Journal of Research in Medical and Dental Science
2022, Volume 10, Issue 12, Page No: 132-136

Available Online at: www.jrmds.in
eISSN No. 2347-2367: pISSN No. 2347-2545

Evaluation of Interleukin-35 role in Fixed Orthodontics Patients

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ABSTRACT

During orthodontic treatment, the immune system targets the teeth through inflammatory processes. Immunological alterations in the gingival crevicular fluid and saliva might occur following fixed orthodontic appliance bonding. Evaluation of Interleukin -35 levels in saliva during initial fixed orthodontic treatment for limited age groups at different time intervals. A total of eighty-eight samples were obtained from saliva (unstimulated) 2–3 mL was collected. The range of ages, from 14 to 35 years from Babylon City, Iraq was included in the case study. during the same time span of study specimen collection from November (2021) to April (2022). The samples were classified into two groups, including 68 patients (orthodontic) as well as a control group of 20 (non-orthodontic). The enzyme-linked immunosorbent assay was used to determine the IL-35 level in saliva. The mean ± SD of IL-35 in orthodontic (patients) and non-orthodontic (control group) is 13.47 ± 11.22 pg/mL. This value is high significant with less than 0.01 according to independent T-test. There was significant difference in interleukin-35 concentration in <1, 1-2, 2-4, and >5 month was (8 ± 1.41; 19.8 ± 7.9; 32.1 ± 10.3; 41 ± 12.2) pg/mL, comparing with it is concentration in control groups (5.9 ± 2.4) pg/mL. It is concluded that the level of IL-35 saliva in fixed orthodontic patients is higher than that of control group (non- orthodontic).

Key words: Interleukin-35, Fixed orthodontic, Immune

HOW TO CITE THIS ARTICLE: Asaad Kareem Al-nafaee, Rasha Jasim Alwarid, Kasem Ahmed Abeas, Evaluation of Interleukin-35 role in Fixed Orthodontics Patients, J Res Med Dent Sci, 2022, 10 (12): 132-136.

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Received: 25-Nov-2022, Manuscript No. JRMDS-22-78143;

Editor assigned: 28-Nov-2022, PreQC No. JRMDS-22-78143(PQ);

Reviewed: 13-Dec-2022, QC No. JRMDS-22-78143(Q);

Revised: 19-Dec-2022, Manuscript No. JRMDS-22-78143(R);

Published: 26-Dec-2022

INTRODUCTION

During orthodontic treatment, the immune system targets the teeth through inflammatory processes, Immunological alterations in the gingival crevicular fluid and saliva might occur following fixed orthodontics appliance bonding, in addition to clinical and microbiological characteristics. In reaction to the presence of germs, pathological situations release cytokines. cytokine levels in gingival crevicular fluid and saliva of orthodontic appliance patient revealed immune alterations [1]. Inflammatory cytokine levels in orthodontic patients may be altered not only by the presence of gingivitis caused by microbes but also by aseptic gingivitis. Previous research has shown that using orthodontics pressure to move teeth might cause sterile inflammatory reactions [2]. Assessing cytokines during orthodontic therapy is critical for determining their relationship and pathways for periodontal health in orthodontic patients [3]. Furthermore, while the significance of inflammatory cytokines in the host's inflammatory response to microbe challenges is well understood, there is a paucity of data on the link between inflammatory cytokines and oral microbe burden [4]. Interleukin -35 (IL-35) is a modern anti-inflammatory cytokine generated by regulatory T cells. IL-35 modulates immune processes and protects against illnesses such as asthma and rheumatoid arthritis. The role of IL-35 in gingivitis and periodontitis, on the other hand, is unknown [5]. Interleukin-35 (IL-35) is the IL-12 family's newest member. that is expressed in a variety of immune cells. This group of interleukines (12, 23, 27, and 35) is a unique collection of heterodimeric proteins with structural similarities but diverse activities [6]. Furthermore, IL-35 has been detected in periodontal tissues and gingival crevicular fluid (GCF) from several types of periodontitis [7,8], and some evidence shows that it plays a crucial role in the pathogenesis of periodontitis [9]. Additionally, iTr35 cells inhibit immune responses mediated by Thl, Thl7, and cytotoxic T-lymphocyte cells. All of these immune

cells have been shown to have a role in the development of periodontitis [10]. A mechanical force causes an aseptic inflammatory reaction in periodontal tissues, followed by coordinated process of bone resorption and placement in the orthodontic movement (OTM), which critically summarizes existing understanding of the immunological systems implicated in OTM inflammation [11]. that most research concentrated on the acute inflammatory phase that initiates alveolar bone resorption. However, the precise processes and immunological response involved in the subsequent OTM stage are unknown. Recent research indicates the presence of natural innate responses of inhabitant and extravagated immune cells, such as granulocytes and NK, dendritic, and γδt cells (Gamma delta T cells) [12]. In addition to microbiological changes, immune system alterations may cause an inflammatory reaction in the tissue and a rise in inflammatory cytokines such as tumor necrosis factor and interleukins (IL-1, IL-1, & IL-6). During an inflammatory reaction, these cytokines and other chemical mediators may tear down collagen through matrix metalloproteinase, resulting in attachment loss and rapid disease development [13].

MATERIALS AND METHODS

This study was conducted at a private orthodontic clinic. Eighty-eight individuals, the range aged from 14 to 35 years from Babylon city, Iraq were included in the case study. During the same time span of study specimen collection from November (2021) to April (2022). Patients gave their consent to take part in the research. Systemic disease patient, autoimmune disease, pregnant women, and smokers to sample collection were also excluded from the study. The sample were classified into two groups include 68 patients (19 male and 49 female). Who underwent fixed orthodontics during the initial stage of treatment. While 20 healthy individuals who were not receiving orthodontic treatment (control group; 10 female and 10 male) that having the same age range.

Sample collection

A saliva sample of 2–3 mL was collected. The patient under study is advised to avoid food or drink for two hours prior to sample collection. Then they asked each subject to rinse with distilled water once before collecting specimens The subjects stayed sitting with their heads angled forward during the sample collection. The operation was carried out in a calm, well-ventilated environment. After clinical evaluations, saliva samples (unstimulated) were taken from subjects using a draining procedure throughout a 5-minute interval in a sterile plastic vial for immunological analysis. The saliva sample was put on ice right away and transported to the lab. Then 10 minutes of centrifuging (Gemmy, Taiwan) at 1000 x g. Until salivary IL 35 levels were measured using an enzyme-linked immunosorbent assay, supernatants were kept at -80°C.

Measurement of IL-35 in saliva

This ELISA kit employs the Sandwich ELISA methodology. IL 35 saliva levels were determined by using the commercial ELISA kit (Elabscience, USA). The principle, procedures, and reagents preparation were prepared according to the manual instructions of the manufacturer's. In a summary, working standards and diluted serum were added to 96-well plates. Just after incubation with biotinantibody at 37 degrees Celsius and washing, the results are analyzed by using a microplate reader at 450 nm (ELISA reader; BioTek, USA) after administration of HRP-avidin as well as TMB substrate. The standard curve was plotted and the concentration was determined. By comparing the OD of the samples to the standard curve, IL-35 concentration in the samples may be calculated.

The statistical analysis

In order to conduct the statistical analysis for this study, IBM SPSS versions 25.0 were employed. the Data should be tested for normal distribution. e homogeneity and randomization were calculated. Additionally, Pearson's chi-square test was used to establish the significant differences for non-parametric data while, mean and standard deviation, T-test, table of ANOVA and Pearson correlation were used to determine the distinctions that are substantial for parametric data.

RESULTS

The distribution result of IL-35 among orthodontic patients and control groups (non-orthodontic) showed outcome prevalence 77.3% of patients and 22.7% of control with female gender (59) 67% and male (29) 33% the percentage refers to the female is more than male. The age group classified to three group (<20, 21-30, and >30 years) the age group < 20 years a high percentage 55.7% from other group as shown in Table 1. The mean ± SD of IL-35 in orthodontic (patients) and non-orthodontic (control group) is 13.47 ± 11.22 pg/mL. This value is high significant with less than 0.01 according to independent T-test as shown in Table 2.

The correlation between the change of concentration of IL-35 and period time of < 1, 1-2, 2-4, and >5 month in fixed orthodontic patients comparing with it is concentration control group as shown in Table 3. The patients one month period time we found mean ± SD (8 ± 1.41). Compared to (5.9 ± 2.4) of control. In addition we found that the mean ± SD increased with time Table 3. The result also show that the P.value of 0.00 of 1-2 month and 2-4 month indicates high significant of those two period time compared to others.

DISCUSSION

The specificity of cytokines was governed by a glycoprotein called IL-35, which was encoded by the p35gene. These glycoprotein is identical to IL-6 and granulocyte colony stimulating factor. The gene EBI3 (Epstein-Barr viral stimulated gene 3) codes for a glycoprotein with a comparative molecular mass of 34,000. Twenty-seven percent of the amino acid sequences in this glycoprotein

are identical to the components of the hematopoietic cytokine receptor superfamily, IL-12 and p40 [14]. The results of the study show that Mean ± S.D of salivary IL-35 value for patients group and controls are (13.47 ± 11.22) pg/mL according to independent T-test which have a different with a significant difference as it is shown in Table 2 and Figure 1.

Ye, et al. [15] reported in his study the number of severe obstacles a comprehensive analysis of this important subject has not been possible. For example, the production of pure IL-35 that is untagged is still a substantial issue that hinders functional analysis. Another significant issue that has not been satisfactorily addressed is the question of how the stability of

Table 1: Distribution of interleukin -35 (IL-35) among orthodontic (Patients) and non-orthodontic (Control Group).

		IL-35		P value
		No.	%	
Gender	Male	29	33.00%	0.58
	Female	59	67.00%	
Outcome	Patients	68	77.30%	0.25
	Control	20	22.70%	
Age group	<20	49	55.70%	0.73
	21-30	29	33.00%	
	>31	10	11.40%	

This table showed distribution of the patients 77.3% (68) and control was 22.7% (20),male 29 (33%) female 59 (67%), and age groups <20 year was 49 (55.7%), 21-30 year was 29 (33%) and >31year was 10 (11.4%).

Table 2: Statistical analysis of interleukin -35 (IL-35) among orthodontic (patients) and non-orthodontic (control group).

	Test Value = 88						
	t	df	Sig. (2-tailed)	Mean ± SD	Mean Difference	95% Confidence Interval of the Difference	
						Lower	Upper
IL35	64.687	87	0	13.47 ± 11.22	76.533	78.88	74.18

The results of the study show that the Mean ± S.D IL-35 value for patients group is (13.47 ± 11.22) which have high significant level < 0.01

Table 3: Correlation between IL-35 and period time.

Time	Patients	Contro1	P. value	Significant
	Mean ± S.D	Mean ± S.D		
<1 month	8 ± 1.41	5.9 ± 2.4	0.014	P *
1-2 month	19.8 ± 7.9		0	P **
2-4 month	32.1 ± 10.3		0	P **
>5 month	41 ± 12.2		0.01	P*
P < 0.01 **				
P >0.01 *				

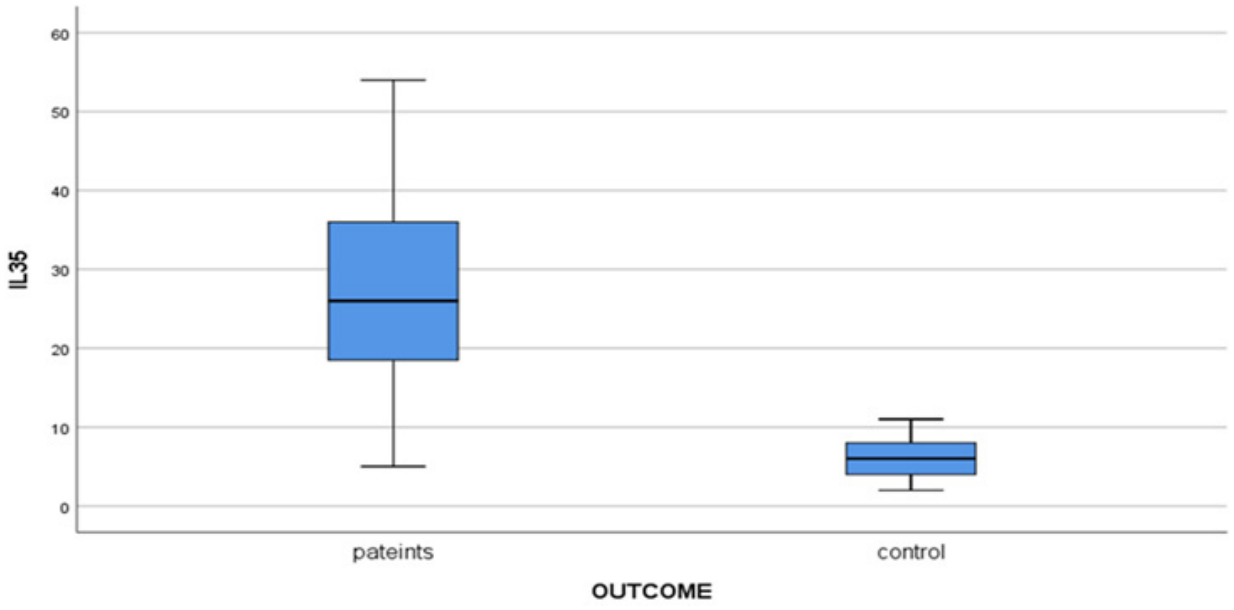

Figure 1: Concentration of interleukin -35 (IL-35) among orthodontic (patients) and non-orthodontic (control group).

heterodimers is maintained and controlled in living organisms. As our knowledge of the biology of IL-35 expands, it is possible that we may discover more unique roles of IL-35 in addition to its role in suppressing the proliferation of T-cells. It is unknown if IL-35 serves any additional roles other those described above. Köseoğlu, et al. [16] which indicated that the gingivitis group had significantly more IL-35 in the GCF than control groups (P<0.01), whereas the chronic periodontitis group had significantly more IL-35 (P. 0.04). There were significant variations between the groups in salivary IL-35 levels (P<0.001), with healthy group having the highest level overall. In this cross-sectional study, healthy people, gingivitis patients, and chronic periodontitis patients' gingival creviculiar fluid, salivary, and serum were examined for IL-35 levels. This is study measure IL-35 levels in periodontal fluid, salivary, and serum. Schmidli, et al. [5] who discovered that the presence of higher amounts of IL-35 in the salvia, gingival crevicular fluid, blood, and gingival biopsies of individuals with inflammatory periodontal disease. In addition, two included clinical studies demonstrated that nonsurgical periodontal treatment may reduce IL-35 production in individuals with chronic periodontitis. Interleukin-35 also has an indisputable function in the pathobiology of inflammatory periodontal illness. Further well controlled research is required to clarify the role of IL-35 in the pathogenesis of gingival and periodontal illness.

There were significant difference in anti-inflammatory in interleukin-35 concentration in <1, 1-2, 2-4, and >5 month was (8 ± 1.41; 19.8 ± 7.9; 32.1 ± 10.3; 41 ± 12.2) pg/mL, comparing with it is concentration in control groups (5.9 ± 2.4) pg/ml.

Interlieukin-35 a Forkhiead boxP3 (Foxp3)+ Tregcell immunosuppressive\anti-inflammatory cytokines, required for Treg cells to have the highest regulatory activity, [17]. In order to prevent periodontitis, IL-35 which acts as a Treg-specific inhibitor of inflammatory cytokines may maintain the equilibrium between bacterial infection and effector cells in patients with chronic periodontal disease. This is accomplished by rebuilding the immune system [18]. Maan, et al. [19] who discovered there were significant differences between orthodontic patients with respect to IL 1β and prostaglandin E2 results respectively at different time points.

CONCLUSION

In current study, we investigated immune system targets the teeth inflammatory during orthodontic treatment. We found that Interleukin-35 is anti-inflammatory cytokine observed to be increased in saliva sample of fixed orthodontic patients, reflecting sign of immune response. In addition, the level of interleukin-35 was significantly evaluation of patients in fixed orthodontic treatment at different time points.

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