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THE EFFECT OF FIXED ORTHODONTIC APPLIANCE THERAPY ON PERIODONTAL HEALTH STATUS AND SALIVARY CYTOKINE LEVELS

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ABSTRACT

BACKGROUND AND OBJECTIVE: Fixed orthodontic appliance therapy (FOAT) is universally recognized and most common method for treating malocclusion. Periodontal complications are frequent consequences of orthodontic therapy that need to be evaluated. The study aimed to evaluate clinical periodontal parameters and salivary cytokines levels among patients undergoing FOAT.

METHODS: 30 systemically healthy subjects (males and females), age ranged from 17-28 years with health periodontium and malalignment teeth attended Khanzad teaching center for FOAT. Clinical periodontal parameters (plaque index, gingival index, sulcus bleeding index, probing depth, debris index simplified, calculus index simplified & oral hygiene index simplified) and unstimulated saliva were assessed at baseline (0 day) before Fixed orthodontic appliance application and after one and three months of therapy. IL-1beta, IL-6, IL-8 and IL-10 levels were assessed in saliva by enzyme-linked immunosorbent assay (ELISA).

RESULTS: The results showed significant increase in the mean values of clinical periodontal parameters and salivary cytokines levels of IL-1beta, IL-6, IL-8 after one and three months of FOAT as compared to base line ($P < 0.05$), as well as significant decrease in the mean value of IL-10 level after one and three months as compared to baseline ($P < 0.05$). No significant correlations were detected between periodontal and immunological parameters.

CONCLUSION: Fixed orthodontic appliance therapy had a negative influence on periodontal health, since it promotes dental plaque accumulation & gingival inflammation. As well as FOAT led to significant increase of pro inflammatory and significant decrease of anti-inflammatory cytokines.

KEYWORD: Orthodontic appliances, Cytokines, Periodontal parameters, Periodontium

1. INTRODUCTION

Fixed orthodontic appliances therapy (FOAT) are one of the common kinds of orthodontic treatment nowadays , which are quite popular among people mainly due to a growing understanding of the importance of teeth arrangement as a support of aesthetic and its role on a person's psychological and career development (Anggraeni et al., 2011). Periodontal health is one of the important factors that can be used to assess the success of orthodontic therapy, and periodontal complications are one of the most prominent adverse effects associated with orthodontics including; gingivitis, periodontitis, gingival recession or hypertrophy, alveolar bone loss, dehiscence, fenestrations, interdental fold, and dark triangle (Dannan, 2010; Solomon, 2019). Presence of microbial plaque is reported to be the most important factor in the initiation, progression, and recurrence of periodontal disease in reduced periodontium (Genco & Borgnakke, 2013).

FOAT creates ‘new’ locations for plaque retention and increases the risk for gingival inflammation. Thus, the increased potential risk for periodontal diseases is among patients undergoing FOAT is of concern for clinicians (Diamanti-Kipioti et al., 1987; Pandis et al., 2010). As a result, oral hygiene precautions are advised since bands, brackets, ligature wires, and elastics promote the accumulation of microbial flora and food residues. In time, the plaque accumulation around orthodontic appliances may cause periodontal disease and caries (Dhami et al., 2013). An abundance accumulation of bacterial biofilms in the presence of fixed orthodontic appliances can cause substantial enamel demineralization, gingival inflammation, and an increase in probing depth (Levrini et al., 2013).

Generally, gingival conditions have been evaluated or observed by clinical examination and represented through several clinical parameters such as plaque Index and gingival Index (Hasegawa et al., 2003). Proinflammatory cytokines have recently received attention as biomarkers in periodontal health evaluations, with the ability to identify early risk of inflammation and periodontal diseases (Miller et al., 2006; Jaedicke et al., 2016).

Various types of orthodontic appliances apply important combined forces to the periodontium (Baeshen, 2021). Orthodontic forces have been found to be a physical agent capable of causing an inflammatory response in the periodontium (Tripuwabhrut et al., 2010). This reaction is necessary for orthodontic tooth movement (Crescini et al., 2007). Although enhanced proinflammatory cytokine production may be attributed to the excess plaque accumulation created by orthodontic appliances, these molecules can also be elevated by biomechanical forces. As a result, orthodontic forces combined with occlusal forces may exacerbate periodontal destruction by promoting periodontal inflammation (Nokhbehsaim et al., 2010).

Orthodontic appliances stimulate the release of certain cytokines, chemokines, and growth factors, which frequently affect the remodeling of periodontal tissues (Baeshen, 2021). Cytokines directly involved in the bone remodeling and inflammatory process during orthodontic tooth movement (OTM), which act directly or indirectly to facilitate bone and periodontal ligament (PDL) cells differentiation, activation, and apoptosis (Andrade et al., 2012). Investigations of their mechanisms of action have recognized their effector (pro-inflammatory) and suppressive (anti-inflammatory) functions during OTM (Al-Ghurabi et al., 2020).

Numerous studies have examined the relationship between fixed appliance and the development of periodontal disease, but only a few systematic reviews reported the results. Therefore, the present study aimed to evaluate clinical periodontal parameters, periodontal health status and salivary cytokines levels at

baseline before FOAT and after 1 and 3 months of treatment with the assessments of the correlation between periodontal parameters and salivary cytokines levels.

2. PATIENTS AND METHODS

2.1 Setting, design and time of the study

A clinical trial study was conducted in periodontics and orthodontics departments of Khanzad teaching center in Erbil city. The data was collected during the period 6th November 2021 to 20th May 2022. The study project was reviewed and approved by the institutional ethical committee (Ethical approval No:70 on 12th September 2021 / College of Dentistry/Hawler Medical University/Erbil/Iraq.

2.2 Subjects

Thirty subjects with healthy periodontium of both sexes, with an average age ranged from 17-28 years were participated in this study. At first subjects with malalignment teeth were attended to the orthodontics department for fixed appliance therapy, then they were recruited to the periodontics department to decide whether they were included in the study or not.

At baseline before fixed orthodontic appliance placements, un stimulated saliva and clinical periodontal parameters were assessed from all participants, then they received fixed orthodontic appliances on the labial (buccal) teeth surfaces (using the same orthodontic buccal tube) by a specialized orthodontist. Then all subjects were reevaluated after 1 and 3 months of therapy for clinical periodontal parameters measurements and salivary samples collection for laboratory estimation of pro and anti-inflammatory cytokines. All the subjects were instructed to brush their teeth at least 2 times daily for 2 minutes, using the same tooth brush (soft bristled orthodontic toothbrush, Wisdom UK), the same type of tooth paste (Colgate tooth paste with Fluoride, USA), the same technique (Bass method brushing technique), and the same interdental brushes (Wisdom UK), which was provided free of cost by the examiner before the treatment and emphasized throughout all planned visits.

The inclusion criteria for selected participants were: systemically healthy, age ranged 17-28 years old, with healthy periodontium (PD≤3, CAL=0, GI<0.6) (Li et al., 2020), and cooperative patients. While exclusion criteria were: smokers, alcoholism, pregnancy and lactation women, patients with systemic disease, previous orthodontic treatment, orthodontic band, extensive dental restorations, presence of fixed crown and bridge, presence of removable

appliance, orthodontic appliance on the lingual surface, periodontal disease or craniofacial anomalies, antibiotics using during or before bonding the brackets to the teeth, periodontal treatment 3 months prior to baseline examination. All subjects were received a verbal explanation about the study (objectives and procedures), and informed written consents were signed by all participants before conduction of the study.

2.3 Comprehensive patient assessment

Comprehensive personal history, medical history and dental history was performed as part of the overall treatment plan, along with a clinical periodontal examination to evaluate periodontal health status. All necessary information regarding each subject was recorded in questionnaire form, which was specially designed for this study including; subjects age, sex, mobile number, occupation, marital status, education level, working status, life style, previous professional dental visits within past year and the patient's oral hygiene habits; frequency of tooth brushing and duration of tooth brushing.

2.4 Clinical periodontal examination

All participants underwent a full mouth periodontal examination by a single trained examiner, using dental mirror, periodontal probe (Williams periodontal probe) and dental explorer. The thickness of plaque was measured according to plaque index (PI) by Silness & Löe, (1964), the extent and severity of gingival inflammation were measured according to the gingival index (GI) by Loe & Silness, (1963), sulcus bleeding upon gentle probing was assessed according to sulcus bleeding index (SBI) by Mühlemann & Son, (1971)**,** probing pocket depth PPD was measured in mm from gingival margin to the base of sulcus/or pocket, no pressure was used and the probe was allowed to fall by its own weight (Lindhe et al., 2008), measurement of oral hygiene health status according to oral hygiene index-simplified (OHI-S) by Greene & Vermillion, (1964). The amount of debris and calculus was measured according to the debris Index-simplified (DI-S) and calculus Index-simplified (CI-S). OHI-S was determined by summing DI-S and CI-S. The interpretation of OHI-S results was as follow: 0-1.2 good oral hygiene, 1.3-3.0 fair oral hygiene and 3.1-6.0 poor oral hygiene (Greene & Vermillion, 1964).

2.5 Saliva sample collection

Five ml of un stimulated saliva was collected from each patient between 9:00 a.m. and 11:00 a.m. according to the protocol described by Navazesh and Kumar in (2008). The subjects were asked to refrain from eating, drinking, smoking or brushing their teeth after mid night on the day of sampling (Navazesh & Kumar, 2008). Saliva samples were collected in plastic test tubes and centrifuged at 3000 rpm for 15 minutes. Then the clear supernatants were immediately aspirated and separated into four aliquots and finally stored at -20°C for subsequent analysis of pro and anti-inflammatory cytokines. Saliva samples were collected at base line before FOAT and after 1 and 3 months of therapy.

The concentration of IL-1beta, IL-6, IL-8 and IL-10 in saliva were assessed by commercially available enzyme-linked immunosorbent assays (ELISA), all assays were conducted according to the manufacturer's instructions (My BioSource, USA).

2.6 Statistical analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS, version 25). Chi square test of association was used to compare proportions. All the data were not normally distributed according to Shapiro-Wilk test. Accordingly, the Friedman test was used to compare the data at different time periods (three periods), and post-hoc test (Wilcoxon signed rank test) was used to compare medians between each two periods of time. Mann Whitney test was used to compare the mean ranks of the variables of two groups. Spearman rho correlation coefficient was calculated to assess the strength of correlation. A p value of ≤ 0.05 was considered as statistically significant.

3. RESULTS

Basic characteristics of the patients were summarized in Table 1, which showed that 30 participants were participated in the study, their mean age (SD) was 22.6 (3.4) years, the median was 21.5 years, and the age range was 17-28 years. The largest proportion (53.3%) of the sample were aged 20-24 years, more than half (60%) were females, and the majority (76.7%) were single. Regarding the educational level,

23.3% were college graduates, and only 10% were illiterate. Around half of the studied samples, brushed their teeth twice daily (53.3%), and (40.0%) one time and less, (6.7%) three times daily. Also 30% of selected participants spent less than two minutes for teeth brushing, (43.3%) spent two minutes, (16.7%) spent three-four minutes, and only (10%) spent five minutes for brushing. However no significant differences were detected between males and females in regard to the frequency of tooth brushing/day ($P = 0.439$), and the duration of tooth brushing ($p = 0.434$), as presented in Table 2.

Table 3 shows the comparison of clinical periodontal parameters at different times of the study, the result shows that there was significant increase in the mean values of PI, GI, SBI, PD, DI-S, CI-S, OHI-S after one (1M) and three months (3M) of FOAT as compared to baseline (0 day) ($p < 0.05$). In regard to two time periods almost all the differences between means values of clinical periodontal parameters were significant, except for the CI-S it was not significant at (0 D vs 1M).

Table 4 shows the comparison of oral hygiene status between males and females at different times of the study, the oral hygiene status of all the participants (males and females) was good at base line of the study (0 day), then after one month, one male and one female with fair oral hygiene was recorded. Then after 3 months of FOAT, some minor changes in the oral hygiene status were observed. Since the oral hygiene status of 6 males and 10 females were changed to fair. No significant differences were detected between males and females at one month ($p = 1.000$) and three months ($P = 0.765$).

Table 5 shows that the mean values of IL-1beta, IL-6, and IL-8 were significantly increased after one month (1M) and three months (3M) of FOAT as compared to baseline (0 day) ($p < 0.05$), in addition, the mean values were decreased after three months (3M) of therapy as compared to one month (1M). While the mean value of IL-10 was significantly decreased after one and three months of FOAT as compared to base line ($p < 0.05$). For the comparison between the three different times in regard to the mean values of pro and anti-inflammatory cytokines, all the differences were significant ($p < 0.001$). While, for the comparison between two times periods, the majority of the differences were significant, with the exception for the differences between 1 and 3 months in regard to IL-1beta, IL-8 and IL-10.

Regarding the correlation between clinical and immunological parameters at different times, spearman rho correlation coefficient was calculated to assess the strength of correlation between the immunological and clinical parameters. Table 6 shows that there was no significant correlation between the immunological and clinical parameters at different time periods of baseline (0 day), 1month(1M) and 3 months (3M) ($p > 0.05$).

Table (1): Basic characteristics of participants.

		No.	(%)
Age (years)	< 20 y	5	(16.7)
	20-24 y	16	(53.3)
	≥ 25 y	9	(30.0)
	Mean (SD)	22.6	(3.4)
Gender	Male	12	(40.0)
	Female	18	(60.0)
Marital status	Single	23	(76.7)
	Married	6	(20.0)
	Divorced	1	(3.3)
Educational level	Ungraduated	3	(10.0)
	Primary school	2	(6.7)
	Intermediate school	8	(26.7)
	High school	10	(33.3)
	College and above	7	(23.3)
Working status	Unemployed	19	(63.3)
	Employed	11	(36.7)
Tooth brushing frequency	1 time and less	12	(40.0)
	2 times	16	(53.3)
	3 times and more	2	(6.7)
Tooth brushing duration	< 2 minutes	9	(30.0)
	2 minutes	13	(43.3)
	3 to 4 minutes	5	(16.7)
	5 minutes	3	(10.0)

No: Number; %: percentage; SD: standard deviation

Table (2): Frequency and duration of tooth brushing by gender.

	Male	Female	Total	*P*-value
	No. (%)	No. (%)	No. (%)	
Frequency of Tooth brushing				
≤ 1 T	4 (33.3)	8 (44.4)	12 (40.0)	
2 T	8 (66.7)	8 (44.4)	16 (53.3)	
≥ 3 T	0 (0.0)	2 (11.1)	2 (6.7)	0.439*
Total	12 (100.0)	18(100.0)	30(100.0)	
Duration of Tooth brushing				
< 2m	3 (25.0)	6 (33.3)	9 (30.0)	
2m	7 (58.3)	6 (33.3)	13 (43.3)	
3-4m	2 (16.7)	3 (16.7)	5 (16.7)	
5m	0 (0.0)	3 (16.7)	3 (10.0)	0.434*
Total	12 (100.0)	18 (100.0)	30 (100.0)	

*By Fisher's exact test; No: number; %: percentage; *P*: probability; T: time; m: minute;
≤: less than and equal; ≥: more than and equal; <: less than.

Table (3): Clinical periodontal parameters at different time intervals of the study.

Indices	Time	Mean ± SD	Mean Rank	Min.	Max.	*P**	Groups	*P***
PI	0D	0.47 ± 0.20	1.00	0.15	0.83	<0.001	0 X 1M	< 0.001
	1M	1.34 ± 0.37	2.03	0.77	2.32		0 X 3M	< 0.001
	3M	1.77 ± 0.29	2.97	1.16	2.50		1M X 3M	< 0.001
GI	0D	0.49 ± 0.18	1.00	0.18	0.77	<0.001	0 X 1M	< 0.001
	1M	1.28 ± 0.27	2.00	0.67	1.76		0 X 3M	< 0.001
	3M	1.69 ± 0.19	3.00	1.25	2.09		1M X 3M	< 0.001
SBI (%)	0D	0.08 ± 0.04	1.00	0.00	0.20	<0.001	0 X 1M	< 0.001
	1M	0.90 ± 0.41	2.00	0.14	1.73		0 X 3M	< 0.001
	3M	1.49 ± 0.39	3.00	0.50	2.39		1M X 3M	< 0.001
PD (mm)	0D	1.84 ± 0.21	1.00	1.41	2.32	<0.001	0 X 1M	< 0.001
	1M	2.22 ± 0.27	2.00	1.88	2.80		0 X 3M	< 0.001
	3M	2.60 ± 0.30	3.00	2.10	3.44		1M X 3M	< 0.001
DI-S	0D	0.25 ± 0.28	1.05	0.00	0.83	<0.001	0 X 1M	< 0.001
	1M	0.74 ± 0.27	1.98	0.33	1.50		0 X 3M	< 0.001
	3M	1.11 ± 0.34	2.97	0.50	1.83		1M X 3M	0.000
CI-S	0D	0.07 ± 0.11	1.82	0.00	0.33	0.002	0 X 1M	0.141
	1M	0.08 ± 0.16	1.90	0.00	0.50		0 X 3M	0.014
	3M	0.17 ± 0.24	2.28	0.00	0.83		1M X 3M	0.024
OHI-S	0D	0.32 ± 0.32	1.05	0.00	1.00	<0.001	0 X 1M	< 0.001
	1M	0.82 ± 0.31	1.97	0.33	1.50		0 X 3M	< 0.001
	3M	1.28 ± 0.44	2.98	0.66	2.33		1M X 3M	< 0.001

SD: Standard deviation; Min: minimum; Max: maximum; *By Friedman test; **By Wilcoxon signed rank test; *P*: probability; PI: plaque index; GI: gingival index; SBI: sulcus bleeding index; PD: probing depth; OHI-S: oral hygiene index simplified; DI-S: debris index simplified; CI-S; calculus index simplified; 0D; 0day; 1M:1month; 3M: 3months; mm: millimeter; %: percentage.

Table (4): Oral hygiene status (OHI-S) by gender at different study periods.

OHS	Time	Male No. (%)	Female No. (%)	Total No. (%)	*P*-value
Good	0 day	12 (100.0)	18 (100.0)	30 (100.0)	NA
Fair		0 (0.0)	0 (0.0)	0 (0.0)	
Poor		0 (0.0)	0 (0.0)	0 (0.0)	
Total		12 (100.0)	18 (100.0)	30 (100.0)	
Good	1month	11 (91.7)	17 (94.4)	28 (93.3)	
Fair		1 (8.3)	1 (5.6)	2 (6.7)	1.000*
Poor		0 (0.0)	0 (0.0)	0 (0.0)	
Total		12 (100.0)	18 (100.0)	30 (100.0)	
Good	3months	6 (50.0)	8 (44.4)	14 (46.7)	
Fair		6 (50.0)	10 (55.6)	16 (53.3)	0.765**
Poor		0 (0.0)	0 (0.0)	0 (0.0)	
Total		12 (100.0)	18 (100.0)	30 (100.0)	

No: Number; %: percentage; *By Fisher's exact test; **By Chi square test. NA: Not applicable.

Table (5): Immunological parameters at different times of the study.

	Time	Mean ± SD	Mean Rank	Min.	Max.	*P**	Groups	*P***
IL-1β (pg/ml)	0D	8.00 ± 12.66	1.02	0.70	62.50		0 X 1M	< 0.001
	1M	33.41 ± 34.71	2.70	3.68	120.51	<0.001	0 X 3M	< 0.001
	3M	30.48 ± 32.13	2.28	1.54	110.51		1M X 3M	0.254
IL-6 (pg/ml)	0D	1.79 ± 1.30	1.00	0.01	6.10		0 X 1M	< 0.001
	1M	5.68 ± 3.12	2.70	1.44	12.09	<0.001	0 X 3M	< 0.001
	3M	4.45 ± 2.66	2.30	1.28	10.06		1M X 3M	0.026
IL-8 (pg/ml)	0D	13.02 ± 9.25	1.00	1.26	36.27		0 X 1M	< 0.001
	1M	29.06 ± 15.24	2.67	1.95	55.15	<0.001	0 X 3M	< 0.001
	3M	26.93 ± 15.31	2.33	5.86	59.15		1M X 3M	0.221
IL-10 (pg/ml)	0D	6.45 ± 7.67	2.60	1.56	33.19		0 X 1M	0.006
	1M	4.07 ± 3.26	1.90	0.88	15.93	<0.001	0 X 3M	< 0.001
	3M	3.93 ± 5.13	1.50	0.09	22.32		1M X 3M	0.056

SD: standard deviation; Min: minimum; Max: maximum; *****By Friedman test; **By Wilcoxon signed rank test; *P*: probability; IL-**1**β: interleukin-1beta; IL-6: interleukin-6. IL-8: interleukin-8; IL-10: interleukin-10; 0D; 0day; 1M:1month; 3M:3months; pg: picogram; ml: milliliter.

. **Table (6):** Correlation between clinical and immunological parameters.

		IL- 1 β		IL-6		IL-8		IL-10	
Indices	Time	rho	*P*	rho	*P*	rho	*P*	rho	*P*
PI	0 Day	-0.134	0.481	-0.004	0.982	0.252	0.180	0.064	0.736
GI		-0.266	0.155	0.051	0.790	-0.038	0.841	-0.015	0.938
SBI		-0.024	0.900	0.257	0.171	0.131	0.490	0.267	0.153
PD		0.190	0.315	0.075	0.692	-0.097	0.609	0.140	0.460
DI-S		0.303	0.103	0.193	0.306	.407*	0.026	0.239	0.203
CI-S		-0.367	0.046	-0.204	0.279	-0.201	0.286	-0.062	0.744
OHI-S		0.210	0.264	0.094	0.623	0.292	0.117	0.173	0.360
PI	1M	0.048	0.802	-0.073	0.701	0.217	0.248	0.067	0.723
GI		0.149	0.432	-0.010	0.960	-0.095	0.616	-0.093	0.625
SBI		0.147	0.438	-0.053	0.782	-0.171	0.368	-0.099	0.601
PD		-0.174	0.357	-0.331	0.074	-0.302	0.105	-0.197	0.296
DI-S		0.395	0.031	0.047	0.804	0.050	0.794	0.226	0.229
CI-S		-0.339	0.067	-0.133	0.483	-0.112	0.557	0.141	0.456
OHI-S		0.179	0.344	0.054	0.776	-0.005	0.979	0.218	0.247
PI	3M	0.123	0.519	-0.115	0.546	0.008	0.968	0.120	0.528
GI		-0.039	0.838	-0.146	0.442	-0.036	0.852	-0.264	0.159
SBI		0.083	0.664	-0.103	0.588	-0.024	0.901	-0.102	0.592
PD		0.193	0.307	0.072	0.705	-0.027	0.889	0.113	0.552
DI-S		0.167	0.379	0.030	0.874	0.140	0.462	-0.018	0.926
CI-S		-0.143	0.452	-0.167	0.377	-0.175	0.356	0.261	0.164
OHI-S		0.054	0.776	-0.076	0.689	0.022	0.910	0.102	0.590

rho: spearman rho correlation coefficient; *P*: probability; 0D: 0day; 1M:1month; 3M: 3 months; IL-**1**β: interleukin-1beta; IL-6: interleukin-6; IL-8: interleukin-8; IL-10: interleukin-10; PI: plaque index; GI: gingival index; SBI: sulcus bleeding index; PD: probing depth; OHI-S: oral hygiene index simplified; DI-S: debris index simplified; CI-S; calculus index simplified.

4. DISCUSSION

4.1. Descriptive characteristics

The present study revealed that 12 participants were males (40%) and 18 were females (60%) with an age ranged from 17 to 28 years and mean the age was 22 years. The age selection was based on the fact that most gingival inflammation & bleeding was observed to be increased at the pubertal age as a result of the hormonal changes that occurred during puberty (Boke et al., 2014). Also, the higher number of females with FOAT placement than males may be due to that fact the young females are more likely to have attractive smile and more seeking for cosmetics and beauty in general. In regard to educational level, the majority of the patients were single and at high school with some particular differences in working status, since 19 of them were unemployed and only 11 individuals were employed with different occupational categories. The lower employment rate may be also account for the fact that the majority of the subjects was in high school and mostly they depend on their parents.

The present study demonstrated that the majority of the selected participants, 16 (53.3%) individuals (8 male and 8 female) brushed their teeth twice daily, with no statistically significant difference between them. The high prevalence rate of tooth brushing among the subjects may be due to the early inclusion criteria for participant's selection with healthy periodontium (no gingivitis nor periodontitis) before conduction of the FOAT, and the majority of the subjects who participated in this study were motivated and educated. Studies reported that frequent tooth brushing on a regular basis could reduce plaque and gingivitis scores (Ainamo, 1971; Warren et al., 2001) . Regarding the duration of tooth brushing, the majority of the participants 13 (43.3%) (7 males and 6 females) of the total 30 subjects reported that their tooth brushing last for two minutes, with no statistically significant differences between them. Studies reported that the significant effect of tooth brushing on plaque removal was achieved after a minimum of 2 minutes (Gallagher et al., 2009; George & John, 2016).

4.2. Clinical periodontal parameters

The present study showed that clinical periodontal parameters: PI, GI, SBI, DI-S &OHI-S were significantly increased after 1 month of FOAT (with the exception for CI-S), reaching their maximum values at 3months as compared to base line. This may be due to the fact that fixed orthodontic appliance encourages dental plaque accumulation and gingival inflammation due to the plaque retentive properties of fixed orthodontic appliance, as previously reported by Ristic et al., (2007). Furthermore, the patient's inability to properly clean his or her teeth around fixed orthodontic devices encourages plaque accumulation, which eventually lead to gingival inflammation (Atassi & Awartani, 2010). These findings of present study are similar to a study, reported that clinical periodontal indices (PI, GI, GBI and PPD) started to increase after the fixed appliances placement, reaching maximum values at 3 months, then followed by a gradual decrease after this period. The authors concluded that fixed appliances might increase all periodontal parameters, but with no destructive effect, due to its transitory situations (Ristic et al., 2007). Our findings are also in the same line with a study conducted by Liu et al., (2011), investigated periodontal tissue alterations before, after 1 and 3 months of fixed appliance placement, and 6 months after the appliance removal. The study revealed a significant increase in PI and GI scores after the first 3 months of therapy. Nevertheless, it appeared that using orthodontic appliances had no long-term impact on periodontal health. Another study by Levrini et al., (2015), reported that after 3 months of fixed orthodontic appliance treatment, a significant increase in PI and bleeding on probing BOP were detected. The increase in PI was directly attributed to neglect oral hygiene procedures by the patients.

In regard to PD, some studies reported that orthodontic treatment leads to significant increase in probing depths (van Gastel, Teughels, et al., 2011; van Gastel, Quirynen, et al., 2011; Karkhanechi et al., 2012), these are corroborating with the present study. While in contrast to our results two studies, revealed no significant changes in PPD after the first 3 months of orthodontic therapy (Liu et al., 2011; Levrini et al., 2015). The significant increase in probing pocket depth after 1 and 3 months of FOAT placements, may be due to the gingival enlargements (pseudopockets) or deep probe penetration into weak connective tissues, as previously reported by van Gastel, Teughels, et al., (2011).

Therefore, the present study showed that FOAT showed a negative effect on periodontal health which is especially performed in patients who have difficulty to maintain appropriate oral hygiene. Therefore, professional oral hygiene combined with education and motivation for adequate plaque control during orthodontic treatment helps patients to avoid periodontal disease and achieve optimal periodontal health.

4.3. Periodontal health status

The results of the present study revealed that at baseline (0 day), oral hygiene status of all participants was good. Moreover, after 1 month of FOAT, only 2 (6.7%) individuals had fair oral hygiene, and the rest 28 (93.3%) participants had good oral hygiene. Whereas some minor changes with no statistically significant difference between males and females were recorded at the end of three month of FOAT. Since 16 (53.3%) individuals had fair oral hygiene and the rest remained with good oral hygiene. However, no subject presented with poor oral hygiene status throughout the entire duration of the study, this could be attributed to the standardization of oral instructions which were emphasized at each visit.

4.4. Immunological parameters

The results of the present study revealed that IL-1beta, IL-6 and IL-8 were significantly increased after FOAT reaching their maximum level at 1 month, followed by 3 months as

compared to baseline ($P<0.05$). These results indicating that the early orthodontic tooth movement leads to an acute-phase response in the periodontium, resulting in an increase production of IL-1 beta, IL-6 and IL-8, as previously reported by three studies (Başaran, Özer, Kaya, & Hamamci, 2006; Ren et al., 2007; Vujacic et al., 2016;). However, salivary levels of IL-1beta, IL-6 and IL-8 were slightly decreased after 3 months as compared to their levels after 1 month, with no statistically significant differences, with the exception of IL-6 ($P = 0.026$), This may be due to the reduction of inflammatory response, as demonstrated by significant down regulation of IL-6 in the saliva.

Our finding is corroborated with the study of Sampaio et al., (2019), reported that the levels of IL-1beta in gingival crevicular fluid were significantly highly increased only at 1 month after bonding brackets when compared to the baseline. They reported that the early stage of orthodontic tooth movement was characterized by acute inflammatory responses that resulted in the production of various inflammatory mediators such as IL-1beta, IL-6 and TNF- α. They also reported that light continuous force tended to sustain relatively high IL-1β levels for a longer duration of time. Regarding the long period (linear stages) of treatment our findings are in disagreement with a study of Başaran, Özer, Kaya, Kaplan, et al., (2006), reported that IL-1beta reached the highest level at 21 days then decreased after 3 months of orthodontic treatment but with no statistically significant differences as compared to base line.

It is well recognized that interleukin-6 (IL-6) is a key mediator of inflammation in chronic local inflammatory responses. It modulates the immune cell recruitment throughout the change from acute inflammation (neutrophil recruitment) to chronic inflammation (monocyte recruitment) (Gabay, 2006) . A study, reported that IL-2, IL-6, IL-8, initiate and maintain tooth movement by cyclic relations when mechanically stimulated. But cytokines have some synergistic actions on each other. This synergism avoids these mediators from increasing excessively (Başaran, Özer, Kaya, & Hamamci, 2006). The present result is in accordance with a study conducted by Gujar et al., (2019), on a comparison of levels of different cytokines among them (IL-6) in GCF of orthodontically treated patient. After 3 weeks of fixed labial appliance, they found that IL-6 level was significantly increased. In contrast to our findings, two studies reported that the levels of IL-6 were significantly increased only 24 hours after FOAT, but with no significant changes after 4 months of force application (Ren et al., 2007), and after 1 month of fixed orthodontic appliance (Sampaio et al., 2019).

IL-8 is a potent proinflammatory cytokine that has a key role in the recruitment and activation of neutrophils during inflammation and is important for regulation of alveolar bone resorption during tooth movement by acting early on the local inflammatory response (Fernandes et al., 2019). The results of the present study is compatible with the findings of Ren et al., (2007), reported that IL-8 level was significantly elevated after one month of orthodontic therapy. Following that, IL-8 levels returned to baseline, indicating that the inflammatory process had subsided. This is also in agreement with the study of Al-Ghurabi et al., (2014), reported a significant increase in the median salivary IL-8 levels after 2 and 4 weeks of orthodontic appliance placement when compared to baseline levels. The variable timing of IL-8 increasing might be attributed to the different treatment mechanisms (Ren et al., 2007). In contrast to our results the study of Sampaio et al., (2019), reported no significant change in IL-8 levels at any time during their investigation.

IL-10 has been describes as an anti-inflammatory cytokine, which potentially antagonize the inflammatory process, and resulting in suppression of inflammatory responses during orthodontic tooth movement (Alhashimi et al., 2000). The results of the present study showed that the mean salivary levels of IL-10 were significantly decreased after 1 and 3 months of FOAT as compared to baseline. With no statistically significant differences in the reduction of IL-10 level between 1and 3 months. This indicating that the presence of IL-10 could play a potential role in regulating the local inflammatory process during orthodontic tooth movement, as previously reported by a study (Karaduman et al., 2015). The relatively low levels of IL-10 may be indicated that the migration of regulatory T-cells which usually produce IL-10 could be impaired, therefore may be a factor in the enhanced activity of bone resorption (Moreira et al., 2008). A study, reported that the concentration of IL-10 declined over the study period (at baseline, 1 h, 24 h, 7 days, and 28 days), with no statistically significant change ($P>0.05$). They concluded that orthodontic forces cause changes in IL-10 levels during the initial stages of force application

(Karaduman et al., 2015). Similarly the study of Nunes et al., (2017), reported some fluctuations in the levels of IL-10 over time with no statistically significant difference.

Regarding the correlation between the immunological and clinical parameters. The findings of the current study revealed no significant correlation between the immunological and clinical parameters at different time periods of the study ($p > 0.05$). The associations between pro-inflammatory cytokines and gingival health status in orthodontic patients are still unknown, despite substantial research into how pro-inflammatory cytokines function as biomarkers in periodontal health evaluation in the general population (Chen et al., 2021).

The main limitations of the current study were as follows: small sample size, absence of long-term potential effect of fixed orthodontic appliance therapy on periodontal parameters after appliance removal, there is no unity across malocclusion types, absence of data in the early stages (hours) of orthodontic tooth movement to determine a specific sequence of cytokine appearance, therefore, these findings should be interpreted with caution.

5. CONCLUSION

The mean values of clinical periodontal parameters were significantly increased after 1 and 3 months of FOAT as compared to baseline. Indicated that FOAT had a negative effect on periodontal health. At the same time, significant increase in the mean levels of IL-1beta, IL-6 & IL-8, and a significant decrease in the mean levels of IL-10 were detected in saliva after 1 and 3 months of fixed appliance application as compared to baseline. In addition, no significant correlations were existed between salivary pro- and anti-inflammatory cytokines with the clinical periodontal parameters among patients undergoing FOAT after 1, and 3 months of treatment.

CONFLICTS OF INTEREST

The authors report no conflicts of interest.

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