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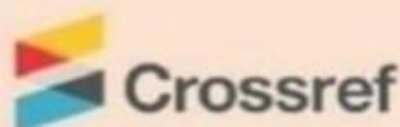
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الصفحة	فهرس البحوث	ت
8 - 1	Determine the bacterial resistance of Streptococcus sobrinus to antibiotics Hanan Saleh Abdulhussain Mithal K.A. Al-Hassani	1
20 - 9	Incidence, pattern and management of mandibular fractures in Al-Anbar governorate in 100 patients Sama Abdulsattar Abd Kamal Turki Aftan	2
29 - 21	Evaluation of salivary IL33 and IL37 in Periodontitis patients with and without type 2 diabetes mellitus Fadya Basil mejbel Heba Fadhil Hassan	3
46 - 30	The Impact of the Waterfall Technique on Spelling Accuracy and Vocabulary Retention among Primary EFL Learners Afrah Munshid Lahad	4
57 - 47	Salivary biomarkers of oxidants and antioxidants for chronic renal disease in patients undergoing maintenance hemodialysis Geehan Nazar Ali Layla Sabri Yas	5
74 - 58	Early detection and segmentation of asphalt pavement cracks: Iraqi highways as case study Shemeam T. Muhey Sinan A. Naji	6
91 - 75	Buzzwords in English Parliament Elections Atyaf Hasan Ibrahim, Narjis Audah Rashk Fatima Raheem Almosawi	7
108 - 92	Strategic Planning to Improve Creativity Using Artificial Intelligence for Islamic University of Minnesota Students USA Raed Mohammad Hanan Sobhi Abdullah Obaid Mohammed Arab Almusawi Helwe jaber Qusquse Fatima Abdurrahman Al-Maraghi	8
116 - 109	The Effect of Crown Fabrication Materials on Wear Resistance and Retention Strength: An Experimental Study Using Statistical Analysis and Magnetic Resonance Imaging Huda Jaafar Naser	9
122 - 117	Structural and Optical Properties of Copper Oxide Nanoparticles Synthesized by Chemical Precipitation Method Uday Ali Sabeeh Al-Jarah	10
145 - 123	Exploring Ideological Positioning in Barack Obama's Speech on Same-Sex Marriage: An Appraisal Theory Analysis Adawiya Jabbar Kadhim Ali Abdulhameed Faris	11
164 - 146	Evaluating the Government Hospitals' Efficiency and Their Impact on Human Development in Iraq Wafaa Hasan Jabur Luma Abdul Manaf Raheem	12
174 - 165	Enzymatic activity of fungi isolated from Otomycosis Azhar Lilo Sayyid Ali A Kasim	13

196 - 175	The Reality of Primary School Teachers' Practice of Professional Accreditation Standards in Light of Approaches to Teacher Professionalization from the Supervisors' Point of View Amera Ali Hasoon Ghasan Kadhim Jabber	14
212 - 197	The relationship of abrogation between the Qur'an and the Sunnah Ali Dhaigham Taher	15
230 - 213	Visual Art Methods and Techniques in Contemporary Art - American Painting as a Model Bayad Abdullah Faqi Ameen Nemat Mohammed Redha Hussein	16
245 - 231	Word-Displacement in The Poetry of Alsa'aleek "Vagabonds" (Selected Examples) Maitham Raheem Shaghati	17
259 - 246	The deficiency of language in perspective the martyr Muhammad Al-Sadr in the book of Menna Al-Mannan in Defense of the Qur'an. Salem Rahim Maaleh	18
272 - 260	The Employment of Historical Symbolism by the Poets of the Seventies Generation:(Khazal Al-Majidi as a Model) Nadam JAbbar Nassr	19
304 - 273	The Level of Employing Professional Technical Skills by Art Education Teachers in Integrating the Relationship Between the Sciences and the Arts, from the Perspective of Specialty Supervisors Zainab Abdul Hussein Jaber Ammar Jabbar Hussein Al-Wahaj Ghassan Kazim Gabr	20
321 - 305	The Impact of a Teaching Strategy Based on TRIZ Theory on Developing Higher-Order Thinking Skills Among Gifted Students in Mathematics Saja Hussein Koma Alaa Ali Hussein	21
335 - 322	The poetic image in the Diwan of Al-Oqaisher Al-Asadi Faten Rajeh Abdel Hameed	22
345 - 336	The efficiency of some Iraqi clays in adsorbing lead using miscible displacement method Abathur Sabar Khalaf Hashim Haneen Kareem Mahdi Wasmy Soheib	23
365 - 346	Effectiveness of the Innovative Matrix Strategy in the Achievement of Students in the Department of Artistic Education in the Subject of Arabic Calligraphy Multaqqa Nassir Jabbar	24
377 - 366	The Intertextuality in Modern Novel: a case study in its origins, manifestations, and Interpretation Raed Radhi Bkheet	25

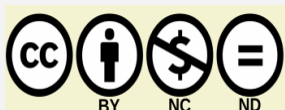


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Evaluation of salivary IL33 and IL37 in Periodontitis patients with and without type 2 diabetes mellitus

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Abstract:

periodontal disease is a long-term inflammatory condition linked to microbes that causes the tooth-supporting structure to deteriorate, periodontitis is a common condition among diabetics and has a strong correlate with the degree and severity of hyperglycemia. The purpose of this study was to compare the amount of the IL-33 and IL-37 in patients both with and without diabetes type 2 mellitus who had periodontitis. a cytokine in periodontal etiology that has not yet been studied.

Keywords: (IL-33), (IL-37), periodontitis and T2DM and without T2DM.

Material & Methods:

The 85 participats in this case control study were split into three groups, Thirty subjects in Group 1 had periodontitis with type 2 diabetes mellitus; thirty patients in Group 2 had periodontitis without type 2 diabetes mellitus; and twenty-five patients made up the control group.Following the collection and centrifugation of saliva, a commercial ELISA kit was used to measure the levels of IL-33 and IL-37. Data analysis was done. The groups were compared using ANOVA and post-hoc ANOVA with least significant distinction (LSD) analysis testing.

Results:

The result of the two groups (Group1: 69.02 ± 6.47 pg/ml, Group2: 60.21 ± 8.39 pg/ml,was elevated compare with the control group's Mean $\pm$ SD of IL-33 was considerably lower (46.46 ± 9.33 pg/ml, $p < 0.05$). There was significant difference ($p < 0.05$) in the mean comparison of IL-33 levels among the groups ($p = 0.0001$), while IL37 the result of two groups (Group1: 55.55 ± 7.95 pg/ml, Group2: 56.18 ± 6.70 pg/ml, the control group's Means of IL-37 was considerably was decreas (75.96 ± 6.76 pg/ml , $p < 0.05$). There was significant difference ($p < 0.05$) in the mean comparison of IL-37 levels among the groups ($p = 0.000$).

Conclusion:

both periodontal and type 2 diabetes are associated with elevated salivary IL-33 and decreased salivary IL-37 levels, respectively.

INTRODUCTION:

Periodontitis, a chronic inflammatory disease that causes vulnerable people's periodontium to be destroyed (Hajishengallis and Lamont 2012; Papapanou et al. 2018) A chronic inflammatory condition linked to microbes, periodontal disease causes the tooth-supporting structure to deteriorate. Globally, it is the main factor contributing to tooth loss and mobility (Devi et al. 2024; Mahdi and Mahmood 2024).

Long-term hyperglycemia leads to a number of metabolic dysfunctions in diabetes mellitus, a complicated disease. Generally speaking, reduced insulin secretion plus action result in hyperglycemia. Diabetes mellitus frequently has a familial history and can develop slowly or subtly (DeFronzo et al. 2015; Tan 2020; Mohammed ; Daily and Mohammed 2017).

Insulin resistance, inflammation, accumulation of advanced glycation end products, and elevated oxidative stress are the hallmarks of type 2 diabetes mellitus (T2DM). Increased belly circumference and overweight/obesity are typically linked to it (Engin 2017; Wu and Tien 2020). showed that the prevalence of type 2 diabetes in individuals with periodontal disorders was roughly twice as high as in diabetic participants who were in good periodontal health in the United States (8). On the other hand, T2DM is brought on by systemic inflammation-induced tissue resistance to insulin and reduced β -cell function (Shoelson, Lee, and Goldfine 2006; King 2008; Abood and Mohammed 2024).

A recently identified proinflammatory cytokine, interleukin-33 (IL-33) belongs to the IL-1 superfamily, which also contains IL-1 α , IL-1 β , and IL-18. When the body mounts an inflammatory defense against bacterial infections, periodontal disease develops. When it comes to periodontal disease, IL-33 most likely plays three roles: systemic cytokine, chemoattractant, and alarmin. When IL-33 is released as an alarmin, it causes necrosis, which kills a number of cells, primarily fibroblasts and epithelial cells (Ballabettu et al. 2019).

A recent addition to the IL-1 family, interleukin-37 (IL-37) is a major regulator with a wide range of anti-inflammatory properties (Palomo et al. 2015). occurring blocker of inflammation and immunological response, interleukin-37 (IL37)(Nsaif and Hassan 2023), an element of the IL-1 family, helps prevent over-activation of these processes and shields tissues from damage caused by inflammation in a variety of illnesses (Bai et al. 2020). There is some evidence that IL-35 and IL-37 have a part in the pathophysiology of periodontitis. (Mitani et al. 2015; Giacoppo et al. 2017). The purpose of this study is to compare the levels of IL-33 and IL-37 in patients who have or do not have type 2 diabetes mellitus who have periodontitis.

Materials and methods:**• Materials and methods:**

Study group: The online tool EPITOOLS (https://epitools.ausvet.com.au/case_controls) was used to determine the sample size, which had a 95% confidence interval and a 5% margin of error. The sample size for the control group with no disease was 25, but the sample size in the periodontitis group was 50, rounded to 60. Therefore, the enzyme immunoabsorbant assay (ELISA) was used to measure the salivary interleukins levels of IL33 and IL37 in a total sample size of 85 patients (25

control, 30 patients with periodontitis who had type 2 diabetes mellitus, and 30 patients with periodontitis patients who did not have type 2 diabetes mellitus).The inquiry was designed as an observational case-control study. Around October 2023 and May 2024, study samples were gathered from the University of Baghdad's Faculty of Dentistry.

- **Ethical approval:** Subjects were recruited from the department of basic sciences at the Teaching Hospital of Collage of Dentistry at the University of Baghdad. The Ethics Board of the University of Baghdad, College of Dentistry, approved the study protocol and determined that it corresponded to the Helsinki and Tokyo standards for human research (Reference number: 864, Project number: 864823, Date: 23\11\2023).

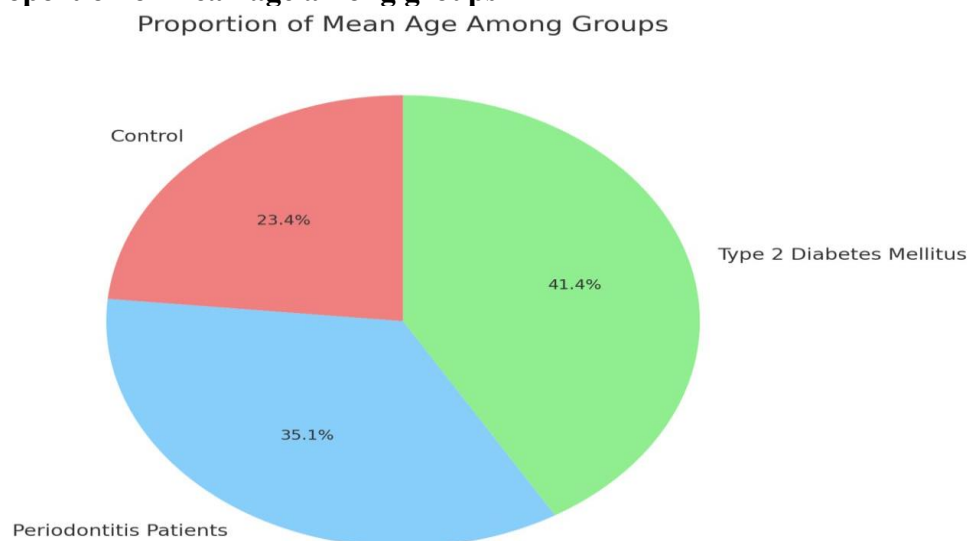
Saliva collection: Whole, unstimulated saliva samples were taken from both healthy participants and suspicious patients (Abd et al. 2023; Mohammed et al. 2024) . A 50 ml Falcon® tube, which is sterile and conical, was used to collect the saliva. After 20 seconds of short vortexing, the saliva specimens were centrifuged for 15 minutes at 2600 X g rpm (Ali, Hassan, and Azeez 2019).

- **Interleukin-33(IL-33)** : The salivary IL33 level was determined by an enzyme-linked immunosorbent assay (ELISA) kit (Sun long,China, 1852710716) .
- **Interleukin-37(IL-37)** : The salivary IL37 level was determined by an enzyme-linked immunosorbent assay (ELISA) kit (Sun long,China, 1742157792) .

Statistical Analysis: Microsoft Excel 2019 and the statistical program for social science edition 26 (SPSS edition-26, Chicago, Illinois, USA) were used to conduct the statistical examination of this prospective study. The formulas for categorical data are count, frequency, and percentage. The connection between these variables is described using the chi-square test.

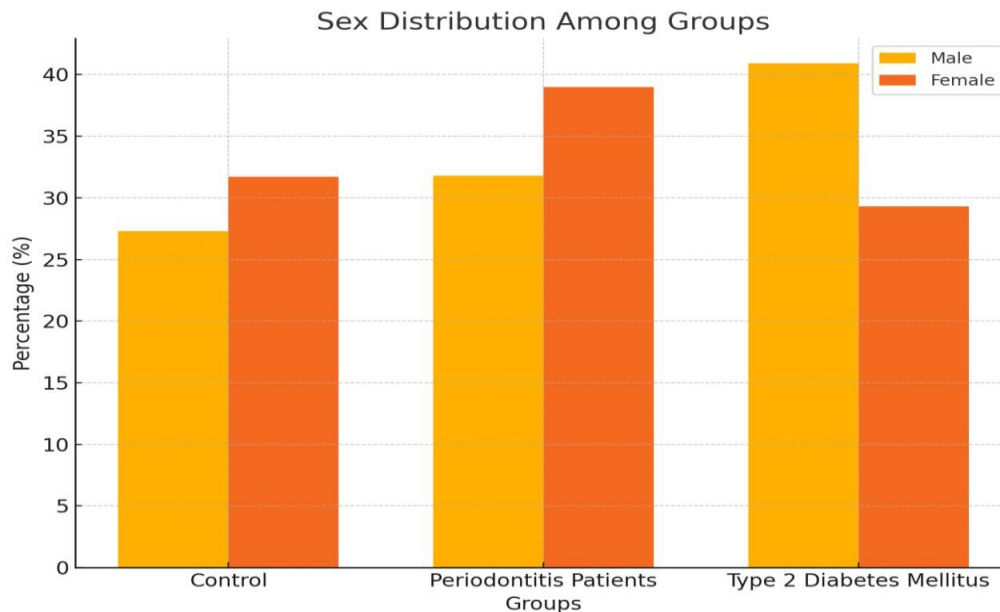
Results: Regarding the age distribution across the control group, periodontal diseases, and diabetes type 2 mellitus groups, there were no significant variations in mean ages ($p = 0.001$). The group used as a control is the the youngest, with a mean age of 32.32 years, following by the periodontal group as 48.47 years and the type 2 diabetes mellitus group around 57.17 years (figure 1).

Figure(1):proportion of mean age among groups



The sex distribution among control, periodontitis, and type 2 diabetes mellitus groups is demonstrated in figure 2. The result showing a relatively balanced distribution with no significant differences among the groups ($p = 0.530$). Males and females are fairly evenly represented across the three groups, with 51.8% males and 48.2% females overall. By using Chi-square analysis the statistical analysis revealed non-significant differences in sex distribution between the groups .

Figure(2);sex distribution among groups.



The current study revealed that the mean level of salivary IL33 was significantly higher in periodontitis and type 2 diabetes mellitus than the healthy control group. The concentration of salivary IL37 was significantly higher in saliva control group than the periodontitis and type 2 diabetes mellitus group figure 2 . Showing significant differences in IL-33 levels among the groups ($p = 0.0001$). The control group has a mean IL-33 level of 46.46 ± 9.33 pg/ml, the periodontitis group has a higher mean of 60.21 ± 8.39 pg/ml, and the type 2 diabetes mellitus group has the highest mean IL-33 level at 69.02 ± 6.47 pg/ml. The statistical revealed significant differences among groups ($P=0.0001$).

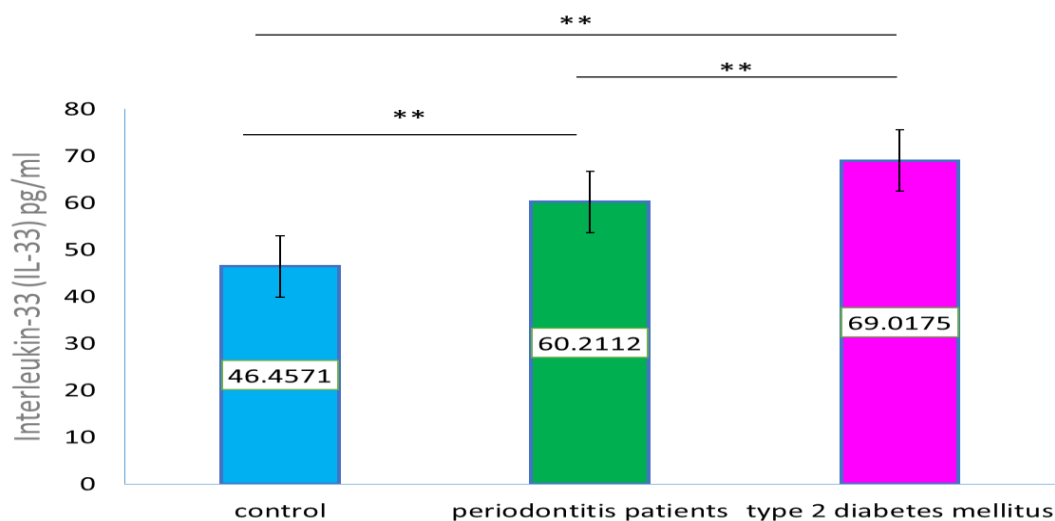


Figure 3: IL33 pg/ml level comparison among the Control group, periodontitis and type 2 diabetes mellitus.

The results of IL-37 levels among groups (control, periodontitis, and type 2 diabetes mellitus) by using ANOVA test are shown in figure 4. The control group has a mean IL-37 level of 75.96 ± 6.76 pg/ml, the periodontitis group has a lower mean of 56.18 ± 6.70 pg/ml, and the type 2 diabetes mellitus group has a similar mean of 55.55 ± 7.95 pg/ml. The ANOVA test reveals a significant difference among groups, ($p= 0.0001$).

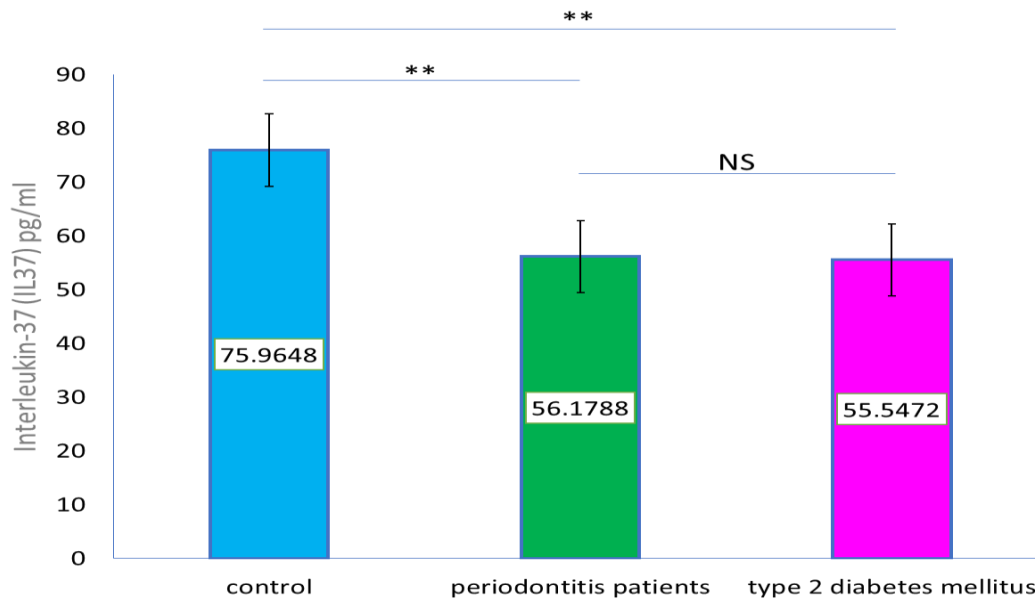


Figure 4: IL37 pg/ml level comparison among the Control group, periodontitis and type 2 diabetes mellitus.

Discussion:

The present study comprised a sample size of 85 individuals diagnosed with periodontitis Iraqi patients with and without type 2 diabetes mellitus and control distribution of both sexes and age. Males and females are fairly evenly represented across the three groups, with (51.8%) males and (48.2%) females overall. This study agree with periodontitis has a documented higher prevalence in men (~57%) compared to women (~39%) signifying a possible sex/gender bias in disease pathogenesis (Ohno et al. 2012; Tarrad, Abdelkawy, and Shaker 2018).

Ignorance of dental hygiene, which is typically seen in men, may account for the higher percentages of men in the group with developed periodontitis (Casanova, Hughes, and Preshaw 2014); Furthermore, it has been demonstrated that both innate and adaptive immunity are influenced by the hormone mediators of the immune response, such as progesterone, testosterone, and estrogens. Progesterone and testosterone generally depress the immune system. However, estrogens also boost the immune system, which helps to explain why males are more likely than females to get infected (Hassan et al. 2024).

While higher male prevalence in the T2DM group was in Dis agreement with (Pastrana et al. 2012; Moslem iPetrodi et al. 2020), Researchers discovered that the prevalence of T2DM was higher in women than in men across the entire study group. This finding could be explained by the fact that women in the present study had higher BMIs than men, which is a significant risk factor for the development of T2DM. In the setting of bone metabolism, IL-33 may have osteoclastogenic effects and contribute to the development of periodontitis. Nonetheless, a number of studies discovered that

in cases of periodontitis, the IL-33/ST2 axis had osteogenic effects and prevented the loss of alveolar bone (Aida et al. 2023). Other studies found that ligature-induced periodontic lesions had greater mRNA levels of TNF α , IL-33, IL-1 α , IL-1 β , and IL-6 than did healthy gingiva. Consequently, IL-33 might be involved in the development of periodontitis (Aida et al. 2023).

Additionally, under pathological circumstances like the microbial response, IL33 can be induced on myeloid cells like neutrophils and macrophages (Ohno et al. 2012) .

The periodontal lesions may become infiltrated by these cells. Thus, it's possible that IL-33 is also produced by invading myeloid cells in the periodontal infections. Our findings are consistent with those of Tarrad et al. (2018), who reported that the CP and CP-DM group had higher levels of IL-33 in both GCF and saliva than the healthy control group. This suggests that IL33 plays a part in the inflammatory response that occurs in periodontitis. Additionally, the data showed that the CP-DM group's GCF and saliva had higher amounts of IL-33 than the CP group, indicating that diabetes has a negative impact on periodontal disease (Tarrad, Abdelkawy, and Shaker 2018).

The current investigation found that there was no way to distinguish between a clinically healthy periodontium and chronic periodontitis based on IL-33 levels in the patients and healthy controls. Different demographics, varying degrees of tissue loss and gingival inflammation, various IL-33 concentrations, different methods, or varying sample sizes could all be contributing factors to this discrepancy.

It is unclear how IL-37 reduces inflammation. Pro-inflammatory cytokines or their receptors are inhibited when IL-37 is released into the extracellular space (Bufler et al. 2002) or go into the nucleus, where they cooperate with Smad3 to prevent the transcription of genes. Low-grade chronic inflammation with aberrant expression and synthesis of several inflammatory mediators, including interleukins and tumor necrosis factor, is a hallmark of diabetes (Wellen and Hotamisligil 2005; Dandona et al. 2004). A recently identified members of the interleukin family having anti-inflammatory and immune-inhibitory properties is interleukin-37 (IL-37) (Nold et al. 2010; Banchereau, Pascual, and O'Garra 2012) (Chen et al. 2015).

It is unknown, therefore, how type 2 diabetes in the elderly and IL-37 expression levels relate to one another. Therefore, we carried out the current study to look into the function of IL-37 in older people with type 2 diabetes and how it relates to dysbiosis of the gut microbiota.

. There is a little evidence that IL-37 might play a part in the pathophysiology of type 2 diabetes. One important anti-inflammatory cytokine that may help reduce inflammation and resistance to insulin brought on by obesity is IL-37 (Ballak et al. 2014). The results of this study concluded that the IL33 acts as proinflammatory cytokines in periodontitis and type 2 diabetes mellitus. And the IL37 acts as an anti inflammatory cytokines in periodontitis and type 2 diabetes mellitus(Maddah and Taha 2024).

Conflicts of Interest:

The writers affirm that they lack competing interests.

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Conflicts of Interest Statement.....

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