	11.2	8.38-8.47	0.2899
3rd month	8.45	8.24	11.27	8.32-9.2	10.45	9.39	12.1	9.46-11.7	0.1986
6th month	8.24	6.45	10.2	6.94-9.84	18.4	12	24.5	14.86-20.2	0.0101

P Value^{A}Mann Whitney U test
*P<0.05 is statistically significant
**P<0.01 is statistically highly significant
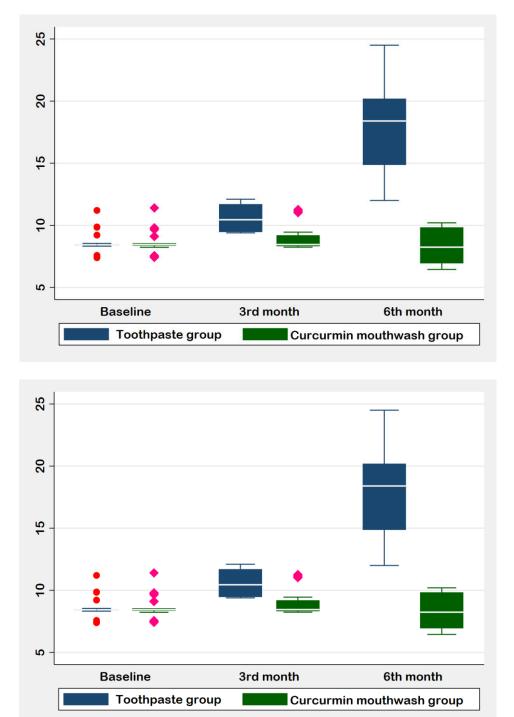***P<0.001 is statistically very highly significant

Figure 3: Box and whisker plot for both the intervention groups for different time periods

DISCUSSION

Oxidative stress occurs whenever there is a shift in balance between the levels of free radicals produced as a result of various metabolic processes and the endogenous antioxidants. Metal ions released from the orthodontic appliances increase the oxidative stress levels in the localised tissues by adding up to the free radicals produced by the body [10].

Recent studies have shown that inflammatory cytokines and pro-oxidants are increased in GCF as a result of periodontal damage and inflammation following orthodontic treatment and have further concluded that there is an increase in these inflammatory cytokines during early

stage of orthodontic treatment [6,11,13,20,]. However, the evidence attained from these studies have assessed biomarker level for a duration of less than 6 months. So, the level of these markers elucidated from these studies are insufficient and unreliable because they may not reflect the actual oxidative stress state of the periodontium. So, in the current study we assessed 8- hydroxide oxyguanosine biomarker for 6 months duration from the start of fixed orthodontic therapy.

Numerous biomarkers such as interleukine-1 beta (IL-1β), tumor necrosis factor alpha (TNF-α), malondialdehyde (MDA) and nitric oxide (NO) have been assessed in previous studies to evaluate oxidative stress levels during fixed orthodontic treatment[1,7,16,17]. In our present study we have assessed the levels of 8-OHdG to check for oxidative stress during fixed orthodontic therapy because it is a marker which is specifically increased during periodontal damage.

Usually markers measured from localised fluids reflect the diseased state of that particular tissue better than other biological fluids. The composition of GCF tends to be more constant when compared with other biological fluids [15]. So, in the current study we have assessed the levels of 8 hydroxydeoxyguanosine from GCF samples as they would better reflect the oxidative stress level of the periodontal tissue[19]. There are two methods for GCF sample collection, using paper strips and micropipettes. In the current study we have utilized micropipettes because collection of GCF with paper strips will cause contamination with blood and saliva which will lead to inaccurate assessment of biomarkers levels.

As most of the orthodontic patients are in levelling and aligning phase during the first 6 months where nickel-titanium archwires is used, the time frame used in the study was first 6 months of orthodontic phase, because it will reflect the true oxidative stress level of the periodontium in a patient undergoing orthodontic treatment.

Turmeric forms an essential part of day to day life and serves as a readily accessible and safe source of antioxidant when compared with the other supplements. Curcumin a phytocolloid extracted from turmeric has been proven to reduce oxidative stress levels in different tissues. Previous studies **done by** SohrabAsefi et al have used curcumin formulations like curcumin gel for bringing down oxidative stress levels in rats with orthodontic appliance [2].

This is the first study done with human subjects. In the current study, orthodontic patients were given curcumin mouthwash to improvise the usage, since toothpaste and gel are laborious for the patient. Hence, the effect of curcumin in reducing oxidative stress in patients undergoing fixed orthodontic therapy by checking the levels of 8 -OHdG from GCF has been assessed.

Results of the current study revealed that the levels of 8 hydroxydeoxyguanosine in GCF in the control group immediately after initial arch wire placement was (median: 8.42 ng/L; IQR=8.38-8.47) and in the experimental group the levels were found to be (median: 8.45ng/L; IQR=8.36-8.56). In a previous study done by Ozcan et al in orthodontic patients assessing the levels of 8 hydroxydeoxyguanosine levels in saliva have shown pre-treatment levels of the marker to be 4.73 ± 4.15 ng/L [3]. As they have taken the marker levels from salivary samples which is an ever-changing medium will not quantify the true oxidative stress levels following orthodontic treatment.

In previous study done by Ozcan et al, has evaluated 8 hydroxydeoxyguanosine levels in saliva at two different time periods: 1 month and 6 months post treatment during which the oxidative stress levels tend to decrease and return back to pre-treatment levels [3]. The level of 8OHdG in their study was done post treatment which means there were no orthodontic materials which would induce the oxidative stress in the local tissues. Hence, in the current study, 8 hydroxydeoxyguanosine levels were assessed during treatment which will quantify the actual oxidative stress state of the periodontium [4,5].

In control group (toothpaste group), the 8 hydroxydeoxyguanosine levels in the GCF showed a steady increase from pre-treatment values of (median: 8.42ng/L; IQR=8.38-8.47) to (median: 10.45 ng/L; IQR=9.46-11.7) after three months of treatment and (median: 18.4 ng/L; IQR=14.86-20.2) after six months of treatment (p<0.001) and the difference was statistically highly significant. Spalj et al in his study showed that there was an increase in oxidative stress levels when using nickel-titanium archwires[18]. The results of our study showed that there is an increase in oxidative stress levels after initiation of orthodontic treatment which is in concordance with the results of the previous studies.

In the current study, after 3 months of fixed orthodontic treatment there was no statistically significant difference between experimental (Curcurmin mouthwash + toothpaste group) and control groups (toothpaste group) with p value =0.1986. Whereas after six months of treatment 8 hydroxydeoxyguanosine levels in control patients (toothpaste group) was (18.4 ng/L; IQR=14.86-20.2) and in experimental patients (Curcurmin mouthwash + toothpaste group) was (median: 8.24 ng/L; IQR=6.94-9.84) showing that there was a slight decrease in the marker level in patients using curcumin mouthwash with p value =0.0101 which is statistically significant. Hence Curcurmin mouthwash could be considered to be an effective modality in reducing the oxidative stress levels in patients undergoing fixed orthodontic treatment.

LIMITATIONS OF THE STUDY
The main limitation of this study is the shorter shelf life period for curcurmin mouthwash of 5 days because shelf life could not be increased without adding preservatives.

FUTURE SCOPE
Further studies should be done to assess the long-term effects of curcurmin mouthwash on oxidative stress levels in patients undergoing fixed orthodontic treatment. Further studies can be conducted using a larger sample to assess whether there is difference in effects of curcumin based on age and gender.

CONCLUSION
Within the limitations of this study, the following conclusions can be drawn:
1. 8-hydroxy deoxyguanosine is a reliable biomarker which reflects the oxidative stress levels of periodontium in patients undergoing fixed orthodontic treatment.
2. 8-hydroxy deoxyguanosine was found to increase above the pre-treatment levels in control patients undergoing fixed orthodontic therapy irrespective of the time period at which the marker is measured.
3. In fixed orthodontic patients who were given curcumin mouthwash, there was significant reduction in the 8-hydroxy deoxyguanosine biomarker levels when measured at the end of 6th month.

Considering the results drawn from the present study, Curcumin mouthwash can be considered as a substitute for conventional mouthwashes by harnessing its antioxidant role. It will serve as a safe, viable and cheaper adjunct for patients undergoing fixed orthodontics in bringing down the level of free radicals.

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