ACTA SCIENTIFIC DENTAL SCIENCES

Volume 9 Issue 2 February 2025

Research Article

Effect of Fixed Orthodontic Treatment on Salivary pH, Flow Rate, *Streptococcus Mutans* Count and Electrolyte Concentration: A Quasi-Experimental Study

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DOI: 10.31080/ASDS.2025.09.1979

Received: January 17, 2025

Published: January 29, 2025

Abstract

Objective(s): To evaluate the salivary pH, flow rate, Streptococcus mutans count, and electrolyte concentration of Calcium (Ca), phosphorus (P), Potassium (K), and Sodium (Na) in patients who are undergoing fixed orthodontic treatment.

Materials and Methods: The study included 20 subjects undergoing fixed orthodontic treatment. Unstimulated salivary samples were collected before the placement of fixed orthodontic appliances and at the 2nd, 4th, and 6th months during the treatment. Salivary pH and salivary flow rate were measured, salivary Streptococcus mutans count (CFU count) was determined with selective microbial agar, and electrolyte concentration in saliva (Ca, P, K, and Na) was determined for 10 subjects using Inductively Coupled Plasma- Optical Emission Spectrometry (ICP-OES) analyzer.

Results: It has been observed that there was a decrease in salivary pH, and an increase in salivary flow rate and Streptococcus mutans count in saliva which was statistically significant($P < .05$) at various time intervals of study ie T2 (2nd month), T4 (4th month), T6 (6th month) during the fixed orthodontic treatment when compared with T0 (before placing orthodontic appliances). The salivary electrolyte concentration of Calcium, Potassium, and Phosphorus was found to be significantly decreasing and the salivary electrolyte concentration of Sodium was observed to be increasing from T0, T2, T4, and T6 time intervals of the study ($P < .05$).

Conclusion: Fixed orthodontic appliances can promote the buildup of plaque, and along with a decrease in salivary pH, an increase in Streptococcus mutans count and a decrease in electrolyte concentration of Calcium and Phosphorus leads to demineralization of enamel gradually leading to white spot lesions and proceeding to dental caries. Understanding the changes in microbial and nonmicrobial parameters of saliva during orthodontic treatment enables the clinician to monitor the progress of treatment and control the factors leading to enamel demineralization from the initial stages by providing adequate oral hygiene guidance in patients undergoing fixed orthodontic treatment.

Keywords: Salivary pH; Salivary Flow Rate; Streptococcus Mutans Count; Salivary Electrolyte Concentration

Introduction

Saliva is the primary defence mechanism of the oral cavity, essential for preserving and maintaining oral tissue health. It is vital for recognizing changes in the oral environment, playing a key role in regulating oral homeostasis. Saliva modulates the ecosystem by lubricating the digestive bolus, protecting against microorganisms, buffering and repairing the oral mucosa, and aiding in the remineralization of teeth. This complex secretion is crucial for the health of the mouth and teeth. However, studying saliva is challenging due to significant intra- and inter-individual differences in its composition and physical characteristics [1].

The regulation of mineral dissolution and deposition in the hydroxyapatite of enamel is influenced by both the inorganic components (calcium, phosphate, and fluoride) and organic components (proline-rich proteins (PRPs), statherins, cystatins, and histatins) of saliva [2]. Salivary quality, characterized by saliva protein content, buffer volume, viscosity, pH, and, along with salivary quantity, which is primarily related to flow rate, plays a crucial role in balancing enamel demineralization and remineralization in a cariogenic environment. Specific changes, such as increased pH, buffer volume, and flow rate, can help reduce susceptibility to caries [3].

Malocclusion is one of the most common dental issues, often increasing the risk of periodontitis and dental caries. Orthodontic treatment can frequently resolve or at least halt the progression of these conditions. However, the intricate design of fixed orthodontic appliances can impact oral hygiene by affecting various parameters, including saliva characteristics and microbial counts [10]. Changes in salivary parameters, such as decreased pH, flow rate, and buffering capacity, can contribute to enamel demineralization and heightened susceptibility to dental caries. These salivary properties are especially critical when using fixed orthodontic appliances, as they increase plaque retention and pose greater challenges for maintaining oral hygiene. Without optimal oral hygiene, these factors promote enamel demineralization and the formation of white spots on teeth [10].

The pH of saliva measures its acidity or alkalinity, typically around 6.3, though this can change due to oral disease. A reduction in salivary pH increases susceptibility to enamel demineralization. The critical pH is the threshold at which enamel demineralization begins. Orthodontic treatment can lead to food retention, which increases bacterial activity and subsequently lowers salivary pH. Assessing the pH value of saliva using pH strips is a useful method for evaluating the oral health of orthodontic patients [2].

Enamel demineralization often results from poor oral hygiene, which can lead to high levels of Streptococcus mutans and lactobacilli. The balance between cariogenic exposure and reparative action is believed to determine whether mineral loss or gain occurs over time. Saliva, a crucial component of the oral self-defense system, is thought to play a significant role in maintaining enamel stability [4].

The biofilm's ability to bind calcium, phosphate, and fluoride from saliva and external sources in the oral cavity facilitates the remineralization of enamel after demineralization. Among the availability of calcium and phosphate ions, calcium remains the limiting factor in enamel remineralization. While resting salivary phosphate levels remain relatively constant, there are significant individual differences in calcium concentrations. These variations in calcium concentration critically impact the critical pH and the potential for remineralization, as remineralization cannot occur when the saliva's degree of saturation with tooth minerals is low. Remineralization can be enhanced by providing small amounts of bioavailable calcium and phosphate ions [2].

Therefore, this study aims to investigate the changes in the oral environment that occur following the placement of fixed orthodontic appliances on permanent dentition. This research seeks to understand how factors such as saliva composition, pH levels, plaque accumulation, and other oral health indicators are affected by the presence of these appliances. Understanding these changes is crucial for optimizing oral hygiene practices and preventing potential complications during orthodontic treatment.

Subjects and Methods

Subjects

Patients from those reporting for routine orthodontic treatment to the Department of Orthodontics, PMS College of Dental Sciences and Research, Trivandrum were selected. They were examined and classified based on Little's Irregularity Index. 20 subjects with Irregularity Index of Mild level (Score 1-3) and Moderate level (Score 4-6) were randomly selected for study, from this only 10 subjects were included for the salivary electrolyte concentration estimation. The Institutional Ethical Committee approved this study and the participants or their guardians signed the informed consent.

The criteria for inclusion were: Subjects in age group of 14-25 years, patients with no previous history of orthodontic treatment, adequate oral hygiene with a Full Mouth Plaque Index score (<20%), patients ready to commence fixed orthodontic treatment after signing informed consent having Class I, Class II Div I and

Class III malocclusions, all treatment plans including extraction and non-extraction cases, patients with no habits of smoking, pan or tobacco chewing and alcohol consumption. The criteria for exclusion were patients with periodontal disease, patients with systemic conditions, patients with proximal caries or restorations, patients with prosthetic rehabilitation in the form of crowns, bridges, or implants, use of antimicrobial solutions or antibiotics.

Materials and Equipment

Conventional stainless steel bracket kit – MBT prescription – 0.022'' slot (American Orthodontics Mini Master Series), bonded with Ormco Enlight light cure adhesive, LED curing light(Woodpecker iLED Plus)elastomeric ligature (Koden), archwires (Garmy)The wire sequence started with 0.014 NiTi, 0.016NiTi, 0.016 x 0.022 NiTi, 0.017 x 0.025 NiTi, and 0.019 x 0.025 NiTi, handheld digital pH meter(Wellon), pipettes, tarson tubes, ICP- OES analyzer (Sree Chitra Tirunal Institute of Medical Sciences and Technology, Poojapura, Trivandrum), Microbial Culture media(Centre for Research on Molecular and Applied Sciences (P) LTD, Trivandrum).

Procedure

The salivary pH, flow rate, and Streptococcus mutans count and electrolyte concentration were assessed before starting the fixed orthodontic treatment(T0) which was taken as the control group, and during 2nd (T2), 4th (T4), 6th (T6) months of treatment.

Saliva collection

Unstimulated saliva was collected from participants by asking them to spit in a sterile beaker and the samples to be tested were refrigerated (-20°C) until transport to the laboratory. The saliva samples were stored in vials and transported aseptically in cryocooler (-4°C) to lab for analysis. The patient was given oral hygiene instructions and instructed not to eat/drink at least 2 hours before saliva collection.

Salivary pH measurement

The salivary pH was assessed at four different time points ie T0, T2, T4, T6. pH value was measured with a handheld digital pH meter.

Salivary flow rate measurement

The salivary flow rate was assessed similar to pH at T0, T2, T4, T6. The unstimulated saliva of patients was collected by asking the patient to spit into a sterile beaker tube for 10 minutes. A calibrated pipette was used to measure the collected saliva. To calculate the salivary flow rate, the collected saliva (milliliter/minutes) was divided by 10 [10].

Evaluation of Streptococcus mutans count

1ml of Patients' saliva was collected for *streptococcus mutans* analysis. Bacterial colonies were analysed and counted from each sample, and the calculation of relative colony-forming units (CFUs) was performed. Using calibrated pipettes, 1 ml of unstimulated saliva was collected and stored in Tarson tubes. The saliva samples were carried to the storage in a cryocarrier kept at - 4°C. The Tarson tubes were kept in refrigerated condition(-80°C) until analysis.

Preparation for *Streptococcus mutans -Mitis salivarius* Agar and enumeration of colony forming units were, the Mitis salivarius Agar media was prepared by dissolving 90.07g MSA in 1000mL distilled water and sterilized by autoclaving at 121°C 15lbs for 15mins. Afterward, the media was allowed to cool to 50°C and were poured onto pre-sterilized, pre-labelled petri plates and were allowed to solidify inside the Laminar airflow chamber with the flow and UV ON. The whole procedure was conducted in a Laminar Air Flow hood. 20µL from each sample were dropped to respective Petri plates and were swabbed onto *Mitis salivarius* Agar. The plates were incubated at 37°C for 24 hours in a microbiological incubator. After incubation, the plates were observed for colony-forming units (CFUs). The CFUs were counted using a Digital Colony counter and were expressed as CFUs/mL.

Evaluation of salivary electrolyte concentration

2ml of unstimulated patient's saliva is collected for analysis of salivary electrolytes concentration for calcium (Ca), phosphorous(P), sodium (Na), potassium (K) by ICP-OES (Inductive Coupled Plasma-Optical Emission Spectrometry) analyser. 2 ml of unstimulated saliva were stored in Tarson tubes. The saliva samples were carried to the storage in a cryocarrier kept at - 4°C. The Tarson tubes were kept in refrigerated condition(-80°C) until analysis.

For high sensitivity and specificity, Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) was adopted as the analytical method to determine electrolyte concentrations of Ca, P, Na, K in saliva. A measured amount of the sample was digested in an acid mixture and analysed according to the procedure for ICP-OES (Inductively Coupled Plasma - Optical Emission Spectroscopy) analysis. The concentration of the element in the solution was determined based on a calibration plot generated by analysing standard solutions. The results were recorded and processed using Win Lab 32 software. The parameter values were duly documented. The obtained values were then comparatively evaluated and statistically analysed.

Results

20 salivary samples were obtained from the patients receiving the fixed orthodontic treatment before the starting of treatment (T0), 2nd month (T2), 4th month (T4), and 6th month (T6) of treatment respectively. Salivary pH, salivary flow rate, and *Streptococcus mutans* count were estimated in 20 salivary samples on the above-mentioned time periods of the study, and electrolyte concentrations of Ca, P, Na, K were estimated in 10 salivary samples on the above-mentioned time periods.

Continuous data was represented as mean and standard deviation. For variable which follow normal distribution, Repeated Measure ANOVA and Tukey post hoc test was used. For non-normal variable Mann Whitney U test, Kruskal Wallis test, Repeated Measure Kruskal Wallis test, and Wilcoxon signed rank test was used.

Data obtained for salivary pH and salivary flow rate for 20 samples before the placement of fixed orthodontic appliances and after the 2nd, 4th, and 6th month of treatment were tested with Repeated Measure ANOVA and Paired T-test with Bonferroni correction. Data

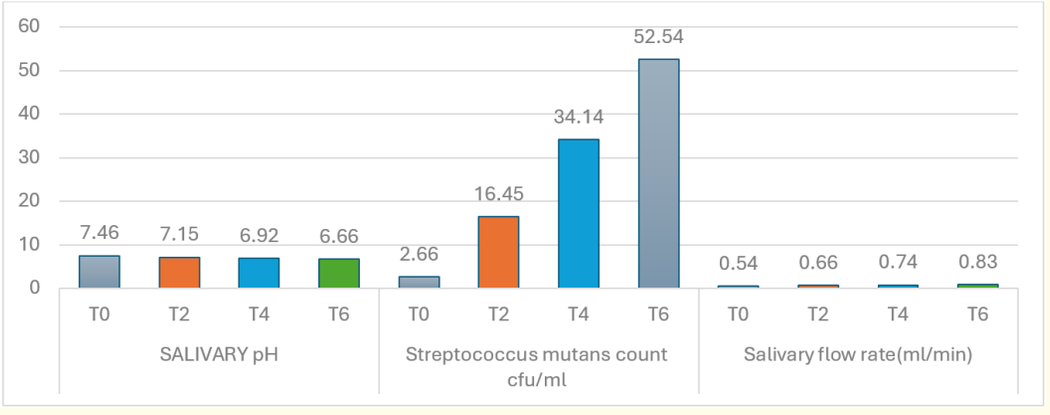
Groups	Group	N	Minimum	Maximum	Mean	Std. Deviation	Significance[ab]
SALIVARY pH	T0 (control group)	20	7.08	7.79	7.46	0.22	*a
	T2 (2nd month)	20	6.91	7.55	7.15	0.24	*a
	T4 (4th month)	20	6.74	7.19	6.92	0.15	*a
	T6 (6th month)	20	6.42	6.95	6.66	0.16	*a
Streptococcus mutans count CFU/ml	T0 (control group)	20	0	24.4×10^3	2.66×10^3	6.02	*a
	T2 (2nd month)	20	5.2×10^3	34.2×10^3	16.45×10^3	9.25	*a
	T4 (4th month)	20	14.3×10^3	62.25×10^3	34.14×10^3	11.91	*a
	T6 (6th month)	20	32.4×10^3	78.15×10^3	52.54×10^3	12.50	*a
Salivary flow rate(ml/min)	T0 (control group)	20	0.4	0.7	0.54	0.10	*b
	T2 (2nd month)	20	0.5	0.8	0.66	0.08	*b
	T4 (4th month)	20	0.6	0.85	0.74	0.07	*b
	T6 (6th month)	20	0.7	0.95	0.83	0.07	*b

Table 1: Mean and Standard Deviation of salivary pH, salivary flow rate, and *Streptococcus mutans* count in saliva at various durations of study T0(Control group), T2, T4, T6.

[a] Repeated Measure ANOVA and Paired T-test with Bonferroni correction was significant at the 0.05 level.

[b] Repeated Measure Kruskal Wallis test and Wilcoxon signed rank test with Bonferroni correction was significant at the 0.05 level.

* $P < 0.05$



Graph 1: Comparison of Salivary pH, Salivary flow rate, and Streptococcus mutans count in saliva at various durations of study T0(Control group), T2, T4, T6.

estimation from the salivary *Streptococcus mutans* count for 20 samples were tested with Repeated Measure Kruskal Wallis test and Wilcoxon signed rank test.

The data summary for salivary pH, flow rate and *Streptococcus mutans* count showed a decrease in the Salivary pH which is statistically significant ($P < 0.05$) when compared with the control T0 and 2nd, 4th and 6th month time period. An increase in the flow rate of saliva during the various durations of study which was statistically significant when compared with respective control T0 and also between T2, T4, T6 ($P < 0.05$). An increase in Streptococcus mutans count observed in T2, T4, T6 of the study which shows statistical significance ($P < 0.05$) when compared with the control T0.

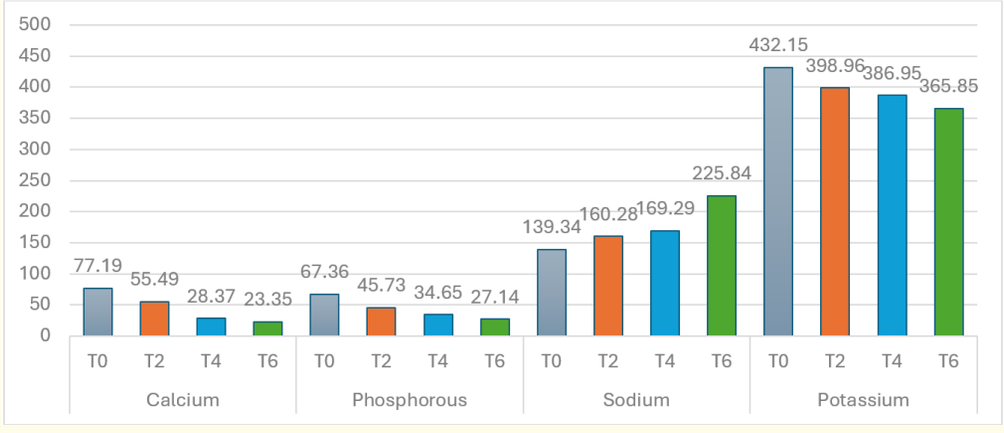
Data obtained for salivary electrolyte concentration for Ca, P, Na, and K for 10 samples before the placement of fixed orthodontic appliances and after the 2nd, 4th, and 6th month of treatment were tested with Repeated Measure Kruskal Wallis and Wilcoxon signed rank test with Bonferroni correction.

Salivary electrolyte concentration (ppm)	Group	N	Minimum	Maximum	Mean	Std. Deviation	Significance[ab]
Calcium	T0 (control group)	10	56.89	98.05	77.19	14.15	*b
	T2 (2nd month)	10	38.63	76.56	55.49	10.91	*b
	T4 (4th month)	10	19.48	39.83	28.37	7.23	*b
	T6 (6th month)	10	17.25	36.4	23.35	6.19	*b
Phosphorus	T0 (control group)	10	40.37	98.2	67.36	22.20	*b
	T2 (2nd month)	10	28.89	62.5	45.73	13.24	*b
	T4 (4th month)	10	22.25	48.6	34.65	9.42	*b
	T6 (6th month)	10	18.9	40.85	27.14	7.02	*b
Sodium	T0 (control group)	10	95.3	185.45	139.34	26.69	*b
	T2 (2nd month)	10	132.54	198.75	160.28	25.32	*b
	T4(4th month)	10	135.52	218.3	169.29	32.35	*b
	T6 (6th month)	10	158.96	375.7	225.84	66.66	*b
Potassium	T0 (control group)	10	317.86	572	432.15	75.37	*b
	T2 (2nd month)	10	308.86	496.5	398.96	65.50	*b
	T4 (4th month)	10	301.75	489.4	386.95	62.89	*b
	T6 (6th month)	10	299.89	460.35	365.85	54.53	*b

Table 2: Mean and Standard Deviation of Salivary Electrolyte of Calcium (Ca), Phosphorus (P), Sodium (Na), and Potassium (K) at various durations of study T0(Control group), T2, T4, T6.

[b] Repeated Measure Kruskal Wallis test and Wilcoxon signed rank test with Bonferroni correction was significant at the 0.05 level.

* $P < 0.05$



Graph 2: Comparative evaluation of Salivary Electrolyte Concentration of Calcium (Ca), Phosphorus(P), Sodium (Na), and Potassium(K) at various duration of study T0(Control group), T2, T4, T6.

The data showed a significant decrease in the Calcium (Ca), phosphorus (P), and Potassium (K) concentration levels and an increase in Sodium (Na) concentration levels from T0, T2, T4, and T6 ($P < 0.05$).

Discussion

The fixed orthodontic appliances not only alter the patients' oral hygiene but also change the salivary properties and microbial count. The present study assessed the salivary pH, flow rate, *Streptococcus* mutans count, and salivary electrolyte concentrations of Ca, P, Na, and K which were analysed before and during fixed orthodontic treatment at various time intervals.

Arab., *et al*. [10] analysed the effect of fixed orthodontic treatment on salivary flow, pH, and microbial counts. The total colony counts of lactobacillus, candida albicans, and Streptococcus mutans increased significantly after 6, 12, and 18 weeks of treatment. During orthodontic treatment, the pH of saliva decreased significantly but there was no significant increase in the flow of saliva.

During fixed orthodontic treatment with stimulated saliva, many previous studies have shown a significant decrease in salivary pH [1,6,12]. In addition, studies have shown that in patients receiving fixed orthodontic treatment the salivary pH was also significantly decreased with unstimulated saliva [17,18].

A significant increase was observed with the stimulated salivary flow rate [1,10,11,18]. in patients undergoing fixed orthodontic treatment and a similar increase was noticed in several studies with salivary flow rate with unstimulated saliva in orthodontic patients [9,15,17]. Kanaya., *et al.* [7]. and Chang [5]., *et al*. observed an increase in salivary flow rate with both stimulated and unstimulated saliva in patients undergoing fixed orthodontic therapy. Alessandri., *et al*. [3] investigated the salivary flow rate in patients undergoing fixed orthodontic treatment and observed an increase in the salivary flow rate over the period of one year of fixed orthodontic therapy.

Several studies were conducted to investigate the microbial colonies in patients with fixed orthodontic treatment causing demineralization of enamel and several acidogenic bacteria namely *Streptococcus mutans,* and *lactobacillus* were found to be significantly increased in the plaque of patients undergoing orthodontic therapy leading to demineralization and white spot lesions [18,20-22].

In the present study, the unstimulated saliva showed a significant decrease in Salivary pH from the T0, T2, T4, and T6 periods of fixed orthodontic treatment, and a decrease was noted between the time intervals when compared to the control group T0 ($P<0.05$) indicating fixed orthodontic treatment making the salivary pH acidic but within the considerable limit. Tooth demineralization occurs when the pH of saliva reaches 5.5.

In this study, unstimulated salivary flow rate and *Streptococcus mutans* count in the saliva of patients undergoing fixed orthodontic treatment were found to statistically increase from the T0, T2, T4, and T6 periods. A relevant increase was also noted between the time intervals when compared with the control group T0 ($P < 0.05$).

The increase in salivary flow rate observed in patients after the placement of fixed orthodontic appliances was attributed to the Mechan sensation caused by the appliances [4]. The orthodontic appliances will act as a mechanical stimulant for the salivary reflexes in the oral cavity.

However, typically an increase in salivary flow rate would raise salivary pH. In this study, however, a decrease in salivary pH was observed. It is crucial to assess the salivary flow rate over an extended period to understand how these changes result from adaptive processes in the human body. This result was also supported by Arab., *et al*. [10].

The decrease in salivary pH can also be associated with an increase in the count of acidogenic bacteria mainly *Streptococcus mutans* [7] and also associated with the increase in plaque retention in patients undergoing orthodontic treatment [12]. Dental plaque contains a mass of acidogenic bacteria which can produce acids and result in a decrease in salivary pH.

A decrease in salivary pH was associated with low Calcium levels in saliva [12]. According to Moussa., *et al*. [19] as the salivary flow rate increases, the concentration of calcium tends to decrease. A decrease in Calcium and phosphorus can be attributed to active caries lesions in orthodontic patients.

Li Y., *et al*. [4]. investigated the effects of fixed orthodontic appliances on salivary flow rate and salivary electrolyte concentration on which the study concluded there was an increase in salivary flow rate and electrolyte concentration of salivary sodium and chlorine and a decrease in the electrolyte concentration of Calcium, Phos-

phorus, and Potassium. Similarly, Cardoso., *et al*. [23] concluded that patients undergoing orthodontic treatment had decreased salivary calcium concentration over a 6-month time period.

Esfehani., *et al*. [13] estimated the salivary Sodium and Potassium levels in patients receiving orthodontic treatment concluded with an increase in Sodium level was noticed with the reduction of Potassium levels after 1 month of fixed orthodontic treatment and returned to base level after 3 months.

In the present study, the salivary electrolyte concentration of Calcium and Phosphorus was significantly reduced from T0 to T2, T4, and T6 respectively and a statistically significant decrease ($p < 0.05$) in difference is found between the time interval of the study.

The salivary electrolyte concentration of sodium was observed to increase and the salivary electrolyte concentration of Potassium was observed to decrease significantly from T0 to T2, T4, and T6 and the difference was found to be significant between the time interval of the study.

The decrease in potassium and increase in sodium could be attributed to the rise in total mixed saliva flow rate. This increase in flow rate leads to reduced sodium absorption by salivary gland ducts. Consequently, there is a corresponding decrease in duct secretion of potassium, resulting in a lower concentration of potassium in saliva [4].

There were only a few studies related to salivary electrolyte concentration in orthodontic patients and were also done for a short duration of time. Complex orthodontic treatment lasts for 1 and a half to 2 years and the salivary properties also change according to that, so further studies need to be done on salivary electrolyte concentration in orthodontic patients.

According to this study a decrease in salivary pH with decrease in Calcium and Phosphorus electrolyte concentration in saliva with an increase in the *Streptococcus mutans* count in the patients receiving fixed orthodontic treatment was highly susceptible for demineralization of enamel which can further leads to white spot lesions and dental caries. So proper oral hygiene instructions should be given for orthodontic patients.

Many studies had evaluated the effectiveness oral rinses in orthodontic patients for plaque control and reducing the *Streptococcus mutans* and *lactobacilli* [8]. Jasso., *et al.* [14] showed bacterial adhesion inhibition with use of silver nanoparticles in brackets to prevent the adhesion of plaque and reducing white spot lesions in fixed orthodontic patients. Krishnaraj., *et al.* [16] in a study stated that by the usage orthodontic brackets with a resin composite containing probiotic bacteria may help lower the levels of Streptococcus mutans in dental plaque.

In the present study, the electrolyte concentration of saliva in patients with orthodontic therapy was done for 10 samples because of the high cost of ICP-OES analysis. This study was followed up to 6 months after the starting of fixed orthodontic treatment. The saliva may show changes over a longer period in its properties. This study was conducted for a limited period. Further studies need to be done to assess the saliva during the entire treatment period to understand both the physical and chemical changes in saliva induced by the fixed orthodontic appliances.

Conclusion

The objective of this study was to evaluate the effect of fixed orthodontic treatment on Salivary pH, flow rate, *Streptococcus mutans* count, and electrolyte concentration.

From our study, it was observed that there was a significant reduction in salivary pH, along with a substantial rise in both salivary flow rate and the Streptococcus mutans count in saliva at various time intervals of study ie T2 (2^{nd} month), T4 (4^{th} month), T6 (6^{th} month) during the fixed orthodontic treatment when compared with T0 (before starting orthodontic treatment). The salivary electrolyte concentration of Calcium, Phosphorus, and Potassium was found to be significantly decreasing and the salivary electrolyte concentration of Sodium was observed to be increasing from T0, T2, T4, and T6 time intervals of the study.

Fixed orthodontic appliances can lead to higher levels of plaque accumulation and along with a decrease in salivary pH, an increase in *Streptococcus mutans* count and a decrease in electrolyte concentration of Calcium and Phosphorous leads to demineralization of enamel gradually leading to white spot lesions and proceeding to dental caries.

Understanding the changes in microbial and nonmicrobial parameters of saliva during orthodontic treatment enables the clinician to monitor the progress of treatment and control the factors leading to enamel demineralization from the initial stages by providing patients receiving fixed orthodontic treatment with more accurate oral hygiene guidance, promoting the usage of interdental cleansing aids, oral rinses, etc. Newly developed brackets with technologies incorporating silver nanoparticles inhibit bacterial adhesion and show a reduction in the demineralization of enamel but further in-vivo studies have to be done on these brackets.

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