EGYPTIAN DENTAL JOURNAL
www.eda-egypt.org
VOL. 71, 1433:1440, APRIL, 2025
PRINT ISSN 0070-9484 • ONLINE ISSN 2090-2360
ORAL MEDICINE, X-RAY, ORAL BIOLOGY AND ORAL PATHOLOGY
Submit Date : 26-02-2025 • Accept Date : 31-03-2025 • Available online: 20-04-2025 • DOI : 10.21608/edj.2025.363185.3399

EVALUATION OF THE LEVELS OF IL-33 IN PERIODONTITIS VERSUS PERI-IMPLANTITIS PATIENTS

Ibrahim El Refai* *and* Mai Zakaria*

ABSTRACT

Aim: The following study was carried out to evaluate comparatively the levels of IL 33 in periodontitis patients and Peri-implantitis patients.

Methods: A total of 60 patients with age ranged between 30 – 55 years, were selected. Detailed dental and medical histories were recorded, intraoral examinations were executed. Radiographs were also taken to evaluate alveolar bone destruction alveolar bone loss was found confirming the clinical periodontitis and Perimplantitis diagnosis. The participants were allocated into two groups. Group I (Periodontitis group) Thirty patients diagnosed having periodontitis. Group II (Peri-implantitis group) Thirty patients diagnosed having peri-implantitis. Samples of GCF were obtained from the selected site of all subjects in both groups. Following the manufacturer's instructions, the obtained commercial enzyme-linked human immunosorbent Assay (ELISA) Kit (Elabscience, USA) was used. Statistical analysis was done using the t-tests and Pearson's correlation analysis.

Results: When comparing the overall IL-33 levels between the two groups regardless of sex, the peri-implantitis group showed slightly higher levels (60.45 ± 19.92) compared to the periodontitis group (58.65±17.90).

Conclusion: Concerning the current results of the study revealing elevated levels of GCF regarding periodontitis and peri-implantitis as disease entities. IL 33 can be used as a biomarker for identification and diagnosis of both diseases.

KEYWORDS: IL-33, Periodontitis,Peri-implantitis

INTRODUCTION

Periodontitis is considered as a widespread health issue and the intial cause for loss of tooth in adults ***(Eke et al. 2020).*** Periodontitis is defined as an inflammatory- infectious disease affecting the gingiva and the supporting structures of the teeth with an etiology and with pathogenesis that are multifactorial ***(Graves 2008).*** An imblanced host response or a disregulation within the microbiota community can produce a disrupted homeostasis related to the periodontium, causing loss of alveolar bone and extracellular matrix degradation ***(Darveau 2010).***

* Oral Medicine and Periodontology Department, Faculty of Dentistry, Cairo University, Cairo, Egypt.

The terminology of peri-implantitis was primarly presented in the year1987 and it was previously clarified as bone loss around dental implant progressively or as a biologic complication ***(Mombelli et al. 1987; Berglundh et al. 2002).***

Peri-implantitis as a disease entitiy is described as progressive pathological state characterized by an inflammatory reaction concerning the peri-implant mucosal tissue and the connective tissue and the loss of the alveolar bone surrounding the implant. The Clinical manifestations regarding peri-implantitis include bleeding on probing and/or pus formation also during probing, increased probing depth and/or mucosal margin recession, also radiographic loss of alveolar bone in comparison to previous findings. Diagnosis of peri-impantitis in absence of previous radiograph can be obtained from the following : bone loss more than 3 mm in the presence of bleeding upon probing and a pocket probing depth greater than 6 mm **(Lindhe and Meyle 2008; Renvert et al. 2018a; Schwarz et al. 2018).**

IL-33 has evolved as an alarm sign for injurious cell damage, activating the cells of immunity ***(Cayrol and Girard 2018).*** Regarding the receptor ST2 of the IL-1 receptor superfamily IL 33 serves as a ligand **(Schmitz et al. 2005).** The binding of IL 33 cause activtation of IL-1R AcP co-receptor, the adaptor protein MyD88, with the association of IRAK. The activation of ST2 cause the stimulation of the MAPK through TNF receptor-associated factor 6 (TRAF6), causing signaling to the activator protein 1(AP-1) through JNKs. TRAF6 allow activation of NF-ϰB and also transcription of proinflammatory gene **(Kakkar and Lee 2008).**

Several cell types express IL-33 these include epithelial cells, smooth muscle cells,macrophages and fibroblasts. Regarding expression of IL33 the major targets incorporated in ST2 stimulation include innate lymphoid cells group 2 , T regulatory cells and Macrophages ***(Cayrol and Girard 2018).*** Intially IL33 not only induce Th2-associated cytokines formation but also can activate NK cells ***(Cayrol and Girard 2018).***

Regarding samples of human gingiva, overproduction of Interlukin 33 was linked to periodontitis as a disease entity and stimulated osteoclastogenesis and the loss of alveolar bone through nuclear factor kappa-β ligand (RANKL) increased receptor activation ***(Laperine et al. 2016).*** IL-33 levels in saliva were higher in Periodontitis patients as well as Systemic lupus erythermatosis patients in comparison to healthy individuals ***(Mendonca et al. 2019).*** A study showed that higher salivary IL-33 levels could be linked to higher bacterial counts recognized in periodontitis patients and systemic lupus erythermatosis patients in comparison to the control group ***(Correa et al. 2017).***

Currently, the most commonly used tools for assessment of peri-implant disease include radiographic measures and clinical data such as bleeding upon probing, probing depth, pus formation and measuring the amount of bone loss and implant mobility ***(Duarte et al., 2016).***Although, it is not that easy that all of such diagnostic methods to be performed or it might not be sufficiently enough to differentiate between the peri-implant onset of disease, activity of the disease and progression of the disease; That's why there is a need of investigation efforts to recognize the different levels of different cytokines helping to reach more accurate diagnosis of the disease also activity and progression. Specifically few studies have been held to explore the significant role of interleukins in peri-implant diseases pathogenesis ***(Adli 2020).***

Aim of the study

The present study was performed to detect comparatively the levels of IL 33 in periodontitis and Peri-implantitis patients.

SUBJECTS AND METHODS

Subject Population

Study Population were selected from the attending patients in the outpatient clinic, Department of Oral Medicine, Periodontology and Oral Diagnosis, Faculty of Oral and Dental Medicine, Cairo and MTI Universities, Cairo, Egypt, between November 2023 and May 2024. Before being part of the study the participants in the study signed out a detailed informed consent and the protocol of the study has an approval from the ethical committee of the faculty of Oral and Dental Medicine Cairo university with reference numbered 64723. A total number of 60 patients with age ranging between 30 – 55 years, were selected. Full detailed medical and dental histories were recorded, intraoral examinations were performed. Radiographs were taken out to evaluate degree of alveolar bone destruction. alveolar bone loss was identified confirming the clinical periodontitis and Perimplantitis diagnosis.

Inclusion and Exclusion Criteria

The criteria included systemically healthy non smoker non diabetic patients, Patients aged > 30 years,absence of diseases or conditions that might have any interference with wound healing such as diabetes mellitus or coagulation disorders and absence of oral mucosal inflammatory conditions such as aphthous ulcers or lichen planus. Patient shouldn't have taken antibiotics within the last three months nor nonsteroidal anti-inflammatory drugs within the last three months also any medications known to interfere healing or periodontal tissue health such as anticonvulsants, calcium channel blockers, or immunosuppressant drugs. Pregnant and lactating mothers as well as Patients who have received periodontal surgery in the last 6 months were excluded.

Participants were splitted into two groups.

Group I (Periodontitis group) Thirty patients were diagnosed with stage IV periodontitis grade B having clinical attachment loss (CAL) ≥5 and probing depth (PD) ≥6 mm and bleeding upon probing.***(Papapanou et al. 2018)***

Group II (Peri-implantitis group) Thirty patients were diagnosed with peri-implantitis having the following: bleeding presence and/or pud formation upon gentle probing. Probing depths of ≥6 mm and Bone levels ≥3 mm apical of the most coronal part of the intraosseous part of the implant. ***(Berglundh et al. 2018)***

Collection of the Gingival Crevicular Fluid (GCF)

Different Samples of GCF were collected out regarding the buccal aspects of the sites which were selected. Criteria for the Selection of the sampling sites were probing depth ≥6mm. Cotton rolls were then used out inorder to isolate selected sites, gently rinsed with water; then air sprayed directly perpendicular to the margin of the gingiva ***(Griffiths 2003).*** Any Salivary contamination of the samples was avoided using a saliva ejector. Regarding supragingival plaque it was gently removed using a dry gauze, and a sterile filter paper strip (Periopaper®, Amityville, NY, USA) was gently incorporated into the entrance of the selected site until recognizing the first sign of resistance for 30 sec. The obtained strips were then placed into sterile Epindorf tubes GCF samples were all stored at -40C until performing laboratory analysis. GCF samples were kept at +4C overnight the day preceeding the analysis. According to the manufacturer's instructions, commercial human enzyme-linked immunosorbent Assay (ELISA) Kit (Elabscience, USA) was used.

SAMPLE SIZE CALCULATION

Sample size which was calculated depending upon a continuous response variable from unmatched pairs in previous study (***Buduneli 2012***). According to this study, matched pairs were normally distributed with standard deviation (68.353) and (55.803) for control and test groups

respectively. If the effect size of matched pairs was (0.8233439), we need to study (30) pairs of subjects to be able to reject the null hypothesis with power 0.87 and type I error probability, associated with this test of this null hypothesis is (0.05).

STATISTICAL ANALYSIS

The study employed two main statistical tests to analyze the data. First, independent t-tests were used to compare levels of IL 33 between periodontitis group and peri-implantitis group, with separate analyses conducted for male and female participants, as well as an overall comparison regardless of sex. The t-tests were appropriate for comparing these continuous variables between two independent groups, with the significance level set at $p < 0.05$. Second, Pearson's correlation analysis was utilized to examine the relationship between age and IL-33 levels in both groups. This test was suitable for assessing the linear relationship between these two continuous variables, providing correlation coefficients (r), confidence intervals, and R-squared values.

RESULTS

Table 1 presents the demographic features of participants in both the periodontitis and peri-implantitis groups, focusing on age and sex distribution. The mean age was similar between the groups, with periodontitis patients averaging 41.30 years and peri-implantitis patients slightly older at 43.20 years. Regarding gender distribution, the periodontitis group showed a higher proportion of males at 60% compared to females at 40%, while the peri-implantitis group demonstrated an even distribution with 50% males and 50% females. This demographic data suggests that while the groups were relatively well-matched in terms of age, there was a slight male predominance regarding the periodontitis group when compared to the balanced gender distribution in the peri-implantitis group.

TABLE (1) Descriptive statistics of age and sex of participants for both groups.

		Group			
		Peri-odontitis		Peri-implantitis	
		M	%	M	%
Age		41.30		43.20	
Sex	Male	18	60.0%	15	50.0%
	Female	12	40.0%	15	50.0%

M; Mean, %; Percentage

Table 2 presenting the results of an independent t-test analysis comparing IL-33 levels between periodontitis and peri-implantitis groups, with data stratified by sex. In male participants, the mean IL-33 levels were slightly higher in the periodontitis group (59.60±15.57) compared to the peri-implantitis group (58.32±18.84), though such difference wasnt statistically significant (p=0.7753). Conversely, among female participants, the peri-implantitis group showed higher mean IL-33 levels (62.58±22.95) compared to the periodontitis group (57.22±23.51), but again, this difference did not reach statistical significance (p=0.3752). When comparing the overall IL-33 levels between the two groups regardless of sex, the peri-implantitis group showed slightly higher levels (60.45±19.92) compared to the periodontitis group (58.65±17.90), though this difference was also not statistically significant (p = 0.7141). These results suggest that IL-33 levels do not significantly differ between

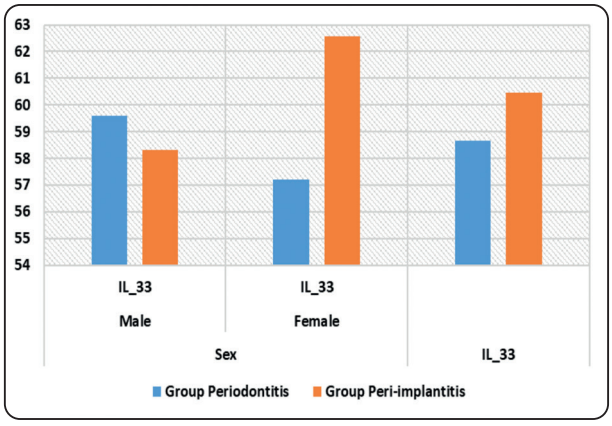


Fig. (1) Comparison of IL-33 Levels Between Periodontitis and Peri-implantitis Groups

periodontitis and peri-implantitis conditions, regardless of the patient's sex.

Table 3 presents the results of Pearson's correlation analysis examining the relationship between age and levels of IL-33 in both periodontitis group and peri-implantitis group. In the periodontitis group, there was a weak negative correlation between age and IL-33 levels (r = -0.2017, 95% CI: -0.5528 to 0.2102), with an R-squared value of 0.04069, indicating that only about 4% of the variance in IL-33 levels could be explained by age. This correlation was not statistically significant (p = 0.3336). In contrast, the peri-implantitis group revealed a moderate positive correlation regarding age and IL-33 levels (r = 0.5082, 95% CI: 0.1414 to 0.7523), though with a very low R-squared value of 0.0095. Interestingly, this correlation was marked as statistically significant (indicated by **) despite the low R-squared value. Both analyses were conducted with 30 pairs of observations. These findings suggest that while age and IL-33 levels show opposing directional relationships in periodontitis and peri-implantitis conditions, the clinical significance of these correlations may be limited given the low R-squared values.

TABLE (2) Independent t-test analysis of DCF IL 33 of participants for both groups.

			Group				P-value
			Peri-odontitis		Peri-implantitis		
			M	SD	M	SD	
Sex	Male	IL_33	59.60	15.57	58.32	18.84	0.7753 (NS)
	Female	IL_33	57.22	23.51	62.58	22.95	0.3752 (NS)
	IL_33		58.65	17.90	60.45	19.92	0.7141 (NS)

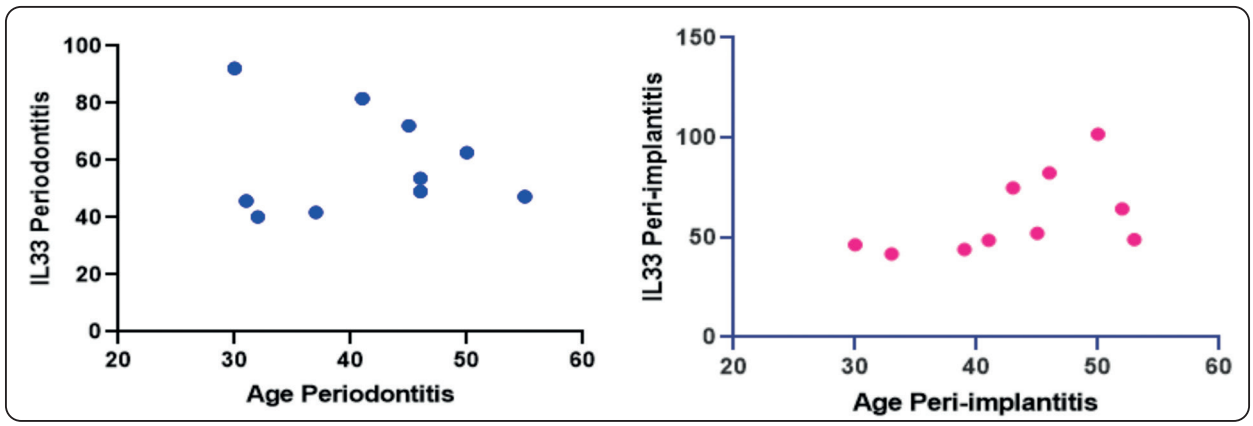
M; Mean, SD; Standard deviation, P; Probability level

(NS); Insignificant difference using Independent t-test

TABLE (3) Pearson's correlation analysis of DCF IL 33 of participants for both groups.

	Age Periodontitis vs. IL33 Peri-odontitis	Age Peri-implantitis vs. IL33 Peri-implantitis
r	-0.2017	0.5082
95% confidence interval	"-0.5528 to 0.2102"	"0.1414 to 0.7523"
R squared	0.04069	0.0095
P (two-tailed)	0.3336	**
Significant? (alpha = 0.05)	No	No
Number of XY Pairs	30	30

*Weak negative Pearson's correlation for periodontitis group Moderate positive Pearson's correlation for peri-implantitis group (NS); Insignificant difference **; Significant difference*



Fig, (2). Correlation Between Age and IL-33 Levels in Periodontitis and Peri-implantitis Groups

DISCUSSION

The present study was formulated to investigate the role of IL 33 as a sole biomarker in diagnosis and interpretation of the progression of both periodontitis and peri-implantitis. Where 60 patient were recruited in the study according to sample size calculation in accordance to ***Buduneli et al. 2012.***

The selection of the gingival crevicular fluid as a detector of cytokines is extensively used. Where **Lamster and Oshrain 1986** concluded that different gingival crevicular fluid parameters (Such as volume, the total count of cytokines and concentration of cytokines) are extensive across the periodontal literature, suggesting that such variations might indicate that progression of periodontal disease having an episodic nature with different inflammation stages and different disease severity**.**

The current study recognized an elevation in the level of IL 33 regarding both periodontitis and peri-implantitis clarifying a recognizable role in both disease entites. the peri-implantitis group showed slightly higher levels (60.45 ± 19.92) compared to the periodontitis group (58.65 ± 17.90), although this difference wasnt statistically significant (p=0.7141).

These findings of elevated IL 33 regarding periodontitis appears to be matching with **Habeeb and Al-Kaabi in 2021** whom identified a noticeable elevation in IL33 levels in the GCF. Several studies support **Habeeb and Al-Kaabi in 2021** findings such as **Sağlam et al.,2017** and **Tarrad et al., 2018**. In contradictory with **Papathanasiou et al. 2014** whom didn't recognize any elevated IL 33 levels in the GCF.

Severino et al. 2016 recognized elevated levels of IL 33 in the GCF in peri-implantitis patients matching the findings of the current study.

Heitz-Mayfield and Lang 2010 concluded that it is clear that periodontitis and peri-implantitis are similar regarding the perspectives of etiology, pathogenesis, diagnosis and therapy and also risk assessment. Identifying some difference in the host responses to these two disease entities explaining the occasional rapid rate of progression of peri-implantitis lesion Clarifing the elevated levels of IL 33 regarding perimiplantitis versus periodontitis in a non statistically significant pattern.

CONCLUSION

Concerning the current results of the study revealing elevated levels of GCF regarding periodontitis and peri-implantitis as disease entities. IL 33 can be used as a biomarker for identification and diagnosis of both diseases.

RECOMMENDATION

Further studies are recommended regarding IL 33 levels especially in peri-implantitis due to limited resource data.

ACKNOWLEDGMENT

The authors would like to deeply appreciate the role of Professor Dr. Mona Hamed Professor of Clinical, National Research Center, for her great efforts and her tremendous technical help.

List of abbreviations

IL 33 Interleukin 33

MYD88 Myeloid differentiation primary response 88

ST2 suppression of tumorigenicity 2

IRAK Interleukin-1 receptor-associated kinase

MAPK Mitogen-activated protein kinases

JNK Jun N-terminal kinase

NF-ϰB Nuclear factor kappa-light-chain-enhancer of activated B cells

REFERENCES

- Adli H 2020. Cytokines and Peri-Implant Disease. Khazar Journal of Science and Technology. Volume 4 №1 2020, 65-69 DOI: 10.5782/2520-6133.2020.4.1.65.
- Berglundh T, Persson L, Klinge B. 2002. A systematic review of the incidence of biological and technical complications in implant dentistry reported in prospective longitudinal studies of at least 5 years. Journal of clinical periodontology. 29 Suppl 3:197-212; discussion 232-193.
- Berglundh T, Armitage G, Araujo MG, Avila-Ortiz G, Blanco J, Camargo PM et al. Peri-implant diseases and conditions: Consensus report of workgroup 4 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. J Periodontol. 2018 Jun;89:S313-8.
- Buduneli N, Özcaka Ö, Nalbantsoy A. Interleukin-33 levels in gingival crevicular fluid, saliva, or plasma do not differentiate chronic periodontitis. J Periodontol. 2012;83(3):362-8. doi: 10.1902/jop.2011.110239.
- Cayrol C, Girard JP. 2018. Interleukin-33 (IL-33): a nuclear cytokine from the IL-1 family. Immunol Rev. 281(1):154-168.
- Correa JD, Calderaro DC, Ferreira GA, Mendonca SM, Fernandes GR, Xiao E,Teixeira AL, Leys EJ, Graves DT, Silva TA. 2017. Subgingival microbiota dysbiosis in systemic lupus erythematosus: association with periodontal status. Microbiome. 5(1):34.
- Darveau, R.P. (2010) Periodontitis: A Polymicrobial Disruption of Host Homeostasis. Nature Reviews Microbiology, 8, 481-490.
- Duarte, P., Serrão, C., Miranda, T., Zanatta, L., Bastos, M., & Faveri, M. (2016). Could cytokine levels in the peri-implant crevicular fluid be used to distinguish between healthy implants and implants with peri-implantitis? A systematic review. Journal of Periodontal Research, 51(6), 689-698.
- Eke PI, Borgnakke WS, Genco RJ. 2020. Recent epidemiologic trends in periodontitis in the USA. Periodontol 2000. 82(1):257-267.
- Graves D. 2008. Cytokines that promote periodontal tissue destruction. J Periodontol. 79 (8, Suppl):1585-1591.
- Griffiths, G.S. (2003). "Formation, collection and significance of gingival crevice fluid." Periodontol 2000 31: 32-42.
- Habeeb SAK, Al-Kaabi SJM. Interleukin-33 level in biological fluids for periodontitis patients in AL-Najaf City, Iraq. Int J Drug Deliv Technol. 2021;11(3):706–710.
- Heitz-Mayfield, Lisa & Lang, Niklaus. (2010). Comparative biology of chronic and aggressive periodontitis vs Peri-implantitis. Periodontology 2000. 53. 167-81. 10.1111/j.1600-0757.2010.00348.x.
- Kakkar R, Lee RT. 2008. The IL-33/ST2 pathway: therapeutic target and novel biomarker. Nat Rev Drug Discov. 7(10):827-840.
- Lamster, I.B., R.L. Oshrain, et al. (1986). "Enzyme activity in human gingival crevicular fluid: considerations in data reporting based on analysis of individual crevicular sites." JClin Periodontol 13(8): 799-804.
- Laperine O, Cloitre A, Caillon J, Huck O, Bugueno IM, Pilet P, Sourice S, Le Tilly E, Palmer G, Davideau JL, et al. 2016. Interleukin-33 and RANK-l interplay in the alveolar bone loss associated to periodontitis. PLoS ONE.11(12):e0168080.
- Lindhe J, Meyle J. 2008. Peri-implant diseases: Consensus report of the sixth european workshop on periodontology. Journal of clinical periodontology. 35(8 Suppl):282-285.
- Mendonca SMS, Correa JD, Souza AF, Travassos DV, Calderaro DC, Rocha NP, Vieira ELM, Teixeira AL, Ferreira GA, Silva TA. 2019. Immunological signatures in saliva of systemic lupus erythematosus patients: influence of periodontal condition. Clin Exp Rheumatol. 37(2):208-214.
- Mombelli A, van Oosten MA, Schurch E, Jr., Land NP. 1987. The microbiota associated with successful or failing osseointegrated titanium implants. Oral microbiology and immunology. 2(4):145-151.
- Papapanou PN, Sanz M,et al. Periodontitis: Consensus report of Workgroup2 of the 2017 World Workshop on the Classificationof Periodontal and Peri-Implant Diseases and Con-ditions. J Periodontol. 2018;89(Suppl 1):S173–S182
- Papathanasiou E, Teles F, Griffin T, Arguello E, Finkelman M, Hanley J, Theoharides TC. Gingival crevicular fluid levels of interferon-γ, but not interleukin-4 or -33 or thymic stromal lymphopoietin, are increased in inflamed sites in patients with periodontal disease. J Periodontal Res.2014;49(1):55–61.
- Renvert S, Persson GR, Pirih FQ, Camargo PM. 2018a. Peri-implant health, periimplant mucositis, and peri-implantitis: Case definitions and diagnostic considerations. Journal of clinical periodontology. 45 Suppl 20:S278-S285.

- Sağlam M, Koseoğlu S, Aral CA, Savran L, Pekbağrıyanık T, Cetinkaya A. Increased levels of interleukin-33 in gingival crevicular fluids of patients with chronic periodontitis. Odontology. 2017;105(2):184–90.
- Severino VO, Beghini M, de Araujo MF, et al. Expression of IL-6, IL10, IL-17 and IL-33 in the peri-implant crevicular fluid of patients with peri-implant mucositis and peri-implantitis. Arch Oral Biol. 2016;72:194-199.
- Schmitz J, Owyang A, Oldham E, Song Y, Murphy E, McClanahan TK Zurawski G, Moshrefi M, Qin J, Li X, et al. 2005. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. Immunity. 23(5):479-490.
- Schwarz F, Derks J, Monje A, Wang HL. 2018. Peri-implantitis. Journal of clinical periodontology. 45 Suppl 20:S246-s266.
- Tarrad N, Abdelkawy M, Shaker O. Interleukin-33 and osteoprotegerin levels in gingival crevicular fluid and saliva in chronic periodontitis and their correlation to diabetes mellitus: a cross-sectional study. Perio J.2018;2(1):1–9.