Ministry of Higher Education
and Scientific Research
Babylon University
Faculty of Dentistry
2021-2022

وزارة التعليم العالي والبحث العلمي
جامعة بابل
كلية طب الاسنان
2021-2022

Association of IL- 17 A with Clinical Periodontal Status

By:

GhofranHaider
Zaianb Ammer
Zahraa Salih,
Hawraa Yassien

supervisor:

Assist.Profa.Dr. Zainb Hadi
lecturer.Dr. Sura Dakhil

ABSTRACT

Periodontitis is characterized by microbial-associated, host mediated inflammation that results in loss of periodontal attachment. Using saliva as a diagnosis body fluid for monitoring various biological alternations in human is attracting many researchers worldwide. Human saliva is an easily accessible biochemical fluid, which is similar to blood in various biological aspects. Besides, it associated with simple and non invasive collection procedures , low-cost storage and easily storage nature. The interleukin-17 family consists of six family members (interleukin-17A- interleukin-17F) and all the corresponding receptors have been identified recently. This family is mainly involved in the host defense mechanisms against bacteria, fungi and helminth infection by inducing cytokines and chemokines, recruiting neutrophils, inducing anti-microbial proteins and modifying T-helper cell differentiation. The aim of the present study was to compare the levels of interleukin-17 a in periodontitis patients with periodontally healthy subjects. One milliliter of salivary samples and full-mouth clinical periodontal recordings (plaque index, bleeding on probing , probing pocket depth and clinical attachment loss) were obtained from thirty eight subjects (fourteen male and twenty four female). Groups of present study include 30 patients with periodontitis (group 1) and 8 periodontaly healthy subjects (groupe 2) with average age range of 19-60 .The participants must have good general health with no history of systemic diseases or smoking , no systemic antibiotic therapy and/or periodontal therapy including scaling or root planning within the last 3 months. After the patient has been selected, and before the base line examination, salivary samples were collected from periodontitis and healthy groups to be examined about Interleukin-17 a. All clinical periodontal parameters showed higher levels in periodontitis group as compared with healthy group. As well as interleukin-17 A levels were (80.26) and (36.01) in periodontitis and healthy groups respectively. As a conclusion interleukin-17 A increased in periodontitis patients .

Key words : periodontitis, interleukin-17 A , salivary samples, clinical periodontal parameters

INTRODUCTION

Periodontitis is characterized by microbial-associated, host mediated inflammation that results in loss of periodontal attachment. The pathophysiology of the disease has been characterized in its key molecular pathways, and ultimately leads to activation of host-derived proteinases that enable loss of marginal periodontal ligament fibers, apical migration of the junctional epithelium, and allows apical spread of the bacterial biofilm along the root surface. The bacterial biofilm formation initiates gingival inflammation; however, periodontitis initiation and progression depend on dysbiotic ecological changes in the microbiome in response to nutrients from gingival inflammatory and tissue breakdown products that enrich some species and antibacterial mechanisms that attempt to contain the microbial challenge within the gingival sulcus area once inflammation has initiated.[(1)]

Current evidence supports multifactorial disease influences, such as smoking, on multiple immune-inflammatory responses that make the dysbiotic microbiome changes more likely for some patients than others and likely influence severity of disease for such individuals. Marginal alveolar bone loss – a key secondary feature of periodontitis – is coupled with loss of attachment by inflammatory mediators. Clinical presentation differs based on age of patient and lesion number, distribution, severity, and location within the dental arch. The level of oral biofilm contamination of the dentition also influences the clinical presentation. In recent decades, attempts to classify periodontitis have centred on a dilemma represented by whether phenotypically different case presentations represent different diseases or just variations of a single disease. Lack of ability to resolve the issue is illustrated in the changes to the classification system that progressively emphasized either differences or commonalities.[(2)]

New periodontitis classification scheme has been adopted, in which forms of the disease previously recognized as “chronic” or “aggressive” are now grouped under a single category (“periodontitis”) and are further characterized based on a multidimensional staging and grading system. Staging is largely dependent upon the severity of disease at presentation as well as on the complexity of disease management.[3]

Periodontitis could be classified into four stages: [3]

- ✓ Stage I periodontitis: Stage I periodontitis is the borderland between gingivitis and periodontitis and represents the early stages of attachment loss.

- ✓ Stage II periodontitis: The incision of this stage associated with clinical attachment loss about 3- 4mm and radiographic bone loss more than 15% and less than 33% in the coronal third, also no tooth loss due to periodontitis associated with this stage.

- ✓ Stage III periodontitis: This stage associated with clinical attachment loss ≥5mm and radiographic bone loss extending to the middle third of the roots, furthermore, tooth loss due to periodontitis ≤ 4 teeth at this stage.

- ✓ Stage IV periodontitis (Advanced): This stage is characterized by the presence of deep periodontal lesions that extend to the apical portion of the root and/or history of multiple tooth loss. while grading provides supplemental information about biological features of the disease including a history-based analysis of the rate of periodontitis progression; assessment of the risk for further progression; analysis of possible poor outcomes of treatment; and assessment of the risk that the disease or its treatment may negatively affect the general health of the patient.

Grades of periodontitis include : [4,5,6]

- ✓ Grade A periodontitis: it presents if the maximum amount of radiographic bone loss in percentage terms is less than half the patient's age in years (for example, less than 40% in an 80-year-old or less than 20% in an 40-year-old).

- ✓ Grade C periodontitis: it presents if the maximum amount of bone loss in percentage terms exceeds the patient's age in years (for example, more than 30% in a 25-year-old or more than 40% in a 39-year-old).

- ✓ Grade B periodontitis: all other situations Grades can be calculated exactly depending on the following formula: If the result of the equation < 0.5 this mean that the patient has grade A (Slow rate of progression) , if the result = (0.5-1.0) this mean that the patient has Grade B (Moderate rate of progression) and if the result > this mean that the patient has Grade C (Rapid rate of progression) According to the latest. Based on pathophysiology, three clearly different forms of periodontitis have been identified: [4,5,6]

1. Periodontitis ,representing the forms of destructive periodontal disease that are generally characterized by slow progression.
2. Necrotising periodontitis disease ,a group of conditions that share a characteristic phenotype where necrosis of the gingival or periodontal tissues is a prominent feature.[7]
3. Periodontitis as a direct manifestation of systemic diseases, heterogeneous group of systemic pathological conditions that include periodontitis as a manifestation.[8]

Saliva is used as a diagnosis body fluid for monitoring various biological alternations in human is attracting many researchers worldwide. Human saliva is an easily accessible biochemical fluid, which is similar to blood in various biological aspects. Besides, it associated with simple and non-invasive collection procedures , low-cost storage and easily storage nature. Biochemical markers plays an important role in the detection of inflammatory changes in short period of time. Saliva contains biomarkers derived from serum, gingival crevicular fluid and mucosal transudate with relatively important diagnostic value, which could be used for detecting periodontal disorders. Salivary components for periodontal diagnosis include enzymes and immunoglobulins, hormones of host origin, bacteria and bacterial products, ions, and volatile compounds.[9]

The interleukin-17 (IL-17) family consists of six family members (IL-17a to IL-17f) and all the corresponding receptors have been identified recently. This family is mainly involved in the host defences mechanisms against bacteria, fungi and helminthic infection by inducing cytokines and chemokines, recruiting neutrophils, inducing anti-microbial proteins and modifying T-helper cell differentiation. IL17a and some other family cytokines are also involved in the development of psoriasis, psoriatic arthritis and ankylosing spondylitis by inducing inflammatory cytokines and chemokines, and antibodies against IL-17a as well as the receptor IL-17Ra are being successfully used for the treatment of these diseases. Involvement in the development of inflammatory bowel disease, multiple sclerosis, rheumatoid arthritis and tumors have also been suggested in animal disease models.IL-17a and its receptor are the founding members of a recently described cytokine family, with unique sequences and functions in the immune system and elsewhere. Consisting of six ligands (IL17a - f) and five receptors (IL-17Ra - IL17Rf) in mammals, these molecules have distinct primary amino acid structures with only minimal homology to other cytokine families. By far the best studied of these cytokines to date are IL-17a and its receptor, IL-17Ra.[10]

IL-17a is produced primarily by T cells, and is the hallmark cytokine of T-helper cell subset that appears to be involved in generation of autoimmunity. Despite its production by the adaptive immune system, IL-17a exhibits pro-inflammatory activities similar to innate immune cytokines such as IL 1beta and TNF-alpha and appears to play important and non-redundant roles in regulating granulocytes in vivo. As a result, IL-17A also plays key roles in host defence. In contrast to the restricted expression of IL-17a, the IL-17Ra receptor is ubiquitously expressed, and thus most cells are potential physiological targets of IL17a.[10]

T-helper 17 (Th17) cells are CD4(+) helper T-cell subset that produces interleukin 17A (IL-17a) and IL-17f. IL-17a plays important roles in allergic responses such as delayed-type hypersensitivity, contact hypersensitivity, and allergic airway inflammation. IL-17a promotes inflammation by inducing various pro-inflammatory cytokines and chemokines, recruiting neutrophils, enhancing antibody production, and activating T cells. IL-6 is required for the development of Th17 cells and tumor necrosis factor functions downstream of IL-17a during the effector phase. IL-1 is important both for developing Th17 cells and eliciting inflammation. Th17 cells, like Th1 and Th2 cells, are involved in host defence against infections, but the contribution of these Th subsets to defense mechanisms differs among pathogens. The roles of IL17F remain largely unknown.[11]

Although IL-17a and IL-17f share the highest amino acid sequence homology, they perform distinct functions; IL-17a is involved in the development of autoimmunity, inflammation, and tumors, and also plays important roles in the host defenses against bacterial and fungal infections, whereas IL-17f is mainly involved in mucosal host defense mechanisms. IL-17E (IL-25) is an amplifier of Th2 immune responses. The functions of IL-17B, IL-17C, and IL-17D remain largely elusive.[12]

Chronic diseases, such as periodontal disease (PD) and rheumatoid arthritis (RA), are characterized by a robust immune response resulting in unresolved inflammation. Inflammation is mediated by pro-inflammatory cytokines; recently inflammatory cytokines are implicated in the progression of localized chronic infections, such as PD, and in serious systemic pathologies, such as diabetes, chronic obstructive pulmonary disease, and cardiovascular disease. Drugs that antagonize inflammatory cytokines are used therapeutically to down-regulate immune mediated pathology in conditions such as RA, although not all patients respond well to this approach. Therefore, identification of potential novel therapeutic targets, such as the IL-17 signaling complex, may be clinically relevant for mitigating inflammatory pathology. However, the manner in which such a therapeutic may influence the onset and progression of PD is poorly understood. Therapeutics that antagonize inflammatory cytokines ameliorate inflammation and bone loss and may have broader implications for individuals with systemic diseases in which inflammation and autoimmunity predominate.[12]

MATERIAL AND METHOD

Thirty eight subjects (fourteen male and twenty four female) were involved in the study . Groups of present study include 30 patients with periodontitis (group 1) and 8 periodontaly healthy subjects (groupe 2) with average age range of 19-60 . The participants were selected from Department of Periodontics ,Collage of dentistry ,University of Babylon . Ethical approval was taken from each participant according to the consent shown in appendix 1. The participants must have good general health with no history of systemic diseases or smoking , no systemic antibiotic therapy and/or periodontal therapy including scaling or root planning within the last 3 months. Questioner involving all demographic criteria shown in appendix 2. After the patient has been selected, and before the base line examination, salivary samples were collected from periodontitis and healthy groups .The following clinical periodontal parameters were recorded: Plaque index (PLI)[13] , BOP , probing pocket depth(PPD) and clinical attachment loss (CAL).

Salivary Sample Collection :

About 1ml sample of non-stimulated whole saliva was collected from each participant in the study . The participant asked to sit in a comfortable position and drok in a sterile test tube after making them rinse his mouth thoroughly with water . Salivary samples were collected at least 1 hour after the last meal and stored at -20 C° till being assessed for IL 17.[14]

Estimation of IL-17 Concentration:

Dilution Method

The dilution method performed as follows: For 100 fold dilution: One-step dilution. 5ml of sample was added to 495 µL sample diluent to yield 100 fold dilution. For 1000 fold dilution: Two-step dilution. 5ml of sample was added to 95 µL sample diluent to yield 20 fold dilution, then 5ml was added 20 fold diluted sample to 245 µL sample diluent, after this, the neat sample has been diluted at 1000 fold successfully. For 100000 fold dilution: Three-step dilution. 5ml of sample was added to 195 µL sample diluent to yield 40 fold dilution, then 5ml was added 40 fold diluted sample to 245 µL sample diluent to yield 50 fold dilution, and finally 5ml was added 2000 fold diluted sample to 245 µL sample diluent, after this, the neat sample has been diluted at 100000 fold successfully.

Reagent preparation

1. All agent brought to room temperature (18-25°C) before use.

2. **Wash Buffer:** 30 mL was added of Concentrated Wash Buffer with 720 mL of deionized or distilled water to prepare 750 mL of Wash Buffer.

3. **Standard working solution:** the standard was centerfuged at 10,000×g for 1 min. 1.0 mL was added. Reference Standard &Sample Diluent allowed to stand for 10 min and was invert gently several times. After it dissolved fully, it was mixed thoroughly with a pipette. This reconstitution produced a working solution of 2000 pg/mL. Then serial dilutions were made as needed. The recommended dilution gradient was as follows: 2000, 1000, 500, 250, 125, 62.5, 31.25, 0 pg/mL. Dilution method: 7 EP tube were taken and 500uL of Reference Standard & Sample Diluent were added to each tube. 500uL of the 2000 pg/mL working solution was pippetted to the first tube and mixed up to produce a 1000 pg/mL working solution. Then 500uL of the solution from the former tube was pippetted into the latter one as shown in figure 1 The illustration below is for reference.

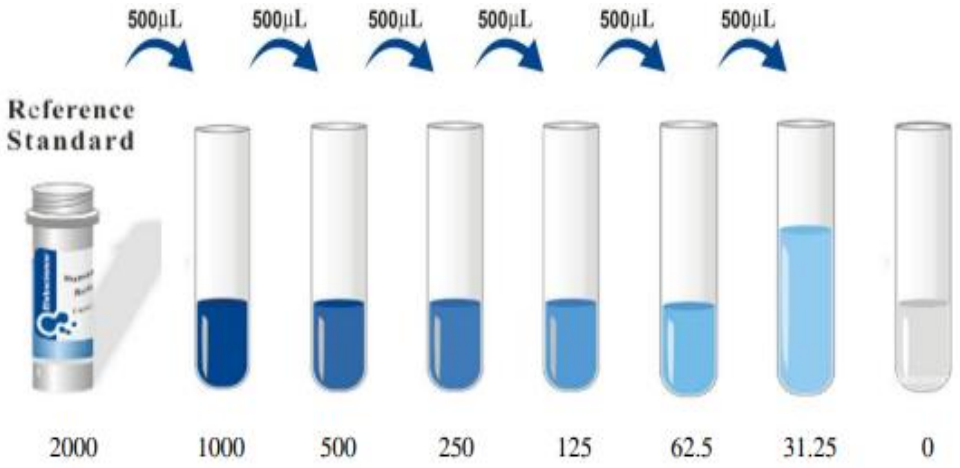

Figure 1 . Dilution steps .

4. **Biotinylated Detection:** Ab working solution: Concentrated Biotinylated Detection Ab was centerfuged at 800×g for 1 min, then the 100× Concentrated Biotinylated Detection Ab was diluted to 1× working solution with Biotinylated Detection Ab

Diluent(Concentrated Biotinylated Detection Ab: Biotinylated Detection Ab Diluent= 1: 99).

5. **Concentrated HRP Conjugate working solution:** the Concentrated HRP Conjugate was centerfuged at 800×g for 1 min, then the 100× Concentrated HRP Conjugate was diluted to 1× working solution with HRP Conjugate Diluent(Concentrated HRP Conjugate: HRP Conjugate Diluent= 1: 99).

Assay procedure

1. Wells for diluted standard, blank and sampe was determined . 100 µL of each dilution of standard, blank and sample were added into the appropriate wells . The plate was covered with the sealer provided in the kit .The plate was incubated for 90 min at 37°C.
2. 100 µL of Biotinylated Detection Ab working solution was added to each well. Then Incubation performed for 1 hour at 37°C.
3. 350 µL of wash buffer was added to each well. Aspiration of the solution from each well was performed for 3 times .
4. 100 µL of HRP Conjugate working solution was added to each well. Incubation was performed for 30 min at 37°C.
5. The wash process was repeated for 5 times as conducted in step 3.
6. 90 µL of Substrate Reagent was added to each well. Incubation performed for about 15 min at 37°C.
7. 50 µLof Stop Solution was added to each well.
8. The optical density (OD value) of each well was calculated with a micro-plate reader set to 450 nm.as shown in figure 2

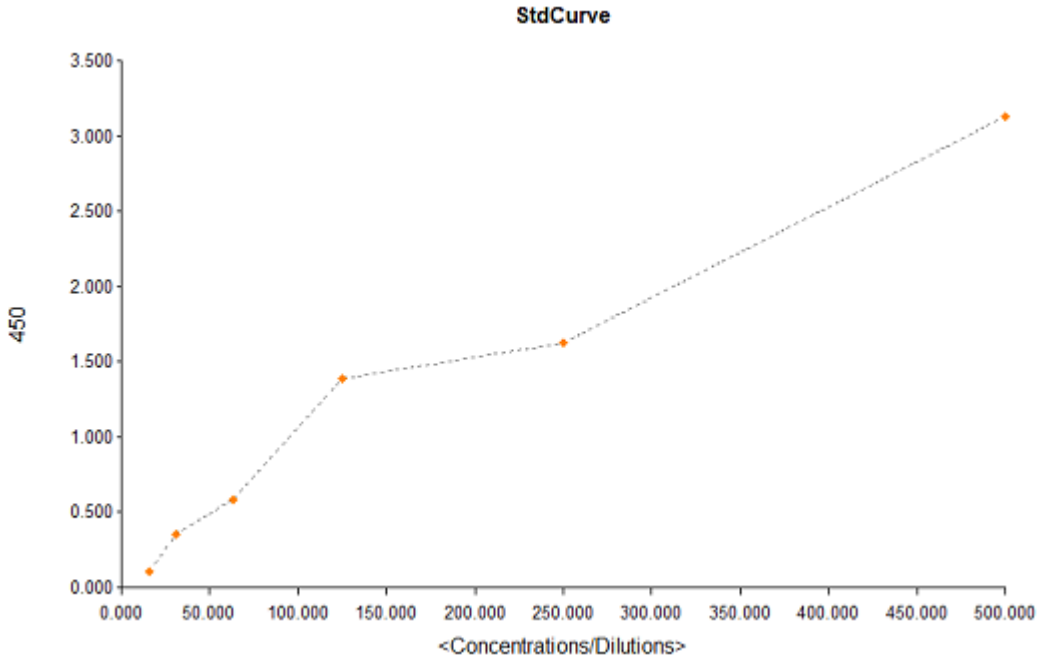

Figure 2. Standard of Il-17a (pg/mL)

Statistical Analysis

Statistical analysis was performed by using SPSS version 23. All data were analyzed using descriptive statistics for normal distribution and homogeneity of variance by the Kolmogorov–Smirnov tests before statistical analyses were managed.Data were expressed as the means ± standard error. P value ($P \leq 0.05$) was considered statistically significant, For comparison between healthy and patients independent t-test was performed for normal distributed data.

Results:

Table number 1 showed the demographic criteria for periodontitis patients group (group 1) and control group (group 2). The means of the age were (42.86 ± 12.26) and (22.5 ± 0.76) in group 1 and 2 respectively . Regarding gender no significant difference was found between groups (p –value = 0.49). Teeth number was (24. 75 ±4.16) in group 1 while it was (27.75 ±0.46) in group 2 . Patient education categorized in to 3 levels (score 1 = primary education , score 2 = secondary education and score 3 = college and higher education) the highest percentage of score 3 was found in healthy group .

Table 1: demographic criteria for test and control groups:

Criteria	Group 1	Group 2
Age	42.8611± 12.25946	22.5000±0.75593
Gender	39% male 61% female	25% male 75% Female
Teeth number	24.75±4.15675	27.75±.46291
Patient education	Primary = 28 % Secondary = 42% Higher education= 30%	Secondary = 13% Higher education= 87 %

Clinical periodontal parameters results showed that the mean of plaque index was (1.2527±.36436) in group 1 while it was (0.6535±.21544) in group 2 with statistically significant difference between them as shown in table 2 .As well as statistically significant difference was found between groups regarding BOP. Probing pocket depth in group 1 was (3.2472±0.62393) with statistical significant difference when compared with group 2. Last not the least CAL mean was (2.6840±1.08523) in group 1 as shown in table 2.

Table 2: clinical periodontal parameters(plaque index , BOP , PPD and CAL) for test and control groups:

Clinical parameters	Group 1	Group 2	t- test	p- value
Plaque index	1.2527±.36436	0.6535±.21544	4.44509.	0.000032.
Bop	0.3306±0.34975	0.00±0.00	2.64855.	0.005712.
Ppd	3.2472±0.62393	1.2163± 0.73868	8.06921.	< .00001.
Cal	2.6840±1.08523	0.00±0.00	6.93066.	< .00001.

Current study found that there was a statistically significant difference between healthy and periodontitis groups as shown in figure 3.

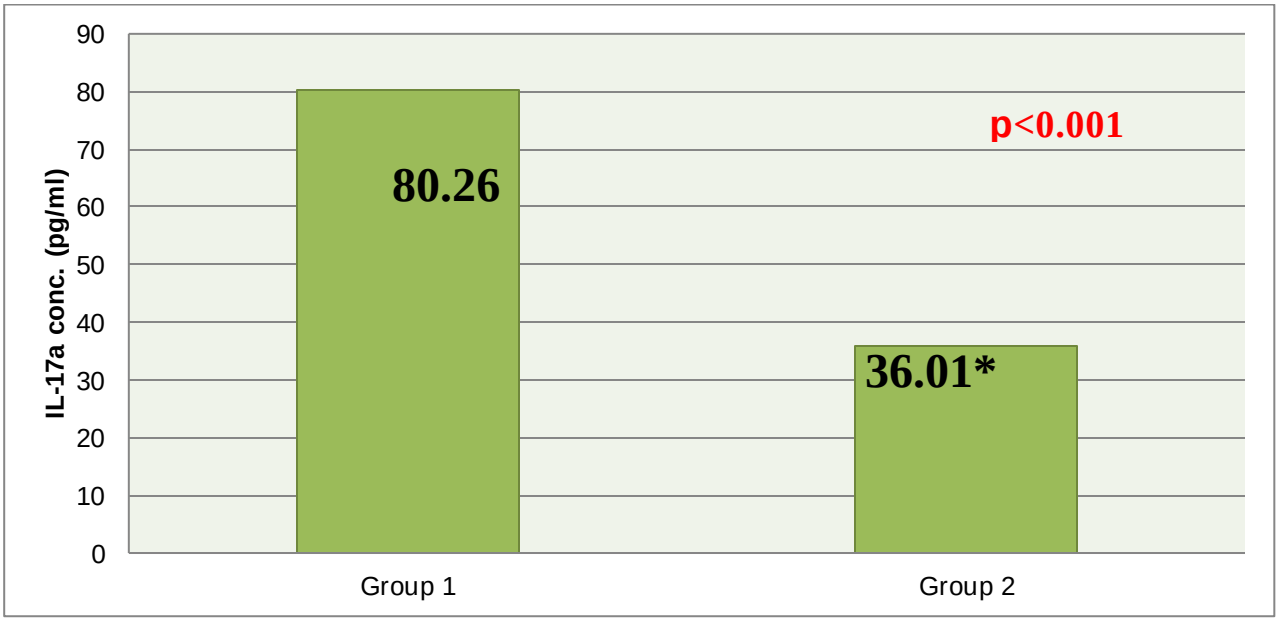

Figure (3): IL-17a concentration in saliva of healthy and patients with periodontitis

Data are presented as mean ± SE. (t-test)

Group 1: patients ; Group 2: Healthy or control

*statistical differences at p<0.001

DISCUSSION

Investigations into the etiology of periodontal disease have suggested that infection by one or more virulent bacteria, interplay of host factors, or an interaction of both may be responsible . These interactions are mediated by cytokines produced by heavy lymphocytic infiltration into periodontal tissues , and these cytokines represent an important component of the immune response to bacterial lipopolysaccharides . IL - 17 may affect osteoclastic bone resorption by stimulating osteoblasts to produce factors that affect the activity and / or formation of osteoclasts ; osteoblasts are IL - 17 - responsive cells and express mRNA encoding the IL - 17 receptor. (15)

Hence it is hypothesized that T cells in periodontal tissues produce and exacerbate inflammatory periodontal disease , activating gingival fibroblasts to produce inflammatory mediators and can playing a role in bone cell metabolism via T - cell derived cytokines.(16)

In this study, IL-17 concentrations were measured in patients with periodontitis and healthy controls, respectively. The mean salivary concentration of IL-17 in patients with periodontitis was 80.26 pg/mL, which was higher than the same concentration in healthy controls (36.01pg/mL), respectively. These results are in agreement with performed by Ozcaka *et al.* (2011) (17)

Several experimental and clinical studies have shown that IL-17 levels are elevated in diseased human periodontal tissues and may play a destructive role in experimental models of periodontal disease. [18,19]

Finally, although little is known about the definite role of IL-17 in the pathogenesis of the periodontal disease, it is suggested that this cytokine, considering that in addition to increasing inflammation, has pro-osteoclastogenic effects, and plays a major role in the pathogenesis of periodontitis. It is hoped that future clinical trials, reveal definite effects of IL-17 in periodontitis using topical interleukin 17-blockers, and more importantly, provide an effective treatment for this inflammatory disease.[20]

Conclusion

Clinical periodontal parameters were higher in periodontitis patients.IL-17 was higher in periodontitis patients and it may be contributed in somehow in the elevation of clinical periodontal parameters.

References

1-Nevins M, Becker W, Kornman K etd. Proceedings of the World Workshop in Clinical Periodontics. Princeton. Chicago American Academy of Periodontology;1989:45(20):149-161.

2-Nevins M, Becker W, Kornman K etd. Proceedings of the World Workshop in Clinical Periodontics. Princeton. Chicago American Academy of Periodontology;1989:45(20):149-161.

3-Tonetti MS, Greenwell H, Kornman KS et al. Staging and grading of periodontitis: framework and proposal of a new classification and case definition. J Periodontol. 2018;89(1):159–172.

4-Fine DH, Patil AG, Loos BG et al. Classification and diagnosis of aggressive periodontitis. J Periodontol. 2018;89(1):103-119.

5-Billings M, Holtfreter B, Papapanou PN, Mitnik GL, Kocher T et al. Age-dependent distribution of periodontitis in two countries: findings from NHANES 2009-2014 and SHIP Trend 2008-2012. J Periodontol. 2018;89(1):140–158.

6-Needleman I, Garcia R, Gkranias N, et al. Mean annual attachment, bone level and tooth loss: a systematic review. J Periodontol. 2018;89(1):120–139.

7. Herrera D, Retamal-Valdes B, Alonso B, Feres M. Acute periodontal lesions (periodontal abscesses and necrotizing periodontal diseases) and endo-periodontal lesions. J Periodontol. 2018;89(1):85–102.

8. Jepsen S, Caton J G, Albandar J M et al. Periodontal manifestations of systemic diseases and developmental and acquired conditions: Consensus report of workgroup 3 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions. J Periodontol.2018; 45(20):219-229.

9-Shu L, Guan SM, Fu SM, Guo T, Cao M, Ding Y. Estrogen Modulates Cytokine Expression in Human Periodontal Ligament Cells. J Dent Res. 2008;87:142–7.

10-R.M. Onishi, S.L. GaffenInterleukin-17 and its target genes: mechanisms of interleukin-17 function in disease Immunology.20101;29 (3): 311-321 .

11-Broxmeyer H.E. Starnes T. Ramsey H. Cooper S. Dahl R. Williamson E. Hromas R. The Interleukin-17 cytokine family members are inhibitors of human hematopoietic progenitor proliferation. 2006; 108(1): 770.

12-Heung-Sik Um, Jae-Young Kim, Youngkyun Lee. Interluekin-17 inhibits osteoblast differentiation and bone regeneration in Archives of Oral Biology.2014;59(9): 897-905.

13-Silness J, Loe H. Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. J Acta Odontol Scand 1964; 22: 121-135.

14- Jassim SD. Effects of non-surgical periodontal therapy on salivary aspartat amino transferase levels in chronic periodontitis patients . Research journal of pharmaceutical, biological and chemical sciences 2017;8(2):2058-2063-

15-Van bezooijen RL , Farih - Sips HC , Papapoulos SE , Löwik , CW (1999) Interleukin - 17 : a new bone acting cytokine in vitro . J Bone Min Res 14 , 1513 1521 .

16- Takahashi K. Azuma T , Motohira H , S:etal .Correlation between IL17 and Periodontal health status. Clinical periodontal journal(2005);J Clin Periodontol 32 : 369-374 .

17.-Ozçaka O, Nalbantsoy A, Buduneli N. Correlation between IL17 and Periodontal health status. Clinical periodontal journal 2011;46 : 592–8.

18-C. M. Queiroz-Junior, M. F. Madeira, F. M. Coelho et al.Correlation between IL17 and Periodontal health status. Clinical periodontal journal 2011;18(7): 3821–3830.

19-T. Honda, Y. Aoki, N. Takahashi et al. Correlation between IL17 and Periodontal health status. Clinical periodontal journal 2008 ;1(2): 137–141.

20-Zenobia C, Hajishengallis G. Basic Correlation between IL17 and Periodontal health status. Clinical periodontal journal 2015 ;69:142–59.

Appendix 1

موافقة للإشتراك في البحث العلمي

اسم الباحث:
عنوان البحث:
مكان إجراء البحث:

أنت مدعو(ة) للمشاركة ببحث علمي سريري سيجرى في ----------.
الرجاء أن تأخذ(ي) الوقت الكافي لقراءة المعلومات التالية بتأن قبل أن تقرر(ي) إذا كنت تريد(ين) المشاركة أم لا. بإمكانك طلب إيضاحات أو معلومات إضافية عن أي شيء مذكور في هذه الاستمارة أو عن هذه الدراسة ككل من طبيبك.

في حال وافقت على المشاركة في هذه الدراسة، سيبقى اسمك طي الكتمان . لن يكون لأي شخص، ما لم ينص القانون على ذلك، حق الاطلاع على ملفك الطبي باستثناء الطبيب المسؤول عن الدراسة ومعاونيه.

موافقة المشترك:

لقد قرأت استمارة القبول هذه وفهمت مضمونها. تمت الإجابة على أسئلتي جميعها. وبناء عليه فأنني، حرا مختارا، أجيز إجراء هذا البحث و أوافق على الإشتراك فيه، وإني أعلم ان الباحث الدكتور _____ وزملاءه ومعاونيه او مساعديه سيكونون مستعدين للإجابة على أسئلتي، وأنه باستطاعتي الإتصال بهم على الهاتف _____
واذا شعرت لاحقا ان الأجوبة تحتاج الى مزيد من الإيضاح فسوف أتصل بأحد اعضاء لجنة الأخلاقيات. كما أعرف تمام المعرفة بانني حر في الإنسحاب من هذا البحث متى شئت حتى بعد التوقيع على الموافقة دون ان يؤثر ذلك على العناية الطبية المقدمة لي.

إسم المشترك:

توقيع المشترك:

Appendix 2

University of Babylon College of Dentistry

Periodontal Department

5th Class Case Sheet(2019-2020)

Name:- Age:- Occupation:-

Body weight/hight:- Marital status:-

Incom (IQD):- <300000.....300000-500000.....>500000.....

Educational achievement:- Primary school / Secondary school/ College or Higher education

History

Chief Complaint:-

Bleeding	Pain	Mobility
Unpleasant test	Dry mouth	Migration of teeth
Halitosis	Altered gingival appearance	Hypersensitivity

Others.....

Past Dental History:-

Visit to dentist.....Regular_____Irregular_____

Tooth brushing.....No_____ Yes_____ Frequency_____

Interdental aids.....No_____ Yes____ toothpicks.....dental floss.....miswak.....

Alcohol dependence .....No.....Yes.....

Smoking status/Current smoker	Cigarette	Water-pipe	Electronic cigarette
No.of smoking /day			
No.of years of smoking			
Duration of each smoking session (minutes)			
Family history of smoking			

Patient's signatur