



ISSN Print: 2394-7489  
ISSN Online: 2394-7497  
IJADS 2022; 8(2): 91-98  
© 2022 IJADS  
[www.oraljournal.com](http://www.oraljournal.com)  
Received: 28-12-2021  
Accepted: 15-02-2022

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## Evaluation of cortisol level in saliva of patient undergoing orthodontic treatment

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DOI: <https://doi.org/10.22271/oral.2022.v8.i2b.1494>

### Abstract

**Objective:** To Evaluate cortisol level before and after insertion of orthodontic appliances at different time interval among the subjects, females, males and in between both the groups on comparison.

**Materials and Methods:** Total number of 20 north Indian subjects of the age group between 15 and 30 years were randomly selected for the study after meeting the study criteria and samples were collected before bonding (T0), 1 hour after bonding (T1) and 4-6 weeks after bonding (T2). The method of analysis used for the study is chemiluminescence assay (CLIA). Evaluation of cortisol level was done using immunoassay kits.

**Result:** There was a significant decrease in serum cortisol from pre-operative to 1 hour and 4 weeks and same for the males group and female group. There is no significant difference in the level of saliva cortisol on comparison in between male and female group.

**Conclusions:** Fixed orthodontic appliances did show a significant effect on salivary parameters in our study that was saliva cortisol level which was related to inflammation or stress.

**Keywords:** Cortisol, Chemiluminescence (CLIA)

### Introduction

Orthodontic treatment though frequently sought for the improvement of both aesthetic and functional aspects of occlusion, has also been considered as an anxiety-producing and stressful treatment modality. According to the literature about 95% of the patients experience some kind of pain associated with orthodontic therapy. This stress is induced not only because of initial use of nickel-titanium wire but, also due to some other orthodontic procedures such as separator placement, activations, application of orthopaedic forces and debonding which produce pain in patients<sup>[10]</sup>.

It has also been suggested that patients treated with fixed appliances experience more pain than removable or functional appliances<sup>10</sup>. Patients describe discomfort associated with orthodontic treatment as feelings of pressure, soreness of the teeth and tension causing pain<sup>[11]</sup>. However, up to 8% of orthodontic patients interrupt their orthodontic treatment because of early pain experiences<sup>[12]</sup>. Furthermore, the thought of having painful experiences discourages some patients from seeking orthodontic treatment, even when it is needed for functional rather than aesthetic reasons<sup>[13]</sup>.

Sergl *et al.*<sup>[9]</sup> reported that individual stress-related factors and anxiety level influence the intensity of discomfort caused by orthodontic appliances. These negative effects can significantly affect patient's daily activity causing difficulty in chewing, sleep disturbances and use of self-medication<sup>[1]</sup> this kind of discomfort, pain and stress associated with the treatment are usually underestimated by the orthodontists and feared by the patient. So, it becomes necessary to evaluate the discomfort and the stress that is produced by the orthodontic therapy. In such cases, pain measurements can be made with an assessment of the patient's anxiety level<sup>10</sup>. But such Subjective assessment of this stress is of limited value as it is incorporated with inherent human bias. Each and every biomarker has a certain level in which it is present in the body in physiological conditions. Under conditions of stress, like orthodontic treatment induced pain, the levels of these biomarkers change, bringing about changes in the body mechanism.

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Hence evaluation of various factors like ACTH, Cortisol in serum, the level of which are indicators of stress, is recommended. But the mere sight of blood and the act of its withdrawal from the body can induce stress and hence influence the readings. Analysis of one's saliva can fortunately overcome these limitations. In the last decade, saliva has gained importance as an important diagnostic aid partially due to its abundance of biomarkers and due to its ease and noninvasive accessibility. An increase in the production of serum cortisol by adrenal cortex, in response to stress, leads to a proportionate increase in its level in saliva. Hence, SC concentrations are closely correlated to serum cortisol concentration [3, 4]. Collection of salivary cortisol is simple, painless, and non-invasive and can be performed at any time kind of a procedure. With this Storage is convenient as the sample can be stored in home freezer and also repeated freeze-thaws do not adversely affect the determination of the cortisol levels [2] Regardless of the precise mechanisms by which the anti-inflammatory effect occurs, this effect of cortisol plays a major role in combating certain types of conditions. Certain conditions are characterized by severe local inflammation, and the harmful effects on the body are caused mainly by the inflammation itself and not by other aspects of the disease which is commonly leads to fluctuation of salivary biomarkers as in here that is cortisol level. The two most important aspects of pain and discomfort in orthodontic treatment are its intensity and duration. Understanding these has clinical implications to improve patient satisfaction and the quality of oral health [10]. In light of the importance of this mostly overlooked issue, this study is performed to evaluate the cortisol level in the patient who are undergoing orthodontic treatment before and after insertion of orthodontic appliances.

### Aim and Objectives

**Aim:** The aim of the study was to evaluate the level of cortisol in saliva of patient undergoing orthodontic treatment before and after insertion of orthodontic appliances at different time interval.

### Objectives

1. Comparison of cortisol level before and after insertion of orthodontic appliances at different time interval.
2. Comparison of cortisol level among males at T0, T1, T2.
3. Comparison of cortisol among females at T0, T1, T2.
4. Comparison of cortisol among females and males at T0, T1, T2.
  - (i) T0 (baseline), pretreatment or before bonding.
  - (ii) T1, 1 hour after initial arch wire insertion and activation.
  - (iii) T2, 4-6 weeks after arch wire insertion and activation.

### Materials and Methods

A total sample of 20 north Indian subjects of the age group between 15 and 30 years were randomly selected from the OPD at the Department of Orthodontics and Dentofacial Orthopedics, RUHS College of Dental Sciences, Jaipur for the study.

### Inclusion criteria

Subjects were included on the basis of the following inclusion criteria-

1. Patient who was not under any medication.
2. Age group of 15-30 years.
3. Patients without any systemic disease.

### Exclusion criteria

Subjects were excluded on the basis of the following exclusion criteria-

1. Patients consuming tobacco in any form.
2. Patients who have already undergone orthodontic treatment.
3. Patients with congenital craniofacial anomalies.

### Standardization of analysis

The analysis of the sample was done by CLIA (Chemiluminescence Immunoassay). The samples were collected at:

- (i) T0 (baseline), pretreatment or before bonding.
- (ii) T1, 1 hour after initial arch wire insertion and activation.
- (iii) T2, 4-6 weeks after arch wire insertion and activation.

### Collection of sample

The patients were randomly selected on the basis of inclusion and exclusion criteria from the OPD of department of orthodontics, who required orthodontic treatment.

### Methodological issues

**Time of collection:** Since there are a number of factors that affect cortisol secretion, there are several important issues to be aware of when measuring salivary cortisol in patient. Since cortisol is secreted in a diurnal pattern, it is imperative to consider time of day in reference to sample collection, to have consistency across subjects. Timing of collection is also relevant depending upon the amount of response to a stressor. Individual variations including, typical sleep, activity, eating, and recent illness need to be considered when planning to use salivary cortisol as a measure of stress [22]. The collection time was always kept between 9:00 and 11:00 a.m., respecting the circadian rhythm of the subject. It was necessary for subjects to abstain from food and drinks (except water). The subjects were also oriented to avoid smoking, physical exercises and tooth-brushing up to 2 hours before sample collection as these can affect the salivary cortisol levels.

### Saliva collection devices

A major advantage of salivary cortisol is, its non-invasive aspect of sampling. The sample were collected in a safe manner with minimal stress (Schwartz *et al.*, 1998). Unstimulated saliva of around 2-4ml of samples was collected with the use of sterile syringes as this was a noninvasive way of collecting the saliva, which was well tolerated by the patient. Evidence of blood contamination was determined by visual inspection. Also, the volume of saliva obtained by this method was sufficient for the cortisol measurements.



**Fig 1:** Collection of Saliva

**Storage of samples**

It was then transferred to a standardized plastic tube, later the clear tubes were kept under cold temperature to prevent distortion until analysis performed in lab. Although, the cortisol in saliva is remarkably stable and would probably survive a month or more at room temperature. However, the saliva will grow mold and acquire a disgusting smell within a few days. So, given that repeated freeze-thaw cycles are not a problem with this molecule.



**Fig 2:** Collected Saliva Sample

**Assays**

Selection of the laboratory and assay for analysis of cortisol requires careful research to identify the most appropriate choice. The method which was selected to perform the analysis was the CLIA (Chemiluminescence Immunoassay) method. The saliva samples were centrifuged upon arrival in the laboratory and stored frozen until analysis.



**Fig 3:** Immunoassay method

**Statistical analysis**

Continuous variables were presented as mean ± SD and median. Normality of data was tested by Kolmogorov-Smirnov test. If the normality was rejected then non parametric test was used. Pre Cortisol (ng/mL) was compared using Paired t test with cortisol after 1 hour and 4 weeks. A p value of <0.05 was considered statistically significant. The data was entered in MS EXCEL spreadsheet and analysis was done using Statistical Package for Social Sciences (SPSS) version 21.0.

**Table 2:** Comparison of pre-Cortisol (mg/mL) with cortisol after 1hour and 4weeks of study subjects.

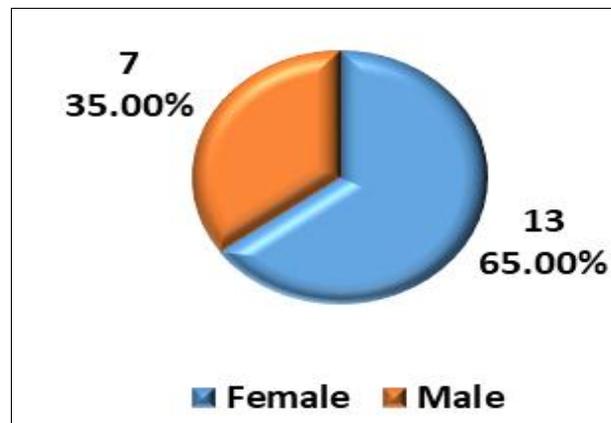
Cortisol(mg/mL)	Mean±Stdev	Median(IQR)	Range	P value	Test performed
Pre	7.72±2.6	7.4(6.48-8.6)	2.4-15.5	-	-
1hour later	4.93±1.69	4.4(3.875-6.6)	2.2-8.8	<.0001	Paired T-test; T value=7.106
4weekslater	3.93±1.21	3.65(3.025-4.85)	2.4-6.7	<.0001	Paired T-test;t value=6.211

**Results**

This study was conducted in Department of Orthodontics and Dentofacial Orthopedics, RUHS College of Dental Sciences, Jaipur (Rajasthan). Total 20 subjects of the age group 15 to 30 years were randomly selected for the study.

**Table 1:** Gender distribution of study subjects.

Gender	Frequency	Percentage
Female	13	65.00%
Male	7	35.00%



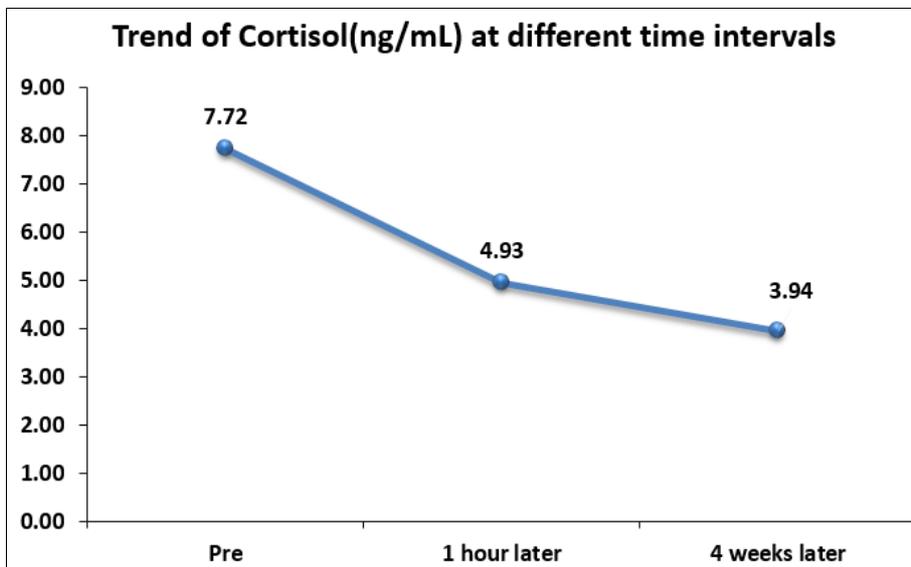
**Fig 4:** Gender distribution of study subjects.

For analysis and study, a minimum 4ml of unstimulated salivary sample was collected in a clear sterile tube. The same method was followed to collect the sample at three different time period that is:- (i) T0 (baseline), pretreatment or just before bonding. (ii) T1, 1 hour after initial arch wire insertion and activation.

(iii) T2, 4-6 weeks after arch wire insertion and activation. The collection of salivary sample was done with great precaution without causing any trauma to the patient’s oral cavity and was strictly kept sterile.

After collection of samples each tube was labeled with the patient name and age, along with the serial number. Samples were stored in the refrigerator and on the same day delivered to the laboratory for analysis. Later results were collected at regular intervals till the final sampling was done that ended up after 4 weeks of first sample collection. Data so obtained was subjected to statistical analysis for obtaining mean, standard deviation and standard mean and significance value for variables. Kolmogorov-Smirnov test was used for normality of data.

Pre Cortisol (mg/mL) was compared using Paired t test with cortisol after 1 hour and 4 weeks. P values <0.05 showed statistically significant results.



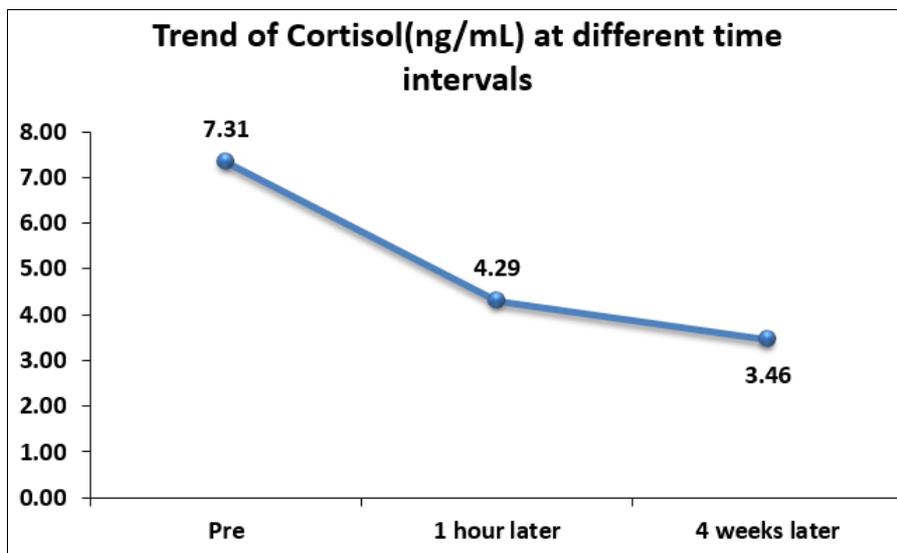
**Graph 1:** Comparison of pre-Cortisol (mg/mL) with cortisol after 1hour and 4weeks of study subjects.

Mean value of Cortisol (ng/mL) preoperatively was 7.72±2.6 with median (IQR) of 7.4(6.48-8.6). 1hour later was 4.93±1.69 with median (IQR) of 4.4(3.875-6.6) 4 weeks later was

3.93±1.21with median (IQR) of 3.65(3.025-4.85) respectively. There was a significant decrease in serum cortisol from preoperative to 1hour and 4weeks. (p value<.05)

**Table 3:** Comparison of Cortisol (mg/mL) of study subjects between follow-up in males.

Cortisol(mg/mL)	Mean±Stdev	Median(IQR)	Range	P value	Test performed
Pre	7.31±1	7.5(6.8-8.1)	5.5-8.4	-	-
1hourlater	4.29±0.8	4.3(3.75-4.8)	3.2-5.4	0.0001	Paired t test; t value=9.04
4weekslater	3.46±0.78	3.1(2.95-3.85)	2.7-4.8	0.0002	Paired t test;t value=7.782



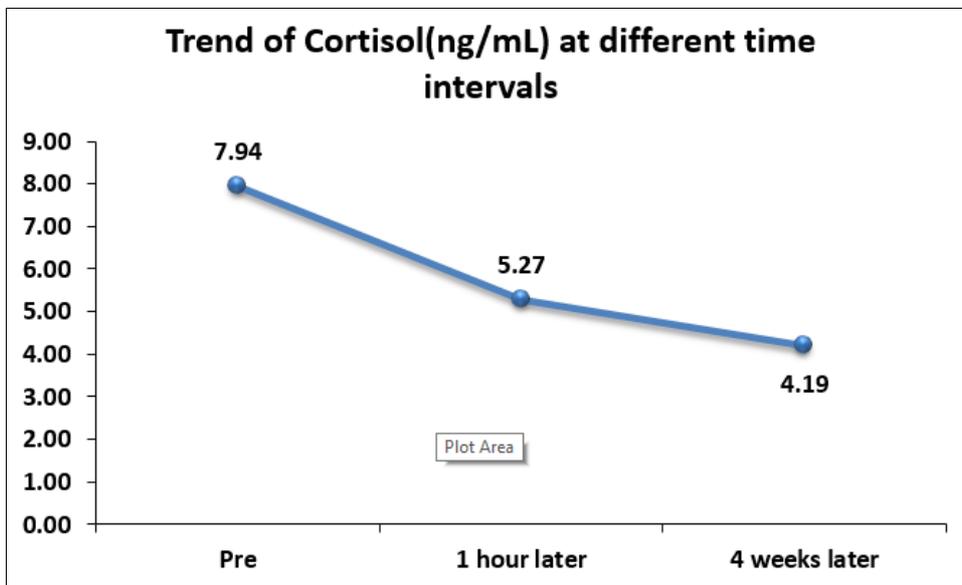
**Graph 2:** Comparison of Cortisol (mg/mL) of study subjects between follow up in males.

Mean value of Cortisol (ng/mL) pre-operatively was 7.31±1 with median (IQR) of 7.5(6.8-8.1). 1hour later 4.29±0.8 with median (IQR) of 4.3(3.75-4.8). 4weeks later in males was

3.46±0.78 with median (IQR) of 3.1(2.95-3.85) respectively. There was a significant decrease in serum cortisol from preoperative to 1hour and 4weeks. (p value<.05)

**Table 4:** Comparison of Cortisol (mg/mL) of study subjects between follow-up in females.

Cortisol(ng/mL)	Mean±Stdev	Median(IQR)	Range	P value	Test performed
Pre	7.94±3.2	7.4(6.4-9.4)	2.4-15.5	-	-
1hourlater	5.27±1.96	4.5(4.1-6.7)	2.2-8.8	0.001	Paired test; t value=4.558
4weekslater	4.19±1.35	3.8(3.6-5.1)	2.4-6.7	0.001	Paired test; t value=4.092



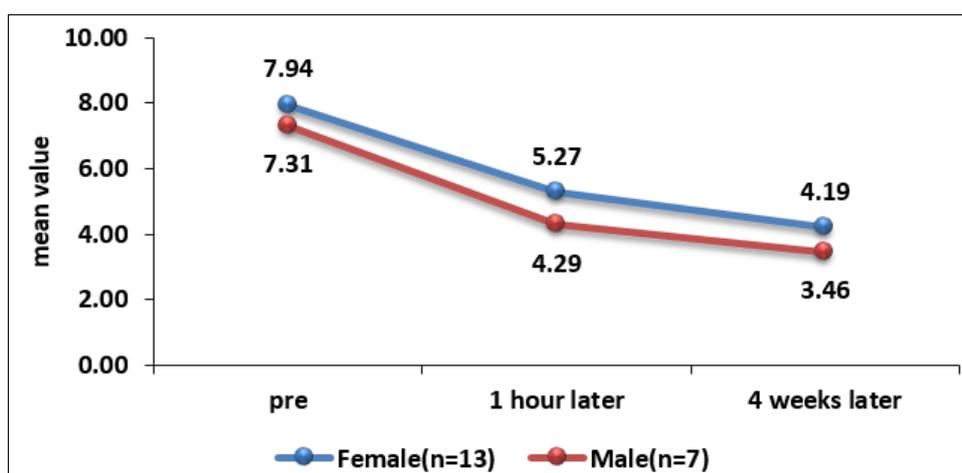
**Graph 3:** Comparison of Cortisol (ng/mL) of study subjects between follow-up in females.

Mean value of Cortisol (ng/mL) preoperatively was 7.94±3.2 with median (IQR) of 7.4(6.4-9.4). 1hour later was 5.27±1.96 with median (IQR) of 4.5(4.1-6.7) 4weeks later in females was 4.19±1.35 with median (IQR) of 3.8(3.6-5.1)

respectively. There was a significant decrease in serum cortisol from preoperative to 1hour and 4weeks. (p value<.05).

**Table 5:** Comparison of Cortisol (mg/mL) between genders.

Cortisol(mg/mL)	Female (n=13)	Male (n=7)	Total	P value	Test performed
<b>Pre</b>					
Mean±Stdev	7.94±3.15	7.31±1.04	7.72±2.59	0.62	ttest;0.504
Median(IQR)	7.4(6.4-9.4)	7.5(6.8-8.1)	7.45(6.475-8.6)		
Range	2.4-15.5	5.5-8.4	2.4-15.5		
<b>1hourlater</b>					
Mean±Stdev	5.27±1.96	4.29±0.8	4.93±1.69	0.132	ttest;1.581
Median(IQR)	4.5(4.1-6.7)	4.3(3.75-4.8)	4.4(3.875-6.6)		
Range	2.2-8.8	3.2-5.4	2.2-8.8		
<b>4weekslater</b>					
Mean±Stdev	4.19±1.35	3.46±0.78	3.94±1.21	0.203	ttest;1.319
Median(IQR)	3.8(3.6-5.1)	3.1(2.95-3.85)	3.65(3.025-4.85)		
Range	2.4-6.7	2.7-4.8	2.4-6.7		



**Graph 4:** Comparison of trend of Cortisol (ng/mL) at different time intervals between Males & Females.

The variable Cortisol (ng/mL) was normally distributed. Thus parametric test was used for the comparison. No significant difference was seen in Cortisol (ng/mL) at pre, 1hour later,4 weeks later between gender.(p value>.05) Mean±Stdev of Cortisol (ng/mL) at pre 7.94±3.15 1hour later

5.27±1.96 4weeks later in female was 4.19±1.35 And in male was Mean±Stdev of Cortisol (ng/mL) at pre 7.31±1.04, Mean±Stdev of Cortisol (ng/mL) at 1hourlater 4.29±0.8, Mean±Stdev of Cortisol (ng/mL) 4weeks later 3.46±0.78 with no significant difference between them.

## Discussion

Dental environment being a new place for someone who comes for treatment, can trigger stress among patients. To counter these stresses, body in response tries to bring the body back to physiological state by certain physiological processes. Major factors which perform these responses against stress factors are the biomarkers which are present in the body. One of them is cortisol, which is a stress-related biomarker. Cortisol is a steroid hormone that regulates a wide range of processes throughout the body, including metabolism and the immune response. It also has a very important role in helping the body respond to stress. This biomarker has been extensively studied in patients with chronic or facial pain conditions [35, 37]. Literature has shown that the correlation in between serum cortisol and saliva cortisol is present and can be proved to be an invasive method for analysis of cortisol without putting patient in unsuitable condition. The cortisol regulation is extremely complex and is not known in detail. Moreover, its circadian cycle is a complex phenomenon, so a more comprehensive evaluation of cortisol secretory activity over different periods of the day is perhaps necessary to reveal any differences in orthodontic patients. To get to know more about the saliva cortisol level we performed a study in which cortisol activity of the patients 1 hour after the appliance activation (T1) and 4 weeks after activation (T2) was noted and compared to the sample at (T0) that was taken before bonding in orthodontic patients. The present results indicate that orthodontic pain is significant to alter the basal cortisol levels and could be related to the evoked characteristic of Pain. With this orthodontic pain can also be spontaneous, it is mainly evoked by unharmed stimuli such as mechanical stimulation of the periodontium during mastication. The clinical pain response induced by stimuli that are usually not harmful is called allodynia [40] and this was clearly observed in our study.

As also demonstrated by Miller *et al.* [46] the level of saliva cortisol was highest in patients undergoing tooth extraction when compared with other procedures, such as prophylaxis, restorative treatments, and examination. They evaluated the adrenal stress response to various dental treatments in healthy adults and found that cortisol levels measured at the start of a dental procedure decreased in patients undergoing noninvasive dental procedures, such as routine examinations. Conversely, cortisol levels at the end of tooth extractions were elevated compared with baseline cortisol. Another study by Padmanabhan *et al.* [28] concluded that saliva cortisol was higher in the study group and within the study group it was raised when extractions were there than in the appointments in which oral prophylaxis and restorations were done respectively. Similarly, Furlan *et al.* [26] did their study on the saliva cortisol, alpha-amylase and heart rate variation in response to dental treatment in children and found higher cortisol and amylase levels before. Similarly M. gecgelen *et al.* [41] reported that there were no statistically significant differences for mean salivary cortisol values between G1 (peak) and G2 (post-peak) skeletal maturity stage groups. David s. Goldstein *et al.* [31] came out with the result that there is small and statistically non-significant changes in cortisol levels in response to the surgery (third molar extraction). C. M. Hill *et al.* [32], according to his study "Salivary cortisol determinations and self-rating scales in the assessment of stress in patients undergoing the extraction of wisdom teeth" showed that, In the GA group, no significant differences were observed when salivary cortisol concentrations in samples collected pre-surgery were compared with those observed in

samples collected post-surgery. Where as In the LA groups there were no statistical differences although the post-surgical levels showed a decreasing trend. According to David A. Jones *et al.* [42]. His study concluded that The TMD group showed a significantly higher cortisol response to experimental stress than the control group. McCartan BE *et al.* [24], showed that Anxiety and salivary cortisol level when measured in two groups of patients with recurrent aphthous ulceration persistent aphthae (Group 1) and others had been relieved of their aphthae following correction of detected haematinic deficiency states {Group 2). Median salivary cortisol levels showed a statistically significant elevation in Group 1.

In our study, patients with age ranging from 15-30 years, both male and female, seeking orthodontic treatment, were evaluated for the salivary cortisol level. The Saliva sample was collected after giving the complete information to the patient and clearing all the doubts about the study. Keeping in mind about the about the circadian cycle of cortisol, morning appointment was selected for sample collection. All the precautions were taken to prevent the contamination during collection of sample, storage and during transfer of it to the lab. Further studies with larger sample size and stringent inclusion criteria may be conducted which may enlighten our knowledge and perception about the biomarkers like cortisol and many others. Continuing with the method that are way more invasive and can impact the psychological condition of patient have to be kept to the procedure or a case which are unavoidable and depending on the condition. As, to be providing same results and same benefits being on the non-invasive side is more comfortable for the patient, like in our case the results have been achieved with non-invasive method without causing any physical or mental discomfort to the patient. Further studies in this area will, no doubt, improve our knowledge about the cost benefit ratios of the newer technologies.

## Conclusion

**The conclusions made from the current study are the following**

1. There was a significant decrease in serum cortisol from pre-operative to 1 hour and 4 weeks.
2. There is significant reduction in the level of saliva cortisol seen in males group.
3. There is significant reduction in the level of saliva cortisol seen in female group.
4. There is no significant difference in the level of saliva cortisol on comparison in between male and female group.

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