Original article

Screening of biochemical parameters in the orthodontic treatment with the fixed appliances: A follow-up study

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A B S T R A C T

The aim of this study was to assess the changes in vital salivary parameters such as calcium, phosphorous, alkaline phosphatase, buffering capacity, pH, flow-rate and Oral Hygiene Index (OHI) in fixed orthodontic treatment patients during the retention period. In this study, saliva samples were collected from 35 patients before de-bonding (T_0) and after 4 to 5 weeks of de-bonding or on retention period (T_1). The biochemical parameters such as calcium, phosphorous and alkaline phosphatase levels were measured with saliva samples. Additionally, flow-rate, buffering capacity, pH and OHI levels was also measured. The current study results showed reduction in calcium, alkaline phosphatase, pH, flow-rate and OHI levels during T_1 ($p < 0.05$). However, phosphorous and buffering capacity levels were increased at T_1. The phosphorous levels showed non statistically significant difference when compared between T_0 and T_1 ($p = 0.42$). The remaining salivary parameters showed statistically significant difference when compared between T_0 and T_1 ($p < 0.05$). The present study concludes that there was a statistically significant decrease in the calcium, alkaline phosphatase, pH, flow rate and OHI values a month after de-bonding and increased in the buffering capacity values.

1. Introduction

Saliva plays an important part in sustaining oral hygiene (Lindawati et al., 2019). Saliva composition varies from person to person and does not show a clear relation to the composition of blood. Low levels of salivary buffering ability, calcium, and phosphate show a connection to caries. Saliva contains a limited proportion of electrolytes and proteins, but they play vital roles in maintaining oral health and integrity of teeth (Bevinagidad et al., 2020). Orthodontic appliances are often associated with dietary changes because of discomfort from masticatory movements. The conventional metal stents are routinely prescribed for patients with extreme narrowing. Orthodontic treatment involves fitting or inserting orthodontic wires to correct an irregular bite. The incidence of orthodontic treatment varies from 10% to 35% in the

developing countries. It is evident from the given evidence that fixed orthodontic appliances are harmful to oral hygiene (Krishnan et al., 2007). Orthodontic appliances cause poor oral hygiene. Retention stage is one of the most critical steps in orthodontic care. There are various retention mechanisms utilized in clinical practice currently, and many of them have both benefits and drawbacks. Set retainers render providing oral hygiene procedures more complicated and encourage the deposition of plaque and calculus (Eroglu et al., 2019).

Biochemical parameters play an important role in assessing individual caries and other tooth demineralization susceptibility (Daniel et al., 2016). Saliva is a complicated biochemical substance, and is a secretion of several glands of the mouth. The end product of drinking water comprises of water, macromolecule proteins and different electrolytes, such as sodium, potassium, calcium, magnesium, bicarbonate, and phosphate. The composition of the saliva can be changed to include increased viscosity; increased level of urea, sodium (Na), potassium (K), and total protein, and decreased level of calcium (Rodrigues et al., 2016).

Calcium is an important mineral for growth of bones and previous studies have indicated that calcium and phosphate in saliva increase with age. Calcium is the only of the electrolytes which do not have a connection with salivary flow rate (Rabiei et al., 2013). Phosphorus is an important element of the body which is

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most often concentrated in bones and teeth, but it is also found in soft tissue, blood and erythrocytes. If salivary flow rises, the concentration of protein, sodium, calcium, chloride, bicarbonate, and pH levels increases, while the concentration of inorganic phosphate and magnesium decreases (Nasution et al., 2018). Alkaline Phosphatase is an enzyme discovered for numerous periodontal cells such as osteoblasts, fibroblasts and neutrophils are a crucial sign involved with the production of bone. It enables mineralization of bones by releasing organic phosphate and inorganic pyrophosphate hydrolyzation (Koppolu et al., 2021). The functional role in alkaline phosphate function together to control shifts in tooth demineralization and remineralization. It is observed that these biochemical parameters play an important role in assessing individual caries and other tooth demineralization susceptibility (Daniel et al., 2016). Saliva flow rate is important to oral health; a higher flow rate improves the cleaning activity of saliva, while a lower flow rate results in plaque production, demineralization, and caries (Arab et al., 2016; Chang et al., 1997). Numerous studies have shown that patients who wear a set orthodontic device encounter improved flow rate (Bonetti et al., 2013). Orthodontic treatment has been shown to cause oral changes, with increased bacterial concentrations and changes in buffer ability, pH acidity, and salivary flow; however, we know nothing about periodontal inflammation contributing to occult saliva blood, and dental plaque acidity (Ceron-Zamora et al., 2020). Upon breaking down carbohydrates, bacteria expel lactic acid, butyric acid, and aspartic acid, which bring down the pH of saliva. As the pH level in the mouth goes below 5.5, the acids begin to decompose the enamel. The longer the teeth are exposed to the low pH in saliva, the more likely dental caries can develop (Hans et al., 2016).

Limited studies have been performed in the global population and in the Saudi population no studies have been performed with all the biochemical parameters. The aim is to see the changes in salivary parameters (Calcium, Phosphorus, Alkaline Phosphatase, flow rate, buffering capacity, pH, and Oral Hygiene Index) after orthodontic treatment with fixed appliances in two different time points, de-bonding visit and 5 weeks during the retention period (T_0 & T_1). The overall goal of the study is to assess the changes in these vital salivary parameters in fixed orthodontic treatment patients during the retention period.

2. Materials and methods

2.1. Sample enrollment

The first procedure in this study was to obtain ethical approval (FPGRP/2020/472/233/229) in order to carry out the research at Riyadh Elm University (REU). In this study, we obtained information from 35 patients who completed orthodontic treatment with fixed appliances at Department of Orthodontics at REU Hospital and a couple of private clinics. A 0.022-in slot MBT bracket system was used to treat all patients (3 M unitek, USA). This prospective

clinical study comprised both genders (21 female and 14 male) with ages ranging from 15 to 35 years old, with participants or their guardians consenting their participation by signing the informed consent form. The inclusion criteria for selecting the patient include permanent dentition with a minimum treatment term of 12 months and treatment with fixed orthodontic appliances. Exclusion criteria for this study subjects include smoking, short-term or long-term antibiotic usage, topical fluoride application, and Patients with periodontitis and patients who reported having received any sort of periodontal therapy during the last 6 months, patients with any of the systemic diseases, or patients with active carious lesions.

2.2. Saliva collection

In this study, 70 saliva samples were collected from the 35 orthodontic patients. From each patient, the saliva sample was collected twice, on day 1 (T_0) before debonding and five weeks after debonding, and on retention (T_1). From each patient, an unstimulated whole saliva sample was collected in sterile tube using the Oragene tube (DNA genotek, Ottawa, Canada) following the manufacturer's instructions (Alharbi et al., 2020; Alshammary and Khan, 2021). Oragene commonly used a sophisticated technique wherein preservation buffers are used to protect the integrity of the sample and stabilizes samples at the point of collection at room temperature until processing and extraction take place (Sindhu and Jagannathan, 2014). Saliva collections were made during morning hours, between 8 and 11 am. The subjects were in the fasting state or two hours after breakfast. Patients were recommended not to eat and drink, or brush their teeth or gargle before 30 mins to 60 mins of saliva sample collection to avoid contamination. The 2 mL of the saliva sample was collected in the spitting method (Priya and Prathibha, 2017). The participants were asked to rinse the mouth out well for 1 min with distilled drinking water then to expectorate the water. Five minutes after this oral rinse, the subjects were asked to spit into the Oragene tube. They were recommended to sit on the dental chair with the position of the right side. The participants were instructed to stop talking and to lower their heads, allowing saliva to flow naturally to the front of their mouths. They were also asked not to cough up mucus as saliva is collected.

2.3. Biochemical parameters

In this study, 4 biochemical parameters such as Calcium, Phosphorous, Alkaline phosphatase and buffering capacity. Additionally, flow-rate and PH were also measured for the saliva samples. Specific parameters were performed with recommended kits and describe in Table 1. The calcium was measured in milligram per deciliter (mg/dL), phosphorous was measured in a millimeter per liter (mmol/L), and alkaline phosphatase was measured in units per liter (U/L). Flow-rate was measured in time, the simplified oral

Table 1
List of tests performed with saliva samples which are present in this study.

Biochemical Test	Method + Machine	Kits used in this study	References
Calcium	Automated Chemistry Analyzer (COBAS)	COBAS (Roche Diagnostics, Germany)	(Iacopetti et al., 2017)
Phosphorous	Automated Chemistry Analyzer (COBAS)	COBAS (Roche Diagnostics, Germany)	(Iacopetti et al., 2017)
Alkaline Phosphatase	Automated Chemistry Analyzer (COBAS)	COBAS (Roche Diagnostics, Germany)	(Baskar and Dharman, 2020)
Flow Rate	Graduate test tube method	Tube method	(Alshahrani et al., 2019)
Buffering Capacity	Hand-held pH meter method	HCl and pH Meter	(Pandey et al., 2015)
pH	pH meter	pH Meter	(Arab et al., 2016)
OHI	Based on recommended values	-	(Lara-Carrillo et al., 2009)

hygiene index (OHI) was confirmed as 0–1.2 as good, 1.3–3.0 as fair and 3.1–6.0 as poor. Finally, pH and buffering capacity were measured using a pH meter which is used to calculate the hydrogen-ion activity in saliva samples. The pH scale ranges from 0 to 14. 0–6.9 is defined as acidic, 7.0–7.9 is defined as neutral and 8.0–14.0 is defined as basic. Salivary parameters were analyzed using Cobas automated biochemistry analyzer for calcium, phosphorous and alkaline phosphatase using human diagnostic kits (Roche Diagnostics, Germany). Each analysis was repeated twice to reconfirm the result.

2.4. 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: 0–12 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 were measured in both T_0 and T_1 patients.

2.5. Statistical analysis

Patient's salivary calcium, phosphorous, alkaline phosphatase, flow rate, pH and buffering capacity levels were tabulated and the estimated values were compared using independent *t-test*. A p-value of < 0.05 was taken as an increased value. Statistical test was applied using SPSS software (Version 25.0) (Khan et al., 2019).

3. Results

3.1. Involvement of patient details

In this study, 35 patients were involved with the diagnosis of orthodontic treatment. The treatment was categorized into T_0 which represents the day of de-bonding of treatment (directly before de-bonding) and T_1 defines as 5 weeks after de-bonding of treatment (on retention). The treatment duration involved in orthodontic treatment was exactly 5 weeks on retention period. The duration of treatment varies depending on the consumption of total time of orthodontic treatment on fixed appliances before the de-bonding ranges between a minimum of 1 year and a maximum of 7 years and 6 months. This study indicates during T_0 phase and T_1 phase, 35 patients (T_0) and 35 patients (T_1) were involved. The mean age of the 35 participants was in the range of 21.5 ± 5.8. The mean age of the female participants was 20.5 ± 6.2 and male participants was 23 ± 4.9. The minimum age of male participants was 15 years and the maximum age was 34 years who were participated in this study. Among women, the minimum and maximum age were 14 years and 39 years. In this study, 40% of subjects involved were males and 60% were females. In this study, there were several types of retainers enrolled and among them, Hawley and fixed (40%) was documented to be high, Hawley was 28.6%, Essix was 11.4%, Essix & fixed was documented to be 14.3%. Finally, Wrap-around and fixed was confirmed to be 5.7%. The mean time of the treatment time was found to be 2.7 ± 1.6 and among males, the estimated time was 2.9 ± 1.9 and in females was 2.5 ± 1.4. The basic details of the patients involved in this study are shown in Table 2, Fig. 1.

Table 2
Details of the involvement of orthodontic patients

	Orthodontic treatment (n = 35)	Prevalence (%)
Age	21.5 ± 5.80	NA
Gender: MaleFemale	14	40
	21	60
Type of retainer	35	100
Essix	04	11.4
Essix & Fixed	05	14.3
Hawley & fixed	14	40
Wrap around and fixed	02	5.7
Hawley only	10	28.6
Treatment Duration	2.71 ± 1.61	NA
Treatment duration in males	2.95 ± 1.90	NA
Treatment duration in females	2.55 ± 1.41	NA

3.2. Biochemical parameter analysis

In this study, 7 parameters were opted, i.e., calcium, phosphorous, alkaline phosphatase, buffering capacity, PH, flow-rate and OHI. Each analysis was repeated twice to reconfirm the results.

3.3. Calcium levels

In this study, calcium levels were measured in both T_0 and T_1 patients. The calcium levels were measured in mg/dL based on the recommendations from the Roche kit. The mean value of calcium in T_0 patients were 9.58 ± 0.27 and the minimum value documented in T_0 patients was 8.9 mg/dL and the maximum value was 10.2 mg/dL. In female subjects, the minimum 8.9 mg/dL and the maximum obtained value is 10.2 mg/dL. The mean value for the female subjects was 9.5 ± 0.3 and in male subjects, 9.4 mg/dL was the obtained minimum value and 9.8 mg/dL was the maximum value. The mean value for the male subjects was 9.6 ± 0.1. Table-2 has described the biochemical values obtained from this study. The mean value of calcium in T_1 patients was 8.45 ± 0.29 and the minimum value documented in T_1 patients was 8.0 mg/dL and the maximum value was 9.2 mg/dL. In female subjects, the minimum obtained value is 8.0 mg/dL and the maximum obtained value is 9.2 mg/dL. The mean value for the female subjects was 8.4 ± 0.3. In male subjects, 8.2 mg/dL was the obtained minimum value and 9.1 mg/dL was the maximum value. The mean value for the male subjects was 8.4 ± 0.29. When the *t*-tests were performed between the T_0 and T_1 values, a statistically signiifcant decrease was obtained (p < 0.0001). The mean difference was documented to be 1.13 and *t*-statistics was found to be 16.87. The upper and lower limit values were 0.99 and 1.26.

3.4. Phosphorous levels

In this study, phosphorous levels were measured in both T_0 and T_1 patients. The phosphorous levels were measured in mmol/L based on the recommendations from the Roche kit. The mean value of phosphorous in T_0 patients was 3.05 ± 0.81 and the minimum value documented in T_0 patients was 1.72 mmol/L and the maximum value was 4.38 mmol/L. In female subjects, the minimum 1.94 mmol/L and maximum obtained value is 4.38 mmol/L. The mean value for the female subjects was 3.1 ± 0.8 and in male subjects, 1.72 mmol/L was the obtained minimum value and 4.38 mmol/L was the maximum value. The mean value for the male subjects was 2.9 ± 0.8. Table 3 has already described the biochemical values obtained from this study. The mean value of phosphorous in T_1 patients were 3.21 ± 0.84 and the minimum value documented in T_1 patients was 2.07 mmol/L and the maximum
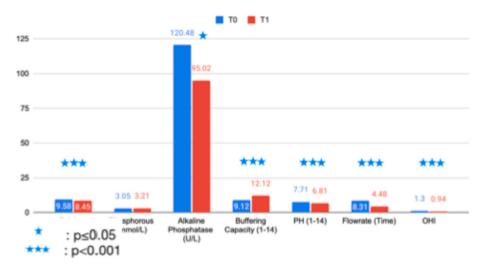
Fig. 1. Comparison between salivary parameters of patients treated with fixed orthodontic appliances at T_0 and T_1.

Table 3
Biochemical Parameters involved in this study

Parameters	T_0 = 35	T_1 = 35	Mean difference	Lower limit	Upper limit	t statistics	P-value
Calcium (mg/dL)	9.58 ± 0.27	8.45 ± 0.29	1.13	0.99	1.26	16.87	<0.0001
Phosphorous (mmol/L)	3.05 ± 0.81	3.21 ± 0.84	−0.16	−0.55	0.23	−0.81	0.42
Alkaline Phosphatase (U/L)	120.48 ± 51.72	95.02 ± 41.54	25.46	3.08	47.83	2.27	0.02
Buffering Capacity (1–14)	9.12 ± 0.12	12.12 ± 0.14	−3	−3.06	−2.93	−96.25	<0.0001
PH (1–14)	7.71 ± 0.26	6.81 ± 0.21	0.9	0.78	1.01	15.93	<0.0001
Flowrate (Time)	8:31 ± 0.74	4:48 ± 0.17	3.83	3.57	4.08	29.84	<0.0001
OHI	1.3 ± 0.52	0.94 ± 0.52	0.36	0.11	0.60	2.89	0.05

value was 5.46 mmol/L. In female subjects, the minimum obtained value is 2.07 mmol/L and the maximum obtained value is 4.26 mmol/L. The mean value for the female subjects were 3.03 ± 0.58 and in male subjects, 2.1 mmol/L was the obtained minimum value and 5.46 mmol/L was the maximum value. The mean value for the male subjects were 3.48 ± 1.10. When the t-tests were performed between the T_0 and T_1 values, no statistically significant difference was obtained (p = 0.42). The mean difference was documented to be −0.16 and t-statistics were found to be −0.81. The upper and lower limit values were −0.55 and 0.23.

3.5. Alkaline phosphatase levels

In this study, Alkaline Phosphatase levels were measured in both T_0 and T_1 patients. The Alkaline Phosphatase levels was measured in U/L based on the recommendations from the Roche kit. The values for male and female varied from certain age groups. The mean value of Alkaline Phosphatase in T_0 patients was 120.4 ± 51.72 and the minimum value documented in T_0 patients was 47 U/L and the maximum value was 251 U/L. In female subjects, the minimum 47U/L and maximum obtained values are 251U/L. The mean value for the female subjects was 110 ± 65.12 and in male subjects, 127U/L was the obtained minimum value and 143U/L was the maximum value. The mean value for the male subjects was 136.2 ± 5.3. The mean value of alkaline phosphatase in T_1 patients was 95.02 ± 41.54 and the minimum value documented in T_1 patients was 38U/L and the maximum value was 192U/L. In female subjects, the minimum obtained value is 38 U/L and the maximum obtained value is 192 U/L. The mean value for the female subjects was 80.95 ± 48.89 and in male subjects, 105 U/L was the obtained minimum value and 126 U/L was the maximum value. The mean value for the male subjects was 116.14 ± 6.06. The t-tests were performed between the T_0 and T_1 values, a statistically significant decrease was obtained (p = 0.02). The upper and lower

limit values were 3.08 and 47.83. The mean difference was documented to be 25.46 and t-statistics was found to be 2.27.

3.6. Buffering capacity

In this study, buffering capacity levels were measured in both T_0 and T_1 patients. The buffering capacity levels were measured using a pH meter considering 0–6.9 as acidic, 7.0–7.9 as neutral and 8.0–14.0 as basic. However, there are no unit values and none of the kits was used. The values for male and female does not vary from any certain age groups. The mean value of buffering capacity in T_0 patients were 9.12 ± 0.12 and the minimum value documented in T_0 patients was 8.9 and the maximum value was 9.4. In female subjects, the minimum 8.9 and maximum obtained value is 9.3. The mean value for the female subjects were 9.1 ± 0.10 and in male subjects, 8.9 was the obtained minimum value and 9.4 was the maximum value. The mean value for the male subjects were 9.13 ± 0.15. The mean value of buffering capacity in T_1 patients were 12.12 ± 0.14 and the minimum value documented in T_1 patients was 11.8 and the maximum value was 12.4. In female subjects, the minimum obtained value is 11.8 and the maximum obtained value is 12.4. The mean value for the female subjects were 12.11 ± 0.14 and in male subjects, 11.9 was the obtained minimum value and 12.3 was the maximum value. The mean value for the male subjects were 12.13 ± 0.14. When the t-tests were performed between the T_0 and T_1 values, a statistically significant increase was obtained (p < 0.0001). The mean difference was documented to be −3 and t-statistics were found to be −96.25. The upper and lower limit values were −3.06 and −2.93.

3.7. pH levels

In this study, pH levels were measured in both T_0 and T_1 patients. The pH levels were measured using a pH meter consider-

ing 0–6.9 as acidic, 7.0–7.9 as neutral and 8.0–14.0 as basic. However, there are no unit values and none of the kits was used. The values for male and female does not vary from any certain age groups. The mean value of pH in T_0 patients were 7.71 ± 0.26 and the minimum value documented in T_0 patients was 7.2 and the maximum value was 8.3. In female subjects, the minimum 7.2 and maximum obtained value is 8.3. The mean value for the male subjects were 7.7 ± 0.30 and in male subjects, 7.3 was the obtained minimum value and 8.1 was the maximum value. The mean value for the male subjects were 7.73 ± 0.21. The mean value of buffering capacity in T_1 patients were 6.8 ± 0.21 and the minimum value documented in T_1 patients was 6.4 and the maximum value was 7.4. In female subjects, the minimum obtained value is 6.5 and the maximum obtained value is 7.2. The mean value for the female subjects were 6.8 ± 0.24 and in male subjects, 6.5 was the obtained minimum value and 7.2 was the maximum value. The mean value for the male subjects were 6.8 ± 0.17. When the *t*-tests were performed between the T_0 and T_1 values, a statistically significant decrease was obtained ($p < 0.0001$). The mean difference was documented to be 0.9 and *t*-statistics was found to be 15.93. The upper and lower limit values were 1.01 and 15.93.

3.8. Flow-rate levels

In this study, flow-rate levels were measured in both T_0 and T_1 patients. The flow-rate levels were measured based on the time required to fill 2 mL of saliva into the tube. However, there are no unit values and none of the kits were used. The time for male and female does not vary from any certain age groups. The mean time for flow-rate in T_0 patients were 8.31 ± 0.74 and the minimum time consumed in T_0 patients was 1:00 min and the maximum time was 25:00 mins. In female subjects, the minimum consumed time was 1:05 mins and the maximum obtained time was 25:00 mins. The mean time for the female subjects were 6.47 ± 0.24 and in male subjects, 1:00 mins were the obtained minimum time and 9:00 mins was the maximum time. The mean time for the male subjects were 3.42 ± 0.10. The mean time of flow-rate in T_1 patients were 4.48 ± 0.17 and the minimum time documented in T_1 patients was 1:00 mins and the maximum time was 20:00 mins. In female subjects, the minimum obtained time was 3:00 mins and the maximum obtained time is 20:00 mins. The mean time for the female subjects were 5.52 ± 0.19 and in male subjects, 1:00 min was the obtained minimum time and 9:00 mins was the maximum consumed time. The mean time for the male subjects were 3.05 ± 0.09. When the *t*-tests were performed between the T_0 and T_1 time frame values, a statistically significant decrease was obtained($p < 0.0001$). The upper and lower limit values were 4.08 and 29.84. The mean difference was documented to be 3.83 and *t*-statistics was found to be 29.84.

3.9. Analysis for OHI levels

The simplified OHI levels were as follows: 0–12 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 were measured in both T_0 and T_1 patients. The mean value of OHI in T_0 patients were 1.3 ± 0.527 and the minimum value documented in T_0 patients was 0.16 and the maximum value was 2.32. In female subjects, the minimum 0.33 and maximum obtained value is 2.32. The mean value for the female subjects were 1.3 ± 0.6 and in male subjects, 0.16 was the obtained minimum value and 1.66 was the maximum value. The mean value for the male subjects were 1.27 ± 0.4. The mean value of OHI in T_1 patients were 0.94 ± 0.52 and the minimum value documented in T_1 patients was 0.25 and the maximum value was 2.5. In female subjects, the minimum obtained value is 0.25 and the maximum obtained value is 2.5. The mean value for the female subjects were

1.0 ± 0.61 and in male subjects, 0.25 was the obtained minimum value and 1.2 was the maximum value. The mean value for the male subjects were 0.8 ± 0.34. When the *t*-tests were performed between the T_0 and T_1 values, a statistically significant decrease was obtained ($p = 0.05$). The mean difference was documented to be 0.36 and *t*-statistics was found to be 2.89. The upper and lower limit values were 0.11 and 0.60.

4. Discussion

The findings of this study confirmed the positive association between T_0 and T_1 groups in orthodontic treatment ($p < 0.05$). The current study's outcomes in buffering capability, pH and flow rate in orthodontic treatment were found to be agreement (Alshahrani et al., 2019; Teixeira et al., 2012; Cardoso et al., 2017; Kanaya et al., 2007) and contradict the results of (Lindawati et al., 2019; Chang et al., 1997) to other studies groups. However, only limited studies showed the association with a combination of buffering capability, pH and flow rate (Anu et al., 2019; Moritsuka et al., 2006). There was a substantial decrease in salivary pH and buffering capacity in the orthodontic appliance group ($p < 0.05$). These findings are consistent with those of (Kanaya et al., 2007) and (Teixeira et al., 2012)studies, who discovered a pH reduction in orthodontic patients. (Chang et al., 1997); on the other side, indicated an improvement in salivary buffering capacity after the placement of orthodontic appliances, but their subjects had the appliances for a shorter period. An impressive study has been conducted to discover the consequences of fixed orthodontic appliances on saliva production (Chang et al., 1997; Teixeira et al., 2012; Kanaya et al., 2007; Maret et al., 2014; Peros et al., 2011). While some authors have indicated an improvement in saliva flow and buffering capacity in caries patients after orthodontic treatment, others have found orthodontic treatment increases caries incidence (Chang et al., 1997; Peros et al., 2011). After being exposed to a cariogenic food, the carious biofilm pH decreases, however, again, it mostly returns to the original because the phosphate and bicarbonate salivary potential return to the resting levels. Saliva acts as a salivary antiseptic and is a potential partner in the development and prevention of dental caries (Cardoso et al., 2017; Peros et al., 2011). Even one month after the placement of orthodontic appliances was a substantial rise in salivary flow rate discovered. (Cardoso et al., 2017) study findings are consistent with the findings of early studies by (Chang et al., 1997) and (Peros et al., 2011), who discovered a substantial increase in salivary flow rate after 3 months relative to the baseline. According to these authors, a transient increase in this salivary parameter was observed after the placement of orthodontic appliances due to the mechanical stimulation offered by the orthodontic appliances. However, no change in this salivary parameter was observed in the subsequent phases of the study, presumably because the salivary flow rate returned to its initial rate after the patient's adaptation to orthodontic appliances (Cardoso et al., 2017). Increased salivary flow also contributes to increased salivary buffer capability. The oral environment can adapt to foreign bodies by increasing salivary flow rate, which has implications for buffer capacity and salivary pH (Arab et al., 2016). 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., 2009). However, in this current study, the treatment was carried out for only 5 weeks. The results of (Lara-Carrillo et al., 2009)study showed that salivary pH was the only property that retained a healthy value during the

treatment period, indicating that it plays an important role even in adverse conditions.

Additionally, other studies are also in accordance with the increased values in the current study with the pH value. (Arab et al., 2016) studies showed a positive association with pH values in orthodontic treatment. However, (LI et al., 2009)studies were not in agreement with our studies. It has previously been documented that patients with fixed orthodontic appliances have lower salivary pH (Bevinagidad et al., 2020; Alshahrani et al., 2019; Teixeira et al., 2012; Lara-Carrillo et al., 2009; Moussa et al., 2017) and our study was in agreement when compared between T_0 and T_1 groups. Additionally, (LI et al., 2009)studies were carried out in calcium, phosphorous, flow rate and pH with orthodontic treatment and this study results showed non-association with calcium, phosphorous and pH. However, the only flow rate was associated.

Bicarbonate, phosphate and protein are the three buffer systems present in the saliva that assists and keep the pH range tolerable within the mouth from 6.0 to 7.5 (Iorgulescu, 2009). The pH body fluid is defined by the protons (H +) content produced in living cells from organic acids. Lactic acid is a typical source of protons and participates in physiological pH regulation. The human body is structured to maintain a healthy acidity and alkalinity balance naturally. In this procedure, lung and kidney play a crucial part. The usual pH level of the blood is 7.40 at a scale between 0 and 14, 0 being the most acidic and 14 the most basic. This value can move in either direction slightly (Aoi and Marunaka, 2014). The median range of pH is between 6.2 and 7.6 for saliva. The pH of saliva changes with food and drink. The consumption of carbohydrate releases lactic acid, butyric acid, aspartic acid, and bacteria in the breakdown of mouth. This lowers the pH levels of saliva. Age might also play a role as adults tend to be more acidic than children in saliva. The imbalance of pH can lead to either acidosis and alkalosis. The acidosis refers to low levels of pH which can leads to diabetes, hypertension and kidney diseases, whereas alkalosis is defined as high pH levels which is associated towards the development of adrenal disease (Baliga et al., 2013). The entire flow rate of the saliva is increased by the tonicity of the saliva. Depending upon the concentration of inorganic constituents the flow rate of saliva varies. The concentration of flow rate was increased with sodium. The saliva in the potassium and iodide concentrations are largely constant at a wide range of flow rates (Burgen and Terroux, 1962). The salivary flow rate is the amount of salivary in a time unit of mL/min or g/min produced by the salivary glands. It can be split into non-stimulated, independent from the presence of stimuli (food, mastication, etc.) and encouraged and separated largely for sensory stimulation, gustatory and chewing purposes (Foglio-Bonda et al., 2017).

Minerals like calcium, alkaline phosphorous 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 this study, statistical association was documented between T_0 and T_1 groups of orthodontic treatment. Our study was found to be in agreement with the calcium levels carried out with saliva samples of other studies (Teixeira et al., 2012; LI et al., 2009; Moussa et al., 2017). However, limited studies have been documented with the inconsistent association (Bevinagidad et al., 2020). This is consistent with Indriana's study, which concluded that increasing the flow rate of saliva will influence the amount of calcium ion concentration in saliva (Indriana, 2011). Salivary calcium levels increased in groups that used fixed orthodontic appliances in this study. The findings of this study are consistent with the findings of Bhavasar et al., (Lindawati et al., 2019) studies who found that changes in the oral environment caused by the presence of orthodontic appliances in the oral cavity influence salivary calcium.

Phosphorus is used in all cells and tissues as an essential component of DNA, RNA, and phospholipids, as a source of high-energy bonds in adenosine triphosphates, as a substrate for various kinases and phosphatases, and as a regulator of intracellular signaling. Approximately 85% of phosphorus, the second most abundant mineral in the human body, is found in bone, especially in hydroxyapatite crystals deposited on the collagen matrix, where it is compounded with calcium, the most abundant mineral. After calcium, phosphorus is the second most essential mineral in the human body. Phosphorus is found in the bones and teeth in the form of hydroxyapatite, with the remainder present in extracellular fluid, soft tissue, and erythrocytes (Foster et al., 2008; Nasution and Amatanesia, 2018). In this study, statistical association was documented between phosphorus levels with both the T_0 and T_1 groups of orthodontic treatment. Our study was found to be in agreement with the phosphorous levels carried out with saliva samples and limited studies have been documented with inconsistent association (Bevinagidad et al., 2020; Chang et al., 1997; Alshahrani et al., 2019; Cardoso et al., 2017; Dallel et al., 2020; Uysal et al., 2011).

Alkaline phosphatase is a glycoprotein that is intended to be involved in processes that contribute to the formation of minerals in tissue such as bone and cement. Alkaline phosphatase, in particular in the dental sector, were of substantial interest to salivary biomarkers. The Alkaline Phosphatase enzyme primarily acts by catalyzing mono-ester hydrolysis and catalyzes a *trans*-phosphorus reaction in high phosphate acceptor levels in various organisms ranging from bacteria to humans. The studies with dental caries showed and showed a relationship to earlier studies with salivary alkaline phosphatase (Farahani et al., 2015; Shetty et al., 2017). In this study, this parameter also showed increased values between alkaline phosphatase levels with both the T_0 and T_1 groups of orthodontic treatment. Our study was found to be in agreement and disagreement with the alkaline phosphatase levels carried out with saliva samples (Bevinagidad et al., 2020; Chang et al., 1997; Alshahrani et al., 2019; Dallel et al., 2020; Abdullah and Abdul, 2011). This study has several strengths and limited limitations such as follows. The current strength of this study was in comparison to serum or tissue, there are several benefits when saliva is used as a diagnostic tool. The benefits of salivary diagnostics are its intrusive collection process, a smaller sample fraction, strong compliance with the patient, cost efficiency, simple storage and transport, greater sensitivity and a correlation with blood level. Moreover, salivary biomarkers for a broad variety of medical conditions such as malignancies, autoimmune disorder, infections and metabolism have been developed thanks to the development of new technologies. One of the limitations of this study was we have opted for only 35 patients with T_0 and T_1 groups. The other limitations of this study were skipping of the diet of the participants and the final limitation of this study was the short time of the retention period which was only 4 to 5 weeks after de-bonding.

5. Conclusion

The present study concludes that there was a statistically significant decrease in the calcium, alkaline phosphatase, pH, flow rate and OHI values a month after de-bonding. However, buffering capacity values were increased in our study group. Our study recommends making the follow up for 3 months, 6 months, 9 months and 1 year of follow-up for longer results and analysis. Thus, additional steps must be taken to maintain oral hygiene and reduce their susceptibility to caries and other periodontal disorders for

patients undergoing fixed orthodontic treatment. Future studies recommend carrying out a large sample size in multiple institutes in all age groups to rule out the disease.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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