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Estimating the salivary levels of IL-35 in smokers with periodontitis: A cross sectional study

P. Yuvashri *, R. Renuka Devi, H. Esther Nalini, P. Arun Kumar Prasad

Department of Periodontology, KSR Institute of Dental Science and Research, Tamil Nadu, India

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ABSTRACT

Background: A refined network and integrated host immune response to bacteria are formed by anti-inflammatory cytokines and the cells that they are associated to IL-35 has been recognized as having strong suppressive activity in chronic inflammatory diseases, together with IL-10 and TGF-β. It is believed that inflammatory diseases like periodontitis trigger the inducible Treg population to express IL-35, expanding regulatory responses by increasing infection.

Aim: The objective is to estimate and compare the salivary IL-35 levels in Periodontally healthy subjects, smokers and non-smokers with Periodontitis in order to educate on the consequences of periodontal health among the diseased patients.

Materials and Methods: Totally 42 subjects were included and they were categorized into Group 1 (n = 14) as Periodontally healthy subjects, Group 2 (n = 14) as systemically healthy non-Smokers with periodontitis and Group 3 (n = 14) as systemically healthy smokers with periodontitis. Each subject was assessed for clinical parameters such as Plaque index, Gingival index, Probing depth, clinical attachment. A polypropylene tube was used to collect unstimulated saliva and centrifuged it at 800 × g for 10 min. Supernatants were collected and stored at −80°C. A commercially available enzyme-linked immunosorbent assay kit was used to analyse levels of human salivary IL-35.

Results: The average age of the subjects in Group 1, Group 2 and Group 3 were 50.53, 52.93 and 52.07 years respectively. All three groups showed a statistically significant difference in clinical parameters including Plaque index, Gingival index, Probing depth and clinical attachment. The salivary IL-35 level was found to be elevated in non-smokers who have periodontitis compared to smokers with periodontitis and healthy individuals. Despite this, the salivary IL-35 levels were found to be statistically significant among three groups at $P < 0.001$.

Conclusion: The salivary levels of IL-35 were found to increase in Periodontitis patients with/without smoking, along with increased clinical parameters. IL-35 is considered a influential biomarker for periodontal disease.

1. Introduction

Periodontitis, an inflammatory disease, leads to inflammation of periodontal soft tissues, subsequently resulting in the breakdown of the periodontal ligament and alveolar bone. Multiple etiological and risk factors have been identified for the initiation and progression of periodontitis with the local microbiota and host immune response emerging as the most pivotal among them. Undoubtedly, cytokines play a crucial role in the progression of periodontitis. Cytokines serve as key modulators of both homeostasis and inflammatory processes, initiating the primary responses against pathogens and stimuli at barrier sites (Vignali et al., 2012).

Cytokines are classified based on structural similarity, gene homology and receptor sharing. Unfortunately, a complex network of cytokines is implicated in periodontitis. The classification of cytokines involved in periodontitis can be divided into three stages: 1) Pro-inflammatory Cytokines: This category includes well-established pro-inflammatory cytokines such as members of the IL-1 family, IL-6 family, TNF family and some recently identified members based on their specific functions. 2) Anti-Inflammatory Cytokines: These encompass cytokines associated with the inhibition of inflammation including Th1 cells, Th2 cells, Th17 cells and cytokines related to regulatory T (Treg)

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* Corresponding author at: 5/1033B new housing unit, Trichy road, singalanthapuram (po), Thuraiyur tk, Trichy 621014, India.
E-mail address: yuvashriragunath@gmail.com (P. Yuvashri).

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cells. 3) Additional Anti-Inflammatory Cytokines: In this stage, other - anti-inflammatory cytokines and their related cells form a complex and integrated host immune response network against bacteria (Dinarello et al., 2007).

Recent studies have reported that single nucleotide polymorphisms in cytokines and their associated receptor-encoding genes are associated with the risk and severity of periodontitis. Currently, numerous research endeavours are underway to investigate the role of cytokines in periodontal tissue with the aim of developing cytokine-targeted therapies for the treatment of periodontal disease (Jing et al., 2019). The IL-12 cytokine family is characterized as the central regulator of immunoregulation. Within this family, IL-12 and IL-23 are classified as pro-inflammatory and pro-stimulatory cytokines, while IL-27 and IL-35 are considered inhibitory cytokines. IL-12 was discovered and recognized for its central role in Th1 responses. Conversely, IL-32 cytokine is exclusively produced by regulatory T cell populations and has been identified as the most potent cytokine at highly inflamed sites, acting as a potent activator of natural Treg cells (Trinchieri et al., 2003).

In conjunction with IL-10 and TGF-β, IL-35 has been identified for its potent suppressive function in both in-vitro and in-vivo studies. Furthermore, IL-35 has been found to expand regulatory responses by promoting the development of IL-35-expressing inducible - Treg populations (Olsonet et al., 2013). To date, only a limited number of studies have investigated the potential role of IL-35 in the aetiology of periodontal disease. Currently, only one study has assessed the levels of salivary IL-35 in subjects with periodontal health and disease. Saliva serves as a valuable diagnostic tool, easily collected and containing a - substantial proportion of the gingival crevicular fluid found in periodontal pockets throughout the oral cavity.

Numerous salivary biomarkers have been suggested as diagnostic tests for periodontal disease. Therefore, saliva can serve as a valuable tool for detecting historical evidence of the disease, confirming current periodontal health and assessing a patient's risk for future periodontal deterioration. Consequently, the objective and focus of this study revolve around quantifying salivary levels of IL-35 in individuals with periodontitis, considering both smokers and non-smokers utilizing bio-immunological analysis. This research aims to enhance our understanding of the implications for oral health.

2. Materials and methods

This cross-sectional study included 42 subjects selected between 18 and 70 years in the Department of Periodontology, K.S.R Institute of Dental Science and Research, Tiruchengode, Namakkal district, Tamil Nadu. The study protocol was approved by the institutional ethical committee board. The study was explained to each patient and the written informed consent was obtained.

Group 1 (n = 14) - Systemically healthy individuals with healthy periodontium.

Group 2 (n = 14) - Systemically healthy individuals with periodontitis.

Group 3 (n = 14) – Systemically healthy individuals with periodontitis who are smokers.

2.1. Inclusion criteria

GROUP:1 (Periodontally Healthy Subjects) Bleeding on probing < 10%, Probing depth ≤ 3 mm, Clinical attachment loss ≤ 1 mm.

GROUP:2 (Non-Smokers with Periodontitis) Patients with Probing depth ≥ 4 mm, Clinical attachment loss ≥ 1 mm and no previous history of periodontal treatment.

GROUP:3 (Smokers with Periodontitis) Patients with Probing depth ≥ 4 mm, Clinical attachment loss ≥ 1 mm and no previous history of periodontal treatment.

- Smokers as per the CDC (Centre's for Disease Control and Prevention) criteria **(**Neuman and Carranza et al., 2019)

2.2. Exclusion criteria

- Individuals with history of systemic diseases, undergone periodontal treatment, undergoing medications like Anti-inflammatory drugs or immunosuppressants ≤ 6 months, Pregnant women and Lactating mothers.

Following clinical parameters were taken**:**

Plaque index – Loe's modification (Loe et al., 1967), Gingival index – Loe's modification (1967)**,** Probing pocket depth (PPD), Clinical attachment level (CAL).

All participants should avoid drinking or eating before 1 h of saliva sample collection. Participants were instructed to rinse the mouth completely with water and then expectorate 5 ml of unstimulated saliva into a polypropylene tube (Fig. 2.3). Salivary samples were immediately placed on ice for transportation to the laboratory. Samples were vortexed for 3 min, followed by centrifuging at 800 × g for 10 min (Fig. 2.4). Supernatants were collected and stored at −80∘C until additional measurement of salivary IL-35 levels was performed using enzyme-linked immunosorbent assay (Elabscience®) (Fig. 2.5).

2.3. Statistical analysis

All analyses were analysed using Statistical Package for Social Sciences [SPSS]. Descriptive analysis of all the explanatory and outcome parameters were done using mean and standard deviation for quantitative variables, frequency and proportions for categorical variables. Kruskal Wallis test followed by Mann Whitney Post hoc analysis was used to compare the mean values of clinical parameters and Sal. IL-35 levels between 3 study groups. Spearman's correlation test was used to assess the relationship between clinical parameters and Salivary IL-35 levels in each study group. The level of significance was set at $P < 0.05$.

3. Results

Table 4.1 presents descriptive statistics comparing clinical parameters among the groups. The mean plaque index (PI) score for Group 1 was 0.57 ± 0.22, for Group 2 it was 2.37 ± 0.35 and for Group 3, it was 1.53 ± 0.26. Multiple comparisons between the groups revealed that Group 1 exhibited a significantly lower mean PI score compared to both Group 2 and Group 3. Furthermore, Group 3 demonstrated a significantly lower mean PI score compared to Group 2.

These intergroup differences were statistically significant at $P < 0.001$. Regarding the mean gingival index (GI) score, Group 1 had a mean score of 0.71 ± 0.20, Group 2 had a mean score of 2.48 ± 0.33 and Group 3 had a mean score of 1.23 ± 0.22. Multiple comparisons among

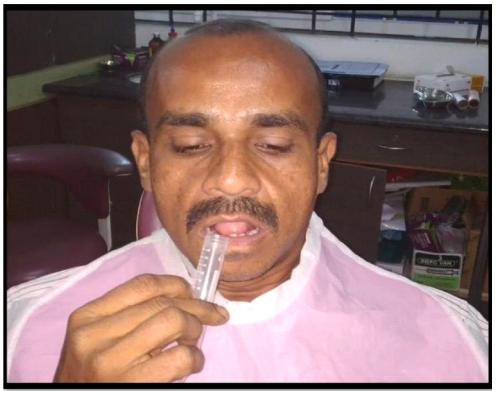

Fig. 2.3. Saliva sample collection.

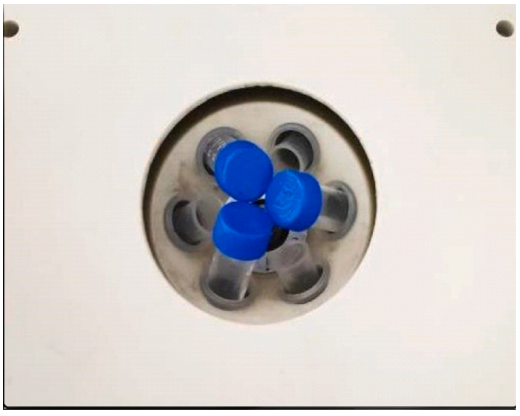

Fig. 2.4. Centrifugation of saliva samples.

Fig. 2.5. Human Salivary IL-35 ELISA Kit.

the groups showed that Group 1 had the lowest mean GI score, significantly lower than both Group 2 and Group 3. Additionally, Group 3 displayed a significantly lower mean GI score compared to Group 2. These differences between groups were statistically significant at $P < 0.001$.

In terms of the mean probing depth (PD) score, Group 1 had a mean score of 0.38 ± 0.17, Group 2 had a mean score of 3.51 ± 0.94 and Group 3 had a mean score of 2.31 ± 0.96. Multiple comparisons between the groups indicated that Group 1 had the lowest mean PD score, significantly lower than both Group 2 and Group 3. Moreover, Group 3 exhibited a significantly lower mean PD score compared to Group 2. These intergroup differences were statistically significant at $P < 0.001$.

The mean Clinical Attachment Loss (CAL) score for Group 1 was 0.38 ± 0.17, for Group 2 it was 4.08 ± 0.86 and for Group 2 it was 2.62 ± 0.79. Multiple comparisons between the groups revealed that Group 1 exhibited a significantly lower mean CAL score compared to both Group 2 and Group 3. Furthermore, Group 3 demonstrated a significantly lower mean CAL score compared to Group 2. These intergroup differences were statistically significant at $P < 0.001$. The comparison of these mean values of clinical parameters was performed using Kruskal-Walli's test followed by Mann-Whitney post hoc test, which showed a statistically significant difference among all three groups.

Table 4.2 displays the mean salivary IL-35 levels, with Group 1 having a mean score of 426.26 ± 71.36, Group 2 with 625.63 ± 68.52 and Group 3 with 535.92 ± 46.99.

Multiple comparisons between the groups indicated that Group 1 had a significantly lower mean salivary IL-35 score compared to both Group 2 and Group 3. Additionally, Group 3 displayed a significantly lower mean salivary IL-35 score compared to Group 2. These differences between groups were statistically significant at $P < 0.001$.

The test results revealed significant positive moderate correlations between salivary IL-35 levels and clinical parameters in Group 2. Specifically, there was a correlation coefficient (rho) of 0.57 with PI at $P = 0.03$, a strong positive correlation with GI (rho = 0.68) at $P = 0.006$, PD (rho = 0.76) at $P = 0.00$ and CAL (rho = 0.73) at $P = 0.001$. In Group 3, salivary IL-35 levels also exhibited significant positive moderate correlations with clinical parameters. There was a correlation coefficient of 0.52 with PI at $P = 0.04$, a strong positive correlation with GI (rho = 0.64) at $P = 0.01$, PD (rho = 0.68) at $P = 0.004$ and CAL (rho = 0.71) at $P = 0.003$. Conversely, Group 1 showed negative correlations between clinical parameters and salivary IL-35 levels.

4. Discussion

Numerous studies have confirmed that the persistent host inflammatory immune response against periodontal pathogens leads to the degradation of both soft and hard periodontal tissues (Cekici et al., 2014). Many of these studies have highlighted common patterns in the immune response, involving specific cytokines, which are generally detrimental in the context of tissue destruction. Interestingly, these same immune response patterns may also play significant roles in controlling periodontal infections (Sakaguchi et al., 2007). It is worth noting that the paradigm of protective and destructive immune responses is often discussed in the context of tissue damage and disease progression (Krauss et al., 2010). The concept of T cell suppressive activity was initially described by Gershon and Kondo in 1970 (Garlet et al., 2010). In line with this, inducible Treg35 (iTR35) cells have emerged as a novel and distinct subset of regulatory T cells, which exert their suppressive effects through the action of interleukin-35 (IL-35) (Collison et al., 2007).

Recent research efforts, particularly in the context of periodontitis, have emphasized the immunomodulatory role of IL-35 in various chronic inflammatory conditions (Kuo et al., 2011). In this context, IL-35 has been found to inhibit experimental colitis and mitigate collagen-induced arthritis in mice by suppressing Th17 cells. Specifically, IL-

Table 4.1
Comparison of mean values of different clinical parameters using Kruskal Wallis test followed by Mann Whitney's post hoc Test.

Parameter	Groups	N	Mean	SD	Min	Max	P-Value [a]	Sig. Diff	P-Value [b]
PI	Group 1	14	0.57	0.22	0.2	0.9	< 0.001*	G1 vs G2	< 0.001*
	Group 2	14	2.37	0.35	1.8	2.9		G1 vs G3	< 0.001*
	Group 3	14	1.53	0.26	1.0	1.9		G2 vs G3	< 0.001*
GI	Group 1	14	0.71	0.21	0.4	1.0	< 0.001*	G1 vs G2	< 0.001*
	Group 2	14	2.48	0.33	1.9	3.0		G1 vs G3	< 0.001*
	Group 3	14	1.23	0.25	0.9	1.8		G2 vs G3	< 0.001*
PD	Group 1	14	0.38	0.17	0.1	0.6	< 0.001*	G1 vs G2	< 0.001*
	Group 2	14	3.51	0.94	1.9	4.7		G1 vs G3	< 0.001*
	Group 3	14	2.31	0.96	0.9	3.8		G2 vs G3	< 0.001*
CAL	Group 1	14	0.38	0.17	0.1	0.6	< 0.001*	G1 vs G2	< 0.001*
	Group 2	14	4.08	0.86	2.5	5.1		G1 vs G3	< 0.001*
	Group 3	14	2.68	0.79	1.5	4.1		G2 vs G3	< 0.001*

* - Statistically Significant a - Kruskal Wallis test b - Mann Whitney Post hoc test.

Table 4.2
Comparison of mean Salivary IL-35 levels (in pg/ml) using Kruskal Wallis test followed by Mann Whitney's post hoc Test.

Parameter	Groups	N	Mean	SD	Min	Max	P-Value [a]	Sig. Diff	P-Value [b]
IL-35	**Group 1**	**14**	**426.26**	**71.36**	**307.6**	**519.8**	**< 0.001***	**G1 vs G2**	**< 0.001***
	Group 2	**14**	**625.63**	**68.52**	**518.0**	**812.7**		**G1 vs G3**	**< 0.001***
	Group 3	**14**	**535.92**	**46.99**	**477.2**	**621.0**		**G2 vs G3**	**0.001***

35 hinders the induction of Th17 cells and the production of IL-17, thus exerting a protective effect in Th17-related diseases. Consequently, IL-35 is emerging as a crucial factor in the regulation of periodontitis (Whirehead et al., 2012). However, the specific involvement of this cytokine IL-35 in the development of periodontal disease remains unexplored.

It is worth noting that despite limited research on this cytokine, several studies have measured the levels of IL-35 in GCF from patients with periodontitis. Accordingly, smoking has been identified as a significant risk factor for the prevalence and severity of periodontal tissue destruction. Smoking exerts various effects, including influencing vascular function, cellular and humoral immune responses, altering cell signalling, disrupting tissue homeostasis, and increasing cytokine production (Pan et al., 2019). In this study, we compared salivary IL-35 levels between smokers and non-smokers with periodontitis, employing enzyme-linked immunosorbent assay (ELISA) for the biochemical analysis of salivary IL-35.

In this study, a total of 42 subjects were enrolled and categorized into three groups: Group 1 (n = 14) comprising periodontally healthy individuals, Group 2 (n = 14) consisting of systemically healthy non-smokers with periodontitis and Group 3 (n = 14 comprising systemically healthy smokers with periodontitis. This categorization was based on their periodontal status and smoking habits. Clinical parameters, including Plaque index, Gingival index, Probing depth, clinical attachment level, and salivary levels of IL-35, were assessed for each subject. The mean age of individuals in Group 1, Group 2, and Group 3 was 50.53, 52.93, and 52.07 years, respectively.

Koseoglu et al., 2015 conducted a study evaluating IL-35 levels in various biological samples, including GCF, saliva, serum and periodontal tissues of patients affected by periodontitis. Their findings indicated that all clinical parameters and GCF IL-35 levels were significantly higher in the chronic periodontitis group compared to both the healthy and gingivitis groups. Additionally, a significant difference was observed among the groups in terms of salivary IL-35 levels ($P < 0.001$) with the highest levels detected in the healthy group and the lowest levels in the chronic periodontitis group. In the present study, Spearman's correlation test was employed to establish the relationship between clinical parameters and salivary IL-35 levels among the groups. Notably, a positive correlation was observed in non-smokers and smokers with periodontitis, who exhibited significantly higher mean PI, GI, PD, CAL and salivary IL-35 scores compared to the healthy group. It is worth mentioning that contrary to previous studies, salivary IL-35 levels were found to be the lowest in healthy group compared to the periodontitis subjects, although this difference between the groups remained statistically significant at $P < 0.001$(Table 4.1).

Jin et al. (2017) conducted a study examining the expression ofi nterleukin-35 (IL-35) in various biological samples, including gingival crevicular fluid (GCF), peripheral blood mononuclear cells, and periodontal tissues, among patients with chronic periodontitis and healthy individuals. Their findings underscored the increased levels of IL-35 in periodontitis subjects, highlighting its significant role in protective mechanisms against periodontal disease. Specifically, IL-35 appears to contribute to maintaining immune system homeostasis and mitigating the inflammatory response. In alignment with these findings, our present study also identified a statistically significant difference between healthy and periodontitis subjects. Notably, periodontitis patients exhibited higher mean salivary IL-35 levels (625.63 ± 68.52) compared to their healthy counterparts (426.26 ± 71.36), as confirmed through statistical analysis using the Kruskal-Walli's test followed by Mann-Whitney's post hoc test (Table 4.2).

In contrast, Koustubb et al., 2017 investigated IL-35 levels in the GCF of patients presenting with chronic gingivitis and periodontitis. Their results revealed elevated IL-35 levels in the chronic gingivitis group - when compared to the chronic periodontitis group. This observation suggests that IL-35 levels tend to decrease with increasing inflam matory status, implying a potential role in suppressing gingival inflammation and preserving periodontal health.

However, our study yielded contrasting findings, as we observed a decrease in IL-35 levels among smokers when compared to non-smokers. Although smoking and related products may induce fewer inflammatory signs in the gingiva, the lower salivary levels of IL-35 in smokers with periodontitis could potentially indicate a higher incidence of disease progression.

In a study conducted by Raj et al., 2018 interleukin-35 (IL-35) levels were estimated and compared in GCF and serum samples obtained from individuals classified as healthy, having gingivitis and suffering from chronic periodontitis (CP). Additionally, the study assessed the impact of non-surgical periodontal treatment (NSPT) on IL-35 levels in CP patients. Their findings revealed that individuals with periodontitis exhibited the highest concentrations of GCF and serum IL-35 among all groups studied. Furthermore, patients who underwent NSPT experienced a significant reduction in IL-35 levels. Our - study similarly identified a consistent association between IL and 35 concentration and the presence of periodontal inflammation (Table 4.3).

IL-35 is a relatively newly identified cytokine that has not been extensively investigated in various disease models, particularly in the context of smoking (Xue et al., 2018). Given the limited scope of parameters explored in the present study, it is conceivable that IL-35 may play a significant role in the pathogenesis of periodontitis. Interestingly, despite the reduced inflammation observed in smokers with periodontitis, our study revealed that salivary IL-35 levels significantly differed from those of non-smokers with periodontitis. Specifically, the highest mean salivary IL-35 score was observed in non-smokers with periodontitis, measuring 625.63 ± 68.52, followed by smokers with periodontitis at 535.92 ± 46.99 and finally, healthy individuals at 426.26 ± 71.36. This difference between the groups was statistically significant at $P < 0.001$. Furthermore, conducting clinical longitudinal follow-ups could help verify whether these cytokines indeed reflect the extent of periodontal inflammation and disease progression.

Table 4.3
Spearman's correlation test to establish the relationship between Clinical parameters and Salivary IL-35 levels in groups.

Group	Marker	Values	PI	GI	PD	CAL
Group 1	**IL-35**	**rho**	**0.36**	**−0.24**	**−0.05**	**−0.05**
		P-Value	**0.19**	**0.39**	**0.87**	**0.87**
Group 2	**IL-35**	**rho**	**0.57**	**0.68**	**0.76**	**0.73**
		P-Value	**0.03***	**0.006***	**0.001***	**0.001***
Group 3	**IL-35**	**rho**	**0.52**	**0.64**	**0.68**	**0.71**
		P-Value	**0.04***	**0.01***	**0.004***	**0.003***

'rho' - the correlation coefficients (-) Minus sign denotes negative correlation.

5. Conclusion

Based on the results of our study, the following conclusions can be drawn: The salivary IL-35 concentration observed in both the periodontitis groups, with and without smokers, exhibited statistical significance at $P = 0.001$. Comparative and correlation analyses indicate a positive association between increased salivary IL-35 levels and elevated levels of clinical parameters. Despite the limited number of studies on IL-35, it holds promise as a potential therapy for controlling alveolar bone resorption during periodontitis (Cafferata et al., 2020). Therefore, investigating the impact of IL-35 inoculations on the suppression of periodontitis besides, contributing to the immunomodulation of periodontal disease, is an intriguing avenue for further research.

In future, additional studies are anticipated to evaluate salivary IL-35 levels in larger populations of smokers and non-smokers. This could serve as a basis for exploring IL-35 as a therapeutic approach for managing periodontal disease, as well as other chronic inflammatory conditions that share similar pathogenesis associated with immunomodulatory imbalances. Such investigations could pave the way for novel therapeutic interventions in these conditions.

Declaration of Competing Interest

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

References

Cafferata, E.A., Terraza-Aguirre, C., Barrera, R., Faúndez, N., González, N., Rojas, C., Melgar-Rodríguez, S., Hernández, M., Carvajal, P., Cortez, C., González, F.E., Covarrubias, C., Vernal, R., Jun 2020. Interleukin-35 inhibits alveolar bone resorption by modulating the Th17/Treg imbalance during periodontitis. J. Clin. Periodontol. 47, 676–688.

Cekici, A., Kantarci, A., Hasturk, H., Van Dyke, T.E., 2014. Inflammatory and immune pathways in the pathogenesis of periodontal disease. Periodontology 2000 64, 80.

Collison, L.W., Workman, C.J., Kuo, T.T., Boyd, K., Wang, Y., Vignali, K.M., Cross, R., Sehy, D., Blumberg, R.S., Vignali, D.A., 2007. The inhibitory cytokine IL-35 contributes to regulatory T-cell function. Nature 22, 566–569.

Dinarello, C.A., et al., 2007. Historical insights into cytokines. Eur. J. Immunol. 37 (2007), S34–S45.

Garlet, G.P., et al., 2010. Destructive and protective roles of cytokines in periodontitis: a re-appraisal from host defense and tissue destruction viewpoints. J. Dent. Res. 89 (2010), 1349–1363.

Jin, Y., Liu, D., Lin, X., 2017. IL-35 may maintain homeostasis of the immune microenvironment in periodontitis. Exp. Ther. Med. 14, 5605–5610.

Jing, L., Kim, S., Sun, L., Wang, L., Mildner, E., Divaris, K., Jiao, Y., Offenbacher, S., 2019. IL-37-and IL-35/IL-37-producing plasma cells in chronic periodontitis. J. Dent. Res. 98, 813–821.

Kaustubh, T.S., Manohar, B.L., Charde, P., Jaiswal, P., 2017. Comparative evaluation of interleukin-35 levels in gingival crevicular fluid in patients with chronic gingivitis and chronic periodontitis. Int. J. Oral Care Res. 5, 83–86.

Koseoglu, S., Saglam, M., Pekbagrıyanık, T., Savran, L., Sutçu, R., 2015. Level of interleukin-35 in gingival crevicular fluid, saliva, and plasma in periodontal disease and health. J. Periodontol. 86, 964–971.

Kuo, J., Nardelli, D.T., Warner, T.F., Callister, S.M., Schell, R.F., 2011. Interleukin-35 enhances Lyme arthritis in Borrelia-vaccinated and -infected mice. Clin. Vaccine Immunol. 18, 1125–1132.

Loe, H., et al., 1967. The gingival index, the plaque index and the retention index systems. J. Periodontol. 38 (1967), 610–616.

B.M. Olson J.A. Sullivan W.J. Burlingham. Interleukin 35: a key mediator of suppression and the propagation of infectious tolerance. Front. Immunol. 18, 2013, 4:315.

Pan, W., Wang, Q., Chen, Q., 2019. The cytokine network involved in the host immune response to periodontitis. Int. J. Oral Sci. 5, 30.

Raj, S.C., Panda, S.M., Dash, M., Patnaik, K., Mohanty, D., Katti, N., Mahapatra, A., Mishra, D., Praharaj, K., 2018. Association of human interleukin-35 level in gingival crevicular fluid and serum in periodontal health, disease, and after nonsurgical therapy: A comparative study. Contemp. Clin. Dent. 9, 293–297.

Sakaguchi, S., Wing, K., Miyara, M., 2007. Regulatory T cells–a brief history and perspective. Eur. J. Immunol. 37, 116–123.

Trinchieri, G., et al., 2003. Interleukin-12 and the regulation of innate resistance and adaptive immunity. Nat. Rev. Immunol. 3 (2003), 133–146.

Vignali, D.A.A., et al., 2012. Mechanisms of T(reg) suppression: Still a long way to go. Front. Immunol. 5, 191.

Vignali, D.A.A., Kuchroo, V.K., 2012. IL-12 family cytokines: immunological playmakers. Nat. Immunol. 13, 722–728.

G.S. Whitehead R.H. Wilson K. Nakano L.H. Burch H. Nakano D.N. Cook. IL-35 production by inducible costimulator (ICOS)-positive regulatory T cells reverses established IL-17-dependent allergic airways disease. J. Allergy Clin. Immunol. 29, 2012, 207–215. e1-5.

Xue, W., Yan, D., Kan, Q., 2019. Interleukin-35 as an emerging player in tumor microenvironment. J. Cancer 10, 2074.