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### RESEARCH ARTICLE

#### EVALUATING CHANGES IN SALIVARY FLOW RATE, PH & MICROBIAL COUNT DURING FIXED ORTHODONTIC TREATMENT - AN IN VIVO STUDY

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#### Abstract

**Aim of the study:** Aim of this study is to assess the changes in salivary flow rate, salivary pH, plaque index, Streptococcus mutans, Lactobacillus, and Candida albicans in patients undergoing fixed orthodontic treatment, before and after placement of fixed orthodontic appliances.

**Materials and Method:** In present study salivary samples from 60 patients (30 Males and 30 Females) with age group of 14-25 years would be undergoing fixed orthodontic treatment at the department of orthodontics. Salivary flow rate, pH, plaque index and microbial colony count were measured. Streptococcus mutans, Lactobacillus and Candida albicans colony counts were measured using mitis salivarius agar, rogosa agar and sabouraud dextrose agar respectively. Saliva collected for 5 mins and measured in ml/min. salivary pH measured with digital pH meter. Salivary samples obtained from the same patients at intervals of 8 weeks and 16 weeks after commencement of orthodontic treatment and evaluated for the same.

**Result:** This study showed fixed orthodontic appliances increases salivary flow rate ( $p=0.0001$ ), decreases salivary pH ( $p=0.03$ ), increases plaque index ( $p=0.0001$ ), also increases Streptococcus mutans, Lactobacillus and Candida albicans colony count ( $p=0.0003$ ,  $p=0.0001$  and  $p=0.019$  respectively). Independent t test reported salivary flow rate significantly increases more in male patients at 16 weeks interval ( $p=0.03$ ).

**Conclusion:** The results of this study confirmed that after placement of fixed orthodontic appliances plaque retention increases, which sequentially decrease the salivary pH and decreased salivary pH promote the growth of microbiota.

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**Introduction:-**

Fixed orthodontic appliances are one of the most commonly used techniques for the treatment of malocclusions. However, complex design of fixed orthodontic appliances alters the normal balance of salivary characteristics and oral microbial flora, which might raise the risk of dental caries and periodontal diseases during fixed orthodontic treatment. The assessment of the factors responsible for development of dental caries depends on the salivary contents and also on its properties.

Saliva is one of the most important environmental factors to maintain the balance between protective factors and caries risk factors during the orthodontic treatment. Saliva is the fluid that may be easily collected, and has been used studied as a toll to assess the risk of caries in orthodontic patients. Fixed orthodontic appliances hamper the oral hygiene maintenance by providing more retentive areas for adhesion of biofilm and plaque formation. Though salivary flow rate increases due to mechanosensation by fixed orthodontic appliances, formation of biofilm and plaque restrict the contact of tooth surface with saliva, which cease the remineralization of enamel.

The additional surfaces provided by fixed orthodontic appliances contribute more areas of environment suitable for the growth of bacteria responsible for initiation and progression of dental caries such as *Streptococcus mutans* and *Lactobacillus*. Unfavourable changes in salivary properties such as decreased salivary pH and increase in these bacteria, increase the risk of dental caries during fixed orthodontic treatment.

Evaluation of the *Streptococcus mutans* and *Lactobacillus* levels in patients before, during and after orthodontic therapy may help to determine caries risk levels and may thus lead to appropriate preventive or antimicrobial therapy. Chang et al. was the first who had examined the effect of the fixed orthodontic therapy on salivary microbiological parameters, pH value and buffering capacity of saliva. Understanding the role of saliva in orthodontic procedures can go long way in improving quality of treatment. With all these considerations this study was undertaken to evaluate changes in salivary parameters: salivary flow rate, pH, plaque index and microbial count of *Streptococcus mutans*, *Lactobacillus* and *Candida albicans* during fixed orthodontic treatment.

**Materials and Method:-**

Salivary samples from 60 patients with the age group of 14-25 years undergoing orthodontic treatment are obtained at intervals, before treatment, eight and sixteen weeks after commencement of orthodontic treatment at the department of Orthodontics. Two hours before the collection of salivary samples, patients are asked to brush their teeth and not to eat or drink after that. The salivary sample is collected in a sterile container. The patient is asked to sit straight in the chair with the head bent down and collect saliva for 5 minutes in a sterile container. Saliva is collected by unstimulated salivary flow in this sterile container. Saliva is collected between 10-12 am to avoid fluctuations due to circadian rhythm. A presterilized digital pH meter was then used to measure the pH of the saliva. Plaque index is measured on 16, 12, 24, 36, 32, and 44 with a periodontal probe according to Silness and Loe plaque index and oral hygiene scaled as excellent, good, fair, and poor.

Microbiological procedure: Culture media mitis salivarius agar, rogosa agar, and sabouraud dextrose agar are prepared for isolation of *Streptococcus mutans*, *Lactobacillus*, and *Candida albicans* respectively. To isolate *Streptococcus mutans*, 90 gm of mitis salivarius agar powder is dissolved in 1000 ml of distilled water, and to increase the selectivity of mitis salivarius agar 1% potassium tellurite is added to the prepared media. *Lactobacillus* anaerobic bacteria are cultured in Rogosa selective medium. For *Candida albicans*, 64 gm of sabouraud dextrose agar powder is dissolved in 1000 ml of distilled water, and to increase selectivity, chloramphenicol is added to the prepared media. Salivary samples are diluted 6 times with 0.9% normal saline (2.5 ml of normal saline added in 0.5 ml of saliva) and cultured on three different plates (mitis salivarius agar, rogosa agar, sabouraud dextrose agar) in a sterile environment. For *Streptococcus mutans* and *Candida albicans* plates are incubated at 37°C for 48 hours in an incubator, whereas for *Lactobacillus* plates are cultured in an anaerobic jar for the anaerobic condition at 37°C for 72 hours.

Same procedure is repeated in all the 60 subjects at the period of eight weeks after orthodontic braces are fixed in their mouth and the same procedure is again replicated after sixteen weeks.

#### Statistical analysis:-

Data was analyzed using the statistical package SPSS.0. Inferential statistics to find out the difference between and within the group done by Repeated measures of ANOVA followed by Tucky's post hoc test. Independent t test was used for two groups comparison.

#### Results:-



Fig. 1 colonies of  
Streptococcus mutans



Fig. 2 colonies of  
Lactobacillus



Fig. 3 colonies of  
Candida Albicans

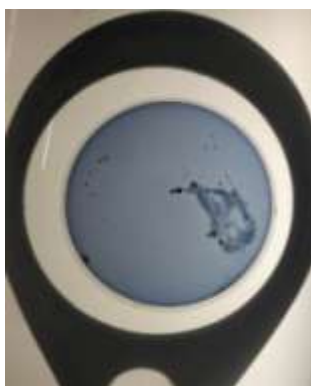


Fig. 4 counting of microbial colonies by manual method with  
magnification glass

**Table1:-** Mean of salivary flow rate, pH, plaque index, Streptococcus mutans, Lactobacillus, and Candida albicans before orthodontic treatment, 8 weeks and 16 weeks after commencement of orthodontic treatment.

	PRE	8WEEKS	16 WEEKS
<b>SALIVARYFLOW RATE</b>	<b>1.51±1.06</b>	<b>2.99±1.13</b>	<b>3.43±1.14</b>
<b>SALIVARY pH</b>	<b>6.46±0.85</b>	<b>6.17±0.80</b>	<b>5.94±0.77</b>
<b>PLA QUE INDEX</b>	<b>1.16±0.22</b>	<b>1.61±0.27</b>	<b>1.94±0.32</b>
<b>S. MUTANS</b>	<b>86.67±60.19</b>	<b>127.7±61.19</b>	<b>213.10±80.32</b>
<b>LACTOBACILLUS</b>	<b>363.38±782.75</b>	<b>7164.03±8073.75</b>	<b>15523.31±12099.75</b>
<b>C.ALBICANS</b>	<b>1.73±3.83</b>	<b>3.58±6.23</b>	<b>5.66±7.57</b>

**Table 2:-** Comparison of salivary flow rate before and after placement of orthodontic appliance .

SALIVARY FLOW RATE		PRE	8WEEKS	16 WEEKS
		1.51±1.08	2.99±1.13	3.43±1.12
P VALUE (REPEATED MEASURES ANOVA TEST)		0.0001*(F=66.37)		
P VALUE (TUKEY'S HSD TEST)	PRE vs 8 WEEKS	0.0001*		
	8 WEEKS vs 16 WEEKS	0.006*		
	PRE vs 16 WEEKS	0.0001*		

**Table 3:-** Comparison of pH of the saliva before and after placement of orthodontic appliance.

SALIVARY PH		PRE	8WEEKS	16 WEEKS
		6.46±0.85	6.17±0.80	5.94±0.77
P VALUE (REPEATED MEASURES ANOVA TEST)		0.03*(F=3.45)		
P VALUE (TUKEY'S HSD TEST)	PRE vs 8 WEEKS	0.09		
	8 WEEKS vs 16 WEEKS	0.23		
	PRE vs 16 WEEKS	0.008*		

**Table 4:-** Comparison of plaque index before and after placement of orthodontic appliance.

PLAQUE INDEX		PRE	8WEEKS	16 WEEKS
		1.16±0.22	1.61±0.27	1.94±0.32
P VALUE (REPEATED MEASURES ANOVA TEST)		0.0001*(F=56.54)		
P VALUE (TUKEY'S HSD TEST)	PRE vs 8 WEEKS	0.0001*		
	8 WEEKS vs 16 WEEKS	0.0001*		
	PRE vs 16 WEEKS	0.0001*		

**Table5:-** Comparison of Streptococcus mutans colony count in saliva before and after placement of orthodontic appliance.

STREPTOCOCCUS MUTANS		PRE	8WEEKS	16 WEEKS
		86.66±60.19	127.8±61.19	213.10±80.32
P VALUE (FRIEDMAN'S TEST)		0.0003*(F=53.28)		
P VALUE (TUKEY'S HSD TEST)	PRE vs 8 WEEKS	0.0001*		
	8 WEEKS vs 16 WEEKS	0.0001*		
	PRE vs 16 WEEKS	0.0001*		

**Table6:-** Comparison of Lactobacillus colony count in saliva before and after placement of orthodontic appliance.

LACTOBACILLUS		PRE	8WEEKS	16 WEEKS
		363.38±782.75	7164.03±8073.75	15523.31±12099.75
P VALUE (FRIEDMAN'S TEST)		0.0001*		
P VALUE (TUKEY'S HSD TEST)	PRE vs 8 WEEKS	0.0001*		
	8 WEEKS vs 16 WEEKS	0.0001*		
	PRE vs 16 WEEKS	0.0001*		

**Table7:-** Comparison of Candida albicans count in saliva before and after placement of orthodontic appliance.

CANDIDA ALBICANS		PRE	8WEEKS	16 WEEKS
		1.73±3.83	3.58±6.23	5.66±7.57
P VALUE (FRIEDMAN'S TEST)		0.019*		
P VALUE (TUKEY'S HSD TEST)	PRE vs 8 WEEKS	0.52		
	8 WEEKS vs 16 WEEKS	0.20		
	PRE vs 16 WEEKS	0.01*		

**Table 8:-** Gender variation - salivary flow rate, before and after placement of orthodontic appliance.

	PRE	8WEEKS	16 WEEKS	P VALUE	POSTHOC	
MALE	1.52±1.08	3.16±1.13	3.78±1.12	0.0001*	PRE vs 8 WEEKS	0.0001*
					8 WEEKS vs 16 WEEKS	0.08
					PRE vs 16 WEEKS	0.0001*
FEMALE	1.54±1.08	2.87±1.13	3.16±1.12	0.0001*	PRE vs 8 WEEKS	0.0001*
					8 WEEKS vs 16 WEEKS	0.13
					PRE vs 16 WEEKS	0.0001*
T TEST	0.92	0.26	0.03*			

**Table9:-** Gender variation - in pH of saliva before and after placement of orthodontic appliance.

	PRE	8WEEKS	16 WEEKS	P VALUE	POSTHOC	
MALE	6.59±0.37	6.25±0.32	6.03±0.29	0.0001*	PRE vs 8 WEEKS	0.04*
					8 WEEKS vs 16 WEEKS	0.02*
					PRE vs 16 WEEKS	0.0001*
FEMALE	6.54±0.31	6.30±0.25	6.05±0.25	0.0001*	PRE vs 8 WEEKS	0.01*
					8 WEEKS vs 16 WEEKS	0.01*
					PRE vs 16 WEEKS	0.0001*
T TEST	0.78	0.81	0.83			

**Table10:-** Gender variation - plaque index before and after placement of orthodontic appliance.

	PRE	8WEEKS	16 WEEKS	P VALUE	POSTHOC	
MALE	1.13±0.17	1.69±0.20	1.90±0.20	0.0001*	PRE vs 8 WEEKS	0.0001*
					8 WEEKS vs 16 WEEKS	0.0001*
					PRE vs 16 WEEKS	0.0001*
FEMALE	1.21±0.45	1.58±0.64	1.94±1.2	0.003*	PRE vs 8 WEEKS	0.18
					8 WEEKS vs 16 WEEKS	0.21
					PRE vs 16 WEEKS	0.002*
T TEST	0.09	0.23	0.86			

**Table 11:-** Gender variation - Streptococcus mutans colonies before and after placement of orthodontic appliance.

	PRE	8WEEKS	16 WEEKS	P VALUE	POSTHOC	
MALE	78.36±62.22	127.7±69.59	210.26±86.89	0.0001*	PRE vs 8 WEEKS	0.02*
					8 WEEKS vs 16 WEEKS	0.0001*
					PRE vs 16 WEEKS	0.0001*
FEMALE	95.96±58.64	129.9±52.71	219.1±72.18	0.0001*	PRE vs 8 WEEKS	0.0001*
					8 WEEKS vs 16 WEEKS	0.0001*
					PRE vs 16 WEEKS	0.0001*
T TEST	0.34	0.78	0.83			

**Table 12:-** Gender variation - Lactobacillus colony count before and after placement of orthodontic appliance.

	PRE	8WEEKS	16 WEEKS	P VALUE	POSTHOC	
MALE	312±711.60	7133.33±7373.02	16156.67±10264.95	0.0001*	PRE vs 8 WEEKS	0.02*
					8 WEEKS vs 16 WEEKS	0.002*

					PRE vs 16 WEEKS	0.0001*
FEMALE	392.76±867.36	6950±8962.95	14548±13965.34	0.0001*	PRE vs 8 WEEKS	0.01*
					8 WEEKS vs 16 WEEKS	0.03*
					PRE vs 16 WEEKS	0.0001*
T TEST	0.68	0.45	0.61			

**Table 13:-** Gender variation - Candida albicans count before and after placement of orthodontic appliance.

	PRE	8WEEKS	16 WEEKS	P VALUE	POSTHOC	
MALE	1.83±3.76	3.06±5.37	5±6.45	0.06	PRE vs 8 WEEKS	0.63
					8 WEEKS vs 16 WEEKS	0.32
					PRE vs 16 WEEKS	0.05
FEMALE	1.63±4.01	4.7±7.07	7.03±8.65	0.01*	PRE vs 8 WEEKS	0.19
					8 WEEKS vs 16 WEEKS	0.38
					PRE vs 16 WEEKS	0.008*
T TEST	0.77	0.31	0.30			

### Discussion:-

Orthodontic devices increase the surfaces of retentive plaque, decreasing the pH level and hydrogen ion concentration in the oral environment. However, physiological responses to protect oral health in challenging circumstances include increased secretion, which raises the concentration of bicarbonate ions, pH elevation, and drastically increased salivary buffering capacity<sup>5</sup>. Hagg et al. reported that fixed orthodontic appliances increase the oral plaque index and alter the oral bacterial population. The same author detected a significant increase in the quantity of Candida<sup>3</sup>. Based on certain studies, orthodontic devices may tend to encourage bacterial development by creating a more favourable environment for bacterial activity, bringing the oral environment at risk for imbalance and potential disease<sup>1</sup>. Streptococcus mutans and Lactobacillus are the most common caries producing and progressing bacteria increases during fixed orthodontic treatment.<sup>1</sup>

Positive variations in the salivary parameters, such as the flowrate of stimulated and unstimulated saliva, pH, and buffering capacity, may contribute to the maintenance of the oral balance<sup>15</sup>. Therefore, this study is conducted to evaluate changes in above mentioned factors during fixed orthodontic treatment. For this study salivary samples were taken from 60 patients, 30 males and 30 females ranging in age from 14-25 years, planning to undergo Orthodontic treatment. In the current study, salivary flow rate, pH, plaque index to evaluate oral hygiene and microbial count of Streptococcus mutans, Lactobacillus and Candida albicans were assessed at various time periods: pre-treatment, eight weeks and sixteen weeks after initiation of fixed orthodontic treatment. Obtained data were analysed using SPSS version 26.0.

Significant difference was observed in mean value of salivary flow rate in pre-treatment and after initiation of orthodontic treatment. Tukey's test shows highly statistically significant difference between all the three variables ( $p=0.0001$ ,  $0.006$  and  $0.0001$ ), showing incremental salivary flow rate successively. Above findings are in correspondence with the study by Anu et al.<sup>15</sup> and Yumi Lindawati et al.<sup>12,13</sup>. The study of Luca Lombardo et al.<sup>6</sup> and Lara-Carrillo et al.<sup>5</sup> also show increase in salivary flow rate, however this difference is statistically non-significant. Above findings suggest physiological response of the body to the fixed orthodontic appliances modifying oral environment thereby altering salivary composition.

Repeated measures of ANOVA test shows, significant decrease in pH of the saliva during fixed orthodontic treatment ( $p=0.03$ ). Tukey's post hoc test shows salivary pH decreased at eight weeks after commencement of fixed orthodontic appliances, but difference found was not statistically significant ( $p=0.09$ ). In the study conducted by Alshahrani et al.<sup>11</sup> statistically significant difference found at 8 weeks interval. This may be due to different method used to measure the pH of the saliva.

Plaque index increased gradually after commencement of fixed orthodontic treatment. These findings are in accordance with the study conducted by H. S. Chang et al.,<sup>2</sup> U. Hagg et al.<sup>3</sup> and Mummolo et al.<sup>7</sup> In which they

found significant increase in plaque index during fixed orthodontic treatment due increased retentive areas because of complex design of appliances.

Result shows significant increase in *Streptococcus mutans* colony count ( $p=0.0003$ ) during fixed orthodontic treatment. Analysis by Tukey's post hoc test indicate colony forming unit of *Streptococcus mutans* increases significantly after eight weeks ( $p=0.0001$ ), after sixteen weeks ( $p=0.0001$ ) of orthodontic treatment and even between eight weeks and sixteen weeks ( $P=0.0001$ ). This can be attributed to habits, more stagnant areas provided by fixed orthodontic appliances for plaque accumulation and lack of proper oral hygiene maintenance.

Result shows highly significant increase in *Lactobacillus* colony count. *Lactobacillus* count increased significantly at 8 weeks interval, from 8 weeks to 16 weeks and from pre-treatment to 16 weeks, analysed with Tukey's HSD post hoc test. These findings are in accordance with the study conducted by **Bloom et al.<sup>1</sup>**, **Mummolo et al.<sup>7</sup>**, **Marda et al.<sup>10</sup>** and **Ivanovic T. et al.<sup>14</sup>** who observed greatest increase in *Lactobacillus* colony count during fixed orthodontic treatment.

Result shows significant increase in *Candida albicans* colonies during study period. Tukey's HSD post hoc test reported statistically non-significant variation from pre-treatment to 8 weeks and from 8 weeks to 16 weeks. However, from pre-treatment to 16 weeks it shows significant difference in *Candida albicans* colony count. This result is in accordance with study conducted by **U. Hagg et al.<sup>3</sup>**, **K. Hibino et al.<sup>4</sup>**, **Arab et al.<sup>9</sup>** and **Khanpayeh et al.<sup>8</sup>** in which significant increase found in *Candida albicans* colonies after fixed orthodontic appliance therapy.

In comparison between male and female, salivary flow rate increased more in males as compared to females at 16 weeks of interval. This can be attributed to smaller salivary gland size in females as compared to males and hormonal pattern of females. In other parameters no significant difference found between male and female patient groups.

### Conclusion:-

The conclusions of this study are:

1. Salivary flow rate significantly increases after the placement of fixed orthodontic appliances.
2. The pH of the saliva decreases significantly after the insertion of fixed orthodontic appliances. This could be due to more retentive areas for plaque and acid production from microbial flora retaining in plaque.
3. Caries-producing and progressing bacteria, *Streptococcus mutans*, and *Lactobacillus* respectively also increased significantly after placement of fixed orthodontic appliances.
4. No any gender variation seen in salivary pH, plaque index, *Streptococcus mutans*, *Lactobacillus*, and *Candida albicans* colonies except salivary flow rate. The salivary flow rate increases more significantly in males as compared to females. This can be attributed to smaller salivary gland size and hormonal patterns in females.
5. With proper oral hygiene measures, plaque retention can be decreased after placement of orthodontic appliances reducing other complications like caries or periodontal damage.

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