Original Article

Estimation and correlation of salivary calcium, phosphorous, alkaline phosphatase, pH, white spot lesions, and oral hygiene status among orthodontic patients

Sangamesha Bevinagidad, Swati Setty, Anand Patil,[1] Srinath Thakur

Departments of Periodontics and [1]Orthodontics, SDM College of Dental Sciences and Hospital, A Constituent Unit of Shri Dharmasthala Manjunatheswara University, Dharwad, Karnataka, India

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Address for correspondence:
Dr. Sangamesha Bevinagidad, Department of Periodontics, SDM College of Dental Sciences and Hospital, A Constituent Unit of Shri Dharmasthala Manjunatheswara University, Sattur, Dharwad - 580 009, Karnataka, India.
E-mail: sangamesh346@gmail.com

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Abstract:

Introduction: The initial carious lesions are the so-called "white spot" lesions (WSLs), which implies that there is a subsurface area with most of the mineral loss beneath a relatively intact enamel surface. It is common in patients who are taking orthodontic treatment. Salivary reservoir of calcium and phosphorous counteracts the demineralization and encourages re-mineralization providing protection against caries challenge. This study was aimed to estimate and correlate the oral hygiene status, WSLs and salivary calcium, phosphorus, alkaline phosphatase (ALP), and pH in patients undergoing fixed orthodontic therapy. **Materials and Methods:** All clinical and biochemical parameters were recorded in 27 patients before starting orthodontic treatment and at 6 months of orthodontic treatment. The salivary calcium, phosphorous, ALP, and pH were determined by Arsenazo III method, Molybdate – UV and the International Federation of Clinical Chemistry method, respectively. International caries detection and assessment system index for WSL, orthodontic plaque index, bleeding on probing, and gingival index were recorded. Shapiro–Wilk's test and Wilcoxon test were applied to the data collected for statistical analysis. **Results:** There was a statistically significant increase with ALP levels, orthodontic plaque index score, gingival index score, and bleeding on probing index score. A statistically significant reduction was seen with respect to levels of calcium and pH. **Conclusion:** Maxillary canines and lateral incisors were the most affected teeth with WSLs (78.84%). There is a need for more stringent prevention programs and oral hygiene practices prior to initiation of orthodontic treatment and also during orthodontic treatment.

Key words:

Oral hygiene index, orthodontic patients, white spot lesions

INTRODUCTION

Orthodontic therapy is accomplished by inducing bone turnover to move the teeth within the alveolus. Orthodontics and periodontics have a complex relationship within the mouth. Oral hygiene maintenance possesses a challenge for the patients with a fixed orthodontic appliance because it will increase the number of retention areas of plaque leading to inflammation of the gingival tissues, demineralization of teeth, and white spot formation which are subsurface demineralization beneath dental plaque. The surfaces of brackets, bands, and wires limit the naturally occurring self-cleansing mechanisms of the oral musculature and saliva.[1]

Saliva is an oral fluid that can be collected by noninvasive means and has beneficial effects on the oral health by virtue of its flow, buffering capacity, pH, and other various factors. The composition of saliva varies between individuals and exhibits no apparent constant relation to the composition of blood. Low salivary buffering capacity, low calcium, and phosphate levels show a pronounced link to increased caries.[2] Salivary electrolytes and proteins account for only a small proportion of saliva, but they play various important roles to maintain the oral health and integrity of teeth. Maintenance of the equilibrium between demineralization and remineralization depends on the ionic concentration of calcium and phosphate in saliva, which in turn is

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influenced by alkaline phosphatase (ALP) levels.[3] It is a nonspecific enzyme that reacts between pH 9 and 10 and associated with the calcification process.[3] Its concentration increases significantly with increasing inflammation and plaque accumulation.[4,5] Hence, this study was aimed to evaluate and correlate the levels of salivary calcium, phosphorous, ALP, pH levels, white spot lesions (WSLs) and oral hygiene status in orthodontic patients. As per the authors' knowledge, there are no studies reported to evaluate and correlate these salivary biomarkers and oral hygiene status.

MATERIALS AND METHODS

A cross-sectional descriptive, analytical clinical-based study was conducted in patients visiting both the Department of Periodontics and Department of Orthodontics. All potential participants were clearly explained regarding the need and design of the study. A signed written informed consent was obtained from all recruits, who visited the orthodontic department and fulfilled the selection criteria to participate in the study. These criteria were patients undergoing fixed orthodontic therapy on labial surfaces of both genders of age 15–30 years, systemically healthy controls, with no other dental problem, nonlactating and nonpregnant females, nonsmokers, patients compliant with the study, no medical (including psychiatric) and pharmacotherapeutic histories that may compromise the study protocol, no patients with rampant caries and no periodontal surgery done in the past 3 months.

The total sample size for this study was 27 determined using G power software. keeping the effect size at 0.707, and an alpha error as 0.05. The calculated sample size was 16. The final sample size has been extrapolated to 27. The final sample size was 26 (8 males and 18 females) as one of the patients discontinued the treatment. All participants were interviewed for personal and sociodemographic variables including name, age, gender, general health status, and also brushing method. The single examiner performed all measurements, who was calibrated against a senior periodontist who represented the gold standard.

The biochemical parameters were calcium, phosphorus, ALP, and pH. The clinical parameters were international caries detection and assessment system (ICDAS) index,[6] orthodontic plaque index,[7] gingival index,[8] and modified sulcular bleeding on probing index.[9] These were recorded at baseline and 6 months of orthodontic treatment. Baseline examination was carried on the day of bonding before bracket placement. Saliva was collected between 10 am–11.30 am according to circadian rhythm. Two milliliters of unstimulated saliva were collected in a sterile saliva collecting tube. The participants were asked to rinse thoroughly with water before the collection of the sample. Five minutes after the oral rinse, the patients were asked to swallow the residual saliva that may be in their mouth. Then, it was collected by a passive drool method[10] and was sent to biochemical laboratory for the estimation of the parameters. The oral hygiene instructions were given to every patient.

Biochemical analysis of saliva

The pH of saliva was determined by pH papers (Prime Surgicals Universal Full Range (1–14) pH Test Strip). Then, saliva was prepared by centrifugation for 10 min at 3000 RPM and the supernatant was used for biochemical estimation of salivary calcium, phosphorus, and ALP. All biochemical estimation tests were done in colorimetric method in ErbaChem5x. Calcium levels were measured by Arsenazo III method.[11] Phosphorus levels were measured by Molybdate – UV.[12] The measurement of ALP levels was done using the International Federation of Clinical Chemistry method.[13]

Statistical analysis

All data collected were entered into Microsoft Excel sheet, and statistically analyzed using statistical package for social sciences software version 19 (IBM corpor., NewYork, USA). The level of significance was set at 5%. Before applying tests of significance normality, the data were assessed using the Shapiro–Wilk test. The parameters were not following normal distribution; hence, the difference in the parameters before and after starting orthodontic treatment was assessed using the Wilcoxon test.

RESULTS

The total number of teeth bonded were 565. The total number of teeth having WSLs were 386. Higher percentage of WSLs (78.84%) was found with maxillary canines and lateral incisors followed by maxillary and mandibular molars (71.15%), maxillary second premolar (69.22%), mandibular lateral incisors (63.46%), mandibular canines and first premolars (61.53%), maxillary central incisors (57.69%), mandibular central incisors (49.98%) maxillary first premolar (46.15%), and least on mandibular second premolar (38.45%) [Table 1].

ALP levels were significantly increased from 47.01 IU/dl to 57.83 IU/L ($P < 0.001$). The clinical parameters, the orthodontic plaque index and bleeding on probing index before orthodontic bracket placement were significantly increased from baseline to 6 months of therapy. The gingival index was significantly increased to 0.75 from 0.05 ($P < 0.001$). pH was significantly reduced to 6 from 7 ($P < 0.001$). The calcium level before orthodontic treatment was 4.24 mg/dl, whereas after 6 months of bracket placement, it significantly decreased to 2.63 mg/dl ($P < 0.001$). The phosphorous level was slightly increased to 9.11 mg/dl from 8.8 mg/dl, but this was not statistically significant [Table 2].

DISCUSSION

Orthodontic therapy has both direct and indirect effects on the periodontium. Areas of local decalcification without cavity formation in tooth surfaces is a major complication during the treatment.[14-16] Our study has revealed a significant increase in

Table 1: Percentage of white spot lesions in each tooth

Teeth	Total number of teeth	WSLs affected teeth	Percentage
Lateral incisor	99	84	75.68
First molar	104	84	71.15
Canine	100	80	71
Second premolar	68	45	66.17
First premolar	64	40	62.48
Central incisor	102	64	62.74

WSLs – White spot lesions

Table 2: Comparison of biochemical and clinical parameters before and 6 months of orthodontic treatment

Parameters	Median (baseline)	Median (6 months of treatment)	*P*
Alkaline phosphatase (IU)	47.01	57.83	0.001**
Orthodontic plaque index	0.0	0.85	<0.001**
Gingival index	0.05	0.75	<0.001**
Bleeding on probing index	0	0.5	<0.001**
Calcium (Ca^{++}) (mg/dl)	4.24	2.63	<0.001**
pH	7	6	<0.001**
Phosphorous (mg/dl)	8.8	9.11	0.534

**Highly significant. *P* value is considered statsistically significant up to 0.05.
P – Probability value

orthodontic plaque index, gingival index, and gingival bleeding index after 6 months of orthodontic therapy. The presence of orthodontic appliances reduces the effectiveness of daily oral hygiene due to the plaque retention.[17] Most of the studies reported increased plaque index scores within 1–3 months after orthodontic appliance placement.[18-21] The orthodontic plaque index was used in this study as it focuses on the plaque in the vicinity of the brackets.

WSLs are common during fixed orthodontic treatment.[22,23] These can be detected clinically within 4 weeks of orthodontic therapy.[24] The overall percentage of WSLs was 68.31% in the present study and its detection was done by ICDAS index, which helps to detect early changes in enamel. Gorelick *et al*.[25] reported 50% prevalence of WSLs by photographic method which may be considered less accurate than direct clinical examination.[26] Some studies showed 84%[27] and 97%[28] prevalence of WSLs.

There was higher percentage of the WSLs (78.84%) affecting the maxillary canines and lateral incisors in our patients after 6 months of orthodontic therapy. It might be due to demineralization with poor oral hygiene.[29] Literature has conflicting results regarding the involvement of teeth. Similar involvement of teeth was found by Geiger *et al*.[30] and Eltayeb *et al*.[26] Gorelick *et al*.[25] reported higher incidence of WSLs in the maxillary lateral incisors. The maxillary and mandibular first molars were the teeth most commonly affected in the study done by Mizrahi.[14] The involvement of maxillary canines and lateral incisors could be because of bracket placement closer to the gingival margin and also anatomic shape of these teeth. This favors plaque retention and difficulty in plaque removal along the gingival margin.[16] The closing loops in the archwires in the region of these teeth have difficulty in plaque removal.[31]

Saliva has multiple important functions in the oral cavity. It has a role in remineralization of the teeth and prevention of demineralization by buffering action. In the present investigation, the passive drool method was used to collect the unstimulated saliva as it provides protection to oral tissues and is present for14 hrs per day.[32]

The results of the present study revealed significantly lower levels of salivary calcium levels in the individuals undergoing fixed orthodontic appliances. Few reports documented similar findings after completion of orthodontic treatment.[33-35] In contrary to our study, Archie *et al*.[36] reported increased salivary calcium concentration in fixed orthodontic treatment.

pH was significantly lowered after 6 months of orthodontic therapy in our patients. After 2 months of orthodontic therapy, Alshahrani *et al*.[37] demonstrated a decrease in salivary pH and calcium levels significantly. Similarly, lower salivary pH in patients with fixed orthodontic appliances has been reported previously.[33,35,38] This could be because of predisposition of the enamel to demineralization.[39]

No significant changes in phosphorous levels were seen in this study. Even Eltayeb *et al*.[26] also reported no changes in phosphorus levels in orthodontic patients, but its reduction after 1 month of treatment was found by Li *et al*.[34] Phosphate ions maintain salivary pH and act as salivary buffer. This may lead to enamel dissolution as there is less saturation of phosphate in saliva.[3] There are limited data available in literature in regard to calcium and phosphorous levels in orthodontic patients.

The present investigation had significantly higher levels of ALP after 6 months of orthodontic treatment compared to baseline. There are no studies estimating salivary ALP levels in orthodontic patients according to our knowledge. Perinetti *et al*.[40] found increased ALP activity in gingival crevicular fluid at various time intervals during orthodontic therapy. It might be due to the bone remodeling by the orthodontic forces and gingival inflammation. ALP levels influence the equilibrium between demineralization and remineralization of the surface of teeth depending on calcium and phosphate concentrations in saliva.[3]

The occurrence of WSLs on the tooth surfaces impacts on simplification of the appliance in orthodontic therapy. The salivary biomarkers may be useful to take preventive measures in these patients. WSLs can be minimized in majority patients by fluoride application, antimicrobial varnish[41] and also using glass ionomer cement instead of composite resin.

The daily use of a 5000 ppm fluoride gel along with a fluoride dentifrice or a 5000 ppm fluoride dentifrice used twice daily was effective in preventing demineralization. Antimicrobial varnish significantly reduced the number of microorganisms in plaque which are caries initiative. Glass ionomer cement helps in re-mineralization of WSLs by fluoride release. Future investigations can be done to evaluate the severity, total mineral loss, and host immunity toward demineralization along with the risk factors associated with caries such as buffering capacity, amylase, total protein concentration, and carbohydrates levels.

CONCLUSION

Orthodontic patients had significantly lower levels of salivary calcium and pH along with higher levels of ALP and significant plaque accumulation, bleeding on probing and gingival inflammation after 6 months of therapy. The most affected teeth with WSLs (78.84%) were maxillary canines and lateral

incisors. These patients should be educated about the risk of developing WSLs and the importance of oral hygiene measures during the treatment.

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Conflicts of interest
There are no conflicts of interest.

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