How to Cite:
Solanki, L. A., & Shantha, S. K. (2022). Comparison of salivary parameters in orthodontic patients with good oral hygiene and bad oral hygiene: An-ex vivo study. *International Journal of Health Sciences*, *6*(S5), 6821–6827. https://doi.org/10.53730/ijhs.v6nS5.11555

Comparison of salivary parameters in orthodontic patients with good oral hygiene and bad oral hygiene: An-ex vivo study

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***Abstract*---**Aim: The aim of the study is to compare the salivary Ph, Buffer capacity, calcium, phosphorus and urea levels in clean and unclean mouths of orthodontic patients. Materials and methods: This ex-vivo study was carried out in orthodontic patients. 40 (group 1: clean mouth; group 2: unclean mouth) patients were included in this study. Unstimulated saliva was collected from both groups using a draining method and was subjected to chemical analysis. Results: Intergroup comparison between clean and unclean mouth revealed that Ph, Buffer capacity, Calcium, phosphorus levels reduced significantly in the unclean mouth as compared to clean mouth patients . Urea levels significantly increased in the unclean mouth group. ($p<0.05$). There was no statistically significant difference between males and females in both groups. Conclusion: Patients undergoing orthodontic treatment have altered chemical salivary parameters. Patients having OHI index levels more than 3, i.e. unclean mouth patients have reduced pH, buffer capacity, calcium and phosphorus levels and increased urea levels in saliva as compared to orthodontic patients having clean mouths.

***Keywords*---**Calcium, Phosphorus, pH, biochemical parameters, urea.

Introduction

Saliva plays an important part in sustaining oral hygiene (Lindawati, Sufarnap, and Munawarah 2019). Low levels of salivary buffering ability, calcium, and

phosphate, urea has connection to caries. Orthodontic appliances are often associated with dietary changes because of discomfort from masticatory movements. It is evident from the given evidence that fixed orthodontic appliances are harmful to oral hygiene (Krishnan and Davidovitch 2015). Orthodontic appliances cause poor oral hygiene. Retention stage is one of the most critical steps in orthodontic care. Set retainers render oral hygiene procedures more complicated and encourage the deposition of plaque and calculus (Eroglu, Baka, and Arslan 2019).

Salivary proteins are adsorbed onto the surface of the enamel and form a protective layer called the pellicle, which regulates the demineralization and remineralization of the enamel in conjunction with calcium and phosphate ions in saliva.(Humphrey and Williamson 2001) Previous studies on changes in the quality and quantity of saliva during orthodontic treatment have yielded conflicting results (Bonetti, Parenti, and Checchi 2008).

All these studies have compared before and after orthodontic treatment. None of the studies have compared between fairly clean mouths and unclean mouths in orthodontic patients. Therefore, the aim of the study is to compare the salivary Ph, Buffer capacity, calcium, phosphorus and urea levels in clean and unclean mouths of orthodontic patients.

Materials and Methods

This ex-vivo study was carried out in a private university setting in the Department of Orthodontics and Dentofacial Orthopaedics after approval by the ethics committee of the university. All the patients signed an informed consent prior to participating in the study.

All the patients were treated with.022 -in-slot MBT bracket system.
The inclusion criteria of the study were patients of 18-35 years of age who were 6-7 months into orthodontic treatment having fixed orthodontic appliances. Exclusion criteria were the patients having antibiotic usage, habit of smoking, active periodontal disease, patients who received periodontal therapy for less than 6 months, any systemic disease or patients with active caries.

There were two groups in this study group 1: Clean mouth 2: Unclean mouth (Figure 1). Sample size calculation was done based on the previous key article with power 85 % ,effect size 0.05 (AlHudaithi and Alshammery 2021). The Sample size was 40 (1: Clean mouth= 20; 2: Unclean mouth = 20).

Procedure of saliva collection

Collection of unstimulated saliva was done between 8- 10 am only to standardize the circadian rhythm. Each patient was asked to swash three times with water to eliminate any food remnants in the mouth. The saliva sample was collected using a draining method. Prior to starting the procedure, the patient was asked to spit for about a minute. The collection of saliva started by asking the patient to have their head bent down and mouth open to allow the saliva to passively collect in the disposable sterile jar. Around 2ml of saliva was collected from both groups

(Edgar and O'Mullane 1990). The collected samples were transferred to a measuring cylinder and the amount required for chemical analysis were poured in sterile plain tubes for centrifugation at 10,000 rpm for 5minutes. The supernatant fluid was frozen at 20 degree Celsius.

Protocol for testing and chemical analysis

Salivary Ph was calculated using a digital ph meter. The ph. meter strip was fully immersed in the saliva sample jar and left for 3 seconds to get a reading which was stable and final. After every sample, the pH meter was washed with distilled water and alcohol. Buffer capacity was also measured using pH meter.

Salivary calcium levels were measured using Calcium Kit (Arsenazo III colorimetric). The colorimetric test for quantitative determination of the total calcium levels in saliva was carried out. The reaction of calcium ions with o-cresolphthalein-complexone in an alkaline medium will form a purple-coloured complex. The calcium ion concentration is proportional to this complex.

Phosphate levels were calculated using Arsenazo III colorimetric kit. The UV test was used for quantitative determination. The principle was based on the reaction of phosphate ions with molybdate in a strong acidic medium to form a complex. The acid formation of phosphomolybdic complex would then reduce in alkaline medium having a blue molybdenum color. The phosphorus concentration is directly proportional to the intensity of the blue color in the near UV medium.
Urea level measurements were done determining the amount of green color iodophenol which was quantified by spectrophotometer at 600 nm. (LiquiCHEK)

Oral hygiene Index levels

The simplified OHI is designed to measure the situation of oral hygiene status using debris and calculus deposition from 2 anterior and 4 posterior teeth. The currently accessible indices do not meet specific criteria for patients with multi-bracket equipment, since only the plaque build-up and signs of inflammation of the marginal gingiva are evaluated on the smooth areas and/or approximate areas of the teeth. The OHI levels were as follows: 0–1.2 is considered as good, 1.3–3.0 is confirmed as fair and 3.1–6.0 is finalized as poor. In this study, OHI levels greater than 3 were considered for unclean mouth patients and less than 1.2 for clean mouth patients.

Statistical analysis

Descriptive statistics were carried out to calculate the mean value of PH, Buffer capacity, calcium, phosphorus and urea levels. Gender distribution was equal in both groups. Shapiro Wilks test revealed non-normal distribution of data. Therefore, Mann-Whitney U test was used for intergroup comparisons of all these parameters.

Results

The mean values have been depicted in Table 1. Intergroup comparison between clean and unclean mouth revealed that Ph,Buffer capacity, Calcium ,phosphorus levels reduced significantly in the unclean mouth as compared to clean mouth patients . Urea levels significantly increased in the Unclean mouth group. ($p<0.05$). There was no statistically significant difference between males and females in both groups.

Discussion

In this study, the salivary chemical parameters were compared between clean and unclean mouth in orthodontic patients. The findings of this study show that orthodontic treatment does have an effect on the salivary parameters. Buffering capacity and pH reduced in orthodontic patients. These parameters were significantly reduced in orthodontic patients with an unclean mouth having an OHI index more than 3 as compared to clean mouth patients. Various studies have shown results similar to us in which they found a reduction in pH and buffer capacity(Kanaya et al. 2007; Teixeira et al. 2012) . Some other studies showed that there was an improvement in these parameters and especially in patients having a high caries index, there was an improvement in pH and buffering capacity(Peros et al. 2011)(Cardoso et al. 2022; Chang, Walsh, and Freer 1997) . Some authors have suggested a gradual increase in the salivary parameters and after a few months due to the adaptation of patients to orthodontic appliances these parameters tend to return to normal. The oral environment can adapt to foreign bodies by increasing salivary flow rate, which has implications for buffer capacity and salivary pH (Imani et al. 2019). Increased salivary flow rate leads to the cleaning process in the oral cavity and modifies the composition of saliva, increasing bicarbonate ions and, as a result, an increase in saliva pH. Saliva's ability to combat acids formed by microorganisms in the oral cavity is influenced by pH and salivary buffer energy (Lara-Carrillo et al. 2010).

Minerals like calcium, alkaline phosphatase and phosphate are essential for the remineralization of teeth with early caries. A decrease in calcium levels promotes the production of dental caries in the oral environment. Calcium in the saliva is essential for maintaining tooth integrity, maintaining body fluid equilibrium, activating secretory gland cells, and aiding in the remineralization process. In our study the calcium levels decreased in patients in both groups but decreased significantly in the unclean mouth group. Some other studies had similar results like our study(Bevinagidad et al. 2020; Moussa 2017) while a few said there was an increase in calcium levels (Lindawati, Sufarnap, and Munawarah 2019).

Phosphorus is the second most essential mineral to be found in bone in the hydroxyapatite crystals in extracellular fluid. In this study phosphorus levels also reduced significantly in unclean mouth patients as compared to clean mouth patients. Felwa et al (AlHudaithi and Alshammery 2021) showed results similar to our study showing that even after removal of appliances there was a decrease in phosphorus levels. Few studies showed results inconsistent with our study (Chang, Walsh, and Freer 1997; Bevinagidad et al. 2020).

Urea levels have been shown to have association with periodontal diseases. They have been reported in periodontally compromised patients and patients having high plaque levels and caries index. This can be the reason for an increased urea salivary levels in unclean mouth as compared to clean mouth. These results were statistically significant.(Wolf 2006; Zawada and Sica 1985).

Decrease in pH, buffer capacity, calcium and phosphorus levels indicate the saliva is acidic and the repair mechanism of saliva is reduced. More severely reduced levels were seen in unclean mouth patients. These patients have a high amount of plaque and calculus and it is indirectly associated with the chemical salivary parameters.

Conclusion

Patients undergoing orthodontic treatment have altered chemical salivary parameters. Patients having OHI index levels more than 3, i.e. unclean mouth patients have reduced pH, buffer capacity, calcium and phosphorus levels and increased urea levels in saliva as compared to orthodontic patients having clean mouths.

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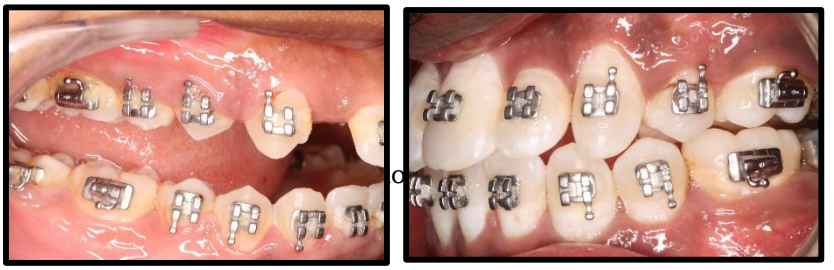
Figures



Tables

Group	pH	Buffer capacity	Calcium (mg/dl)	Phosphorus (mg/dl)	Urea (mg/dl)	Mann-Whitney U
Clean mouth	7.4	6.9	13.5	5.5	36.1	0.00*
Unclean mouth	6.6	6.2	12.1	7.5	49.9	0.00*

*Statistically significant