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Immunological Biomarkers IL-34, MCP-1, and MIP-1α: Age and Sex Associations in Periodontitis Patients

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Abstract

Periodontitis is a common osteolytic disease and the leading cause of adult tooth loss. The disease's susceptibility can be influenced by systemic factors, with age and sex being significant factors. The current study assessed the correlations between interleukins and age and sex in periodontitis patients. A total of 85 periodontitis patients and 40 healthy individuals aged between 18 and 67 years were included to examine statistical correlations between interleukins and age/sex in periodontitis. Using ELISA techniques, levels of IL-34, MCP-1, and MIP-1α in serum and gingival crevicular fluid (GCF) were assessed. Statistical analyses, including the Kruskal-Wallis Test, Mann-Whitney Test, and Chi-square Test, were conducted to ascertain relationships among immunological markers, age, and sex, with significance set at $P \leq 0.05$. The results revealed significant age correlations with IL-34 concentrations in serum and GCF, notably increasing in patients aged 40-54. Conversely, no substantial age-based variances were observed in MCP-1 or MIP-1α levels within the patient group. Sex effect on IL-34 production, demonstrating higher levels in patients compared to healthy individuals. Conversely, MCP-1 and MIP-1α levels showed no significant sex-related variations within patients. In conclusion, this study underscores a positive association between age and IL-34 levels in serum and GCF among periodontitis patients. Additionally, it underscores the sex impact on IL-34 production. These findings contribute to comprehending the immunological mechanisms of periodontitis and the role of age and sex in disease susceptibility. Further exploration is needed to assess these findings' implications in tailoring personalized treatment approaches for periodontitis.

Keywords: periodontal diseases, MCP-1, IL-34, MIP-1α

Резюме

Пародонтитът е често срещано остеолитично заболяване и водеща причина за загуба на зъби при възрастни. Възприемчивостта към заболяването може да бъде повлияна от системни фактори, като възрастта и полът са особено значими. Настоящото проучване оценява корелациите между интерлевкините, възрастта и пола при пациенти с пародонтит. Включени са общо 85 пациенти с пародонтит и 40 здрави лица на възраст между 18 и 67 години, за да се изследват статистическите корелации между интерлевкините и възрастта/пола при пародонтит. С помощта на ELISA техники бяха са оценени нивата на IL-34, MCP-1 и MIP-1α в серума и гингивалната кревикуларна течност (GCF). За установяване на връзките между имунологичните маркери, възрастта и пола са проведени статистически анализи, включващи теста на Kruskal-Wallis, Mann-Whitney и Chi-square, като значимостта е определена при $P \leq 0,05$. Резултатите разкриват значими възрастови корелации с концентрациите на IL-34 в серума и GCF, които се увеличават особено при пациенти на възраст 40-54 години. Обратно, не се наблюдават съществени възрастови различия в нивата на MCP-1 или MIP-1α в рамките на групата пациенти. Влияние на пола върху производството на IL-34 демонстрира по-високи нива при пациентите в сравнение със здравите индивиди. Обратно, нивата на MCP-1 и MIP-1α не показват значителни вариации, свързани с пола. В заключение, това проучване подчертава положителната връзка между възрастта и нивата на IL-34 в серума и GCF сред пациентите с

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пародонтит. Освен това то подчертава влиянието на пола върху производството на IL-34. Тези констатации допринасят за разбирането на имунологичните механизми на пародонтита и ролята на възрастта и пола за податливостта към заболяването. Необходимо е по-нататъшно проучване, за да се оцени значението на тези констатации за адаптирането на персонализирани подходи за лечение на пародонтит.

Introduction

Periodontal disease is a multifactorial condition caused by complicated interactions between bacterial plaques, immune system components, and genetic variation in the host (Bhuyan *et al*., 2022). One of the primary causes of periodontal diseases is bacterial complexes that colonize oral tissues. Secondary causes include the presence of cavities, calculus, and dental plaques, as well as anatomical traits such as a short tooth root. Additionally, genetic and risk factors like systemic illness and smoking contribute to the development of periodontal diseases. (Shi and Zhang, 2015). After inflammation happens in periodontitis and tissue damage, Gingival Crevicular Fluid (GCF) is released. GCF contains electrolytes, fibrinogen, albumin, enzymes, as well as immunological laments like immune system cells, cytokines, and various types of bacteria. It transforms from low-concentration discharges into the gingival crevices or gingival sulcus, which are the areas of the gums that are not affixed to the teeth directly (Khurshid *et al*., 2017). There are two forms of immunological responses to bacterial pathogens: innate immunity and acquired immunity. In periodontitis patients, inflammatory cell infiltration of T and B cells, macrophages, and neutrophils, in addition to the inflammatory mediators' release, increases in the gingival connective tissue (Yucel-Lindber and Båge, 2013). Cytokines and chemokines carry out cell-to-cell communication (chemotactic cytokines). The immune response to infection is controlled by cytokine and chemokine signals, Cytokines are tiny, low-molecular-weight proteins that influence the severity and duration of an inflammatory response. nuclear factor transcriptional regulation in the genome Through the toll-like receptor pathway, kappa-B must be engaged to control the release of proinflammatory cytokines from distinct cell types in response to particular pathogen-associated chemical patterns, such as lipopolysaccharide (Hanada and Yoshimura, 2002), as well as epithelial and fibroblast cells, phagocytes (neutrophils and macrophages) are responsible for the production of cytokines during acute and early chronic phases of inflammation, impacts cell motility and extravasation by encouraging neutrophil rolling and adherence through increased adhesion molecules, and cell migration to infected and inflamed sites is further facilitated by chemokine synthesis (Kalaczkowska and Kubes, 2013). One of the hematological cytokines that regulates, differentiates, and causes the development of blood cells, such as monocytes, phagocytes, and osteoblasts is IL-34, which shares a receptor with CSF-1(Muñoz-Garcia *et al*., 2021). Although IL-34 and MCSF perform some of the same activities, they have different characteristics and utilize different routes (Chang, 2015). During the 1989 workshop, the variables of age and rate of disease progression were added to the other clinical manifestations of the disease because it is believed that these variables are closely related to the development of the disease, and periodontal disease is classified into pre-pubertal (Localized and Generalized), juvenile (Localized and Generalized), and rapidly progressive (Adult Periodontitis, Necrotizing Ulcerative Periodontitis (Caton *et al*., 2018). Innate immune responses vary with age, including diminished antigen presentation capability, alteration in chemotactic phagocytes, and natural killing function, also as well as disrupted inflammatory reaction and reduced growth and number of antigen-naïve T cells, and rise in antigen-experienced memory T cells number, The reduction in the population of naïve T cells is linked to immunological compromise and a diminished ability to respond to novel or modified infectious agents (Mahbub *et al*., 2011). Furthermore, higher MCP-1 levels have been linked to getting older (Tapp *et al*., 2013). However, there is immune system dysregulation with aging, which is frequently characterized by a chronic inflammatory state coupled with decreased cell-mediated processes. Furthermore, systemic factors such as hormonal and metabolic changes that occur with aging may lead to changes in the immune system's intrinsic functioning (Michaud *et al*., 2013). This research offers new insights into how immunological markers, age, and sex intertwine in periodontitis. These findings might revolutionize periodontitis management by guiding personalized diagnostics and targeted treatments for better patient care.

Materials and methods

Specimens' collection

Under artificial lighting, an examination of the mouth was conducted in the periodontics de-

partment. Under the supervision of a periodontitis specialist, the same examiner assessed and collected clinical variables. Before getting gingival crevicular fluid (GCF) from dental pockets, the supragingival calculus was eliminated using a sickle scalar. Then teeth were separated using cotton pads to avoid contamination from saliva and blood. Following teeth scaling, the depth of the pocket was determined using a periodontal probe. A sterilized absorbent paper point with size (30-45 mm) was used to collect GCF. A periodontal pocket with a depth of over four millimeters was selected, and the two paper points were entered within until moderate resistance was observed. The paper points were allowed to remain in place for thirty seconds put into an empty Eppendorf tube and kept frozen at -80°C to determine IL-34, MIP-1α, and MCP-1 concentration.

Serum collection

Five ml of venous blood was obtained from each person participant's patient and healthy in the study three ml of it was carefully inserted into the gel tube, left to coagulate for thirty minutes at the temperature of the room, and then centrifuged for around fifteen minutes at 3000 rpm. The resulting serum was obtained in a sterilized Eppendorf tube and kept frozen at -80°C to determine the concentration of IL-34, MCP-1, and MIP-1α.

Immunological study (Estimation of IL-34α, MCP-1 and MIP-1α Concentration by ELISA)

ELISA kits (Sunloge, USA) were used for the purpose of measuring the concentrations of human IL-34 a, MIP-1α, and MCP-1Concentration in serum and GCF for periodontitis and healthy groups. According to the method provided by the company manufacturer for each test kit.

Statistical analysis

The statistical software SPSS version 24 was used for all statistical analyses. For every variable that observed the distribution's normality, the T-independent test was used; for variables that did not observe the distribution's normality, non-parametric analyses such as the Mann-Whitney and Kruskal-Wallis (multiple comparisons) tests were used in additionally to the test of Chi-square. The results were statistically significant at a P value ≤0.05, displayed as mean ± SEM.

Results

Relationship between the IL-34, MCP-1, and MIP-1α levels and age in clinical samples of patients and control

The present study investigates the levels of IL-34 according to patient age groups, the results indicated the different levels, in some groups the level was increased while decreasing in others.

Age was observed to have a significant effect on the IL-34 concentration in periodontitis patients. The statistical analysis revealed that the mean IL-34 level in serum, and GCF increased in patients of 40–54 years old, followed by the group 28 – 40 and ≤ 27.00 years old. On the other hand, the findings indicate a higher mean level of IL-34 in patient serum than GCF of +50 within the patient group. Also, the results showed significant differences between patients and control (Table 1).

Table 2 indicates that there aren't any significant differences in the serum and GCF level of MCP-1 at P≤0.05 based on age progression within the patient group, Additionally, when patients were compared with the control, no significant differences were found in the MCP-1 level in the samples collected from patients and for all age groups, but from other hand the mean of serum MCP-1 increased in the age groups (28–40, 41–53) compared within the same groups, and this indicate for presence of internal differences but the limitation of sample size may be attributed leads for current results.

The current results did not agree with the find-

Table 1. Comparison of IL-34 levels in clinical samples according to age

IL-34 [pg/ml]	Age group	Patients	Mean± SEM	P value	Control	Mean± SEM	p-value
Serum	≤ 27	22	451.15±49.52	0.04*	8	169.62±13.30	0.73
	28 - 40	32	443.95±43.21	0.02*	15	163.62±8.11	0.73
	41 - 53	20	608.48±65.09	0.04*	11	175.59±13.90	0.74
	54+	11	562.18±59.46	0.22	6	169.47±20.08	0.99
GCF	≤ 27	22	305.52±66.31	0.27	8	192.78±16.00	0.50
	28 - 40	32	230.55±15.60	0.02*	15	202.52±7.50	0.07
	41 - 53	20	402.79±82.50	0.02*	11	178.67±10.19	0.07
	51+	11	211.71±34.58	0.04*	6	182.20±7.51	0.55

*Significant differences at p≤0.05

Table 2. Comparison of MCP-1 levels in clinical samples according to age

MPC-1 [pg/ml]	Age group	Patients	Mean± SEM	P value	Control	Mean± SEM	p-value
Serum	≤27	22	288.73±16.99	o.995	8	219.32±37.92	0.143
	28 - 40	32	288.85±15.39	0.072	15	149.66±28.53	0.143
	41 - 53	20	329.14±12.47	0.096	11	172.51±27.38	0.350
	54+	11	280.10±23.44	0.764	6	147.70±49.34	0.648
GCF	≤27	22	274.25±24.75	0.731	8	134.49±26.52	0.198
	28 -40	32	287.05±25.69	0.683	15	185.22±19.97	0.198
	41 - 53	20	271.40±29.45	0.945	11	152.47±30.84	0.356
	54+	11	321.75±41.15	0.340	6	152.34±42.09	0.446

*Significant differences at p≤0.05

ing of Vickers (2017) who showed a significant P value comparing the groups' ages demonstrated statistically. The current investigation discovered that the mean MCP-1 concentrations in GCF increased proportionately from periodontitis to health, which made this study in agreement with the findings by Babu *et al*. (2017). Numerous other research had revealed that periodontitis patients had increased GCF and serum MCP-1 levels in comparison to healthy individuals (Pradeep *et al*., 2009a; Pradeep *et al*., 2009b). The heterogeneity in MCP-1 levels across patients from every group might be linked to the role of MCP-1 at various phases of the illness process when GCF and serum samples have been collected (Babu *et al*., 2017).

The investigation showed that there are no significant differences in the serum and GCF levels of MIP-1α at P≤0.05 based on age progression within the patient group. Additionally, when patients were compared with the control, no significant differences were found in the MIP-1α level in the clinical samples and for all age groups (Table 3).

The results agree with Emingil (2004) who reported that the concentration of MIP-1a in GCF appears not to be associated with periodontitis.

Relationship between the IL-34, MCP-1, MIP-1α levels, and sex in clinical samples of patients and control

For investigating the concentration of IL-34 based on sex, the current study indicates that patients have higher IL-34 levels than healthy, furthermore, the level of IL-34 production is influenced by female and male hormone variations in study participants according to study findings which indicates there are no significant differences in serum concentration of IL-34in both male and female in both patients and control at P value 0.21, 0.139 respectively, whereas the GCF level of IL-34 varied significantly P 0.02 between male and female in the healthy control group, but there is no significant in patient groups. The present study suggests that in research groups, sex had slightly effect on IL-34 production (Table 4).

Table 5 showed no significant differences between levels of MCP-1 in both serum and GCF of the male and female patient groups, additionally, the absent significant differences were found in control groups. This finding suggested there is no relation between MCP-1 production and sex and the inflammatory response of MCP-1 not affected by sex.

Table 3. Comparison of MIP-1α levels in clinical samples according to age

MIP-1a [pg/ml]	Age group	Patients	Mean± SEM	P value	Control	Mean± SEM	p-value
Serum	≤27.00	22	80.06±10.16	0.229	8	67.20±10.67	0.421
	28. - 40	32	100.93±11.18	0.229	15	78.63±9.15	0.142
	41 - 53	20	106.60±15.88	0.171	11	59.50±6.82	0.609
	54+	11	122.03±20.43	0.071	6	74.78±5.03	0.664
GCF	≤27.00	22	92.23±8.09	0.453	8	64.22±11.05	0.329
	28 - 40	32	103.28±11.07	0.453	15	81.40±12.03	0.329
	41 - 53	20	86.65±10.60	0.273	11	65.57±12.70	0.942
	54+	11	111.83±16.97	0.318	6	53.54±7.54	0.155

*Significant differences at p≤0.05

Table 4. Comparison of IL-34 levels in clinical samples based on sex

IL-34	Sex	Patient n=85	Mean± SEM	P-value	Control n=40	Mean± SEM	P-value
Serum	Female	35	541.28±46.97	0.21	23	176.78±8.46	0.139
	Male	50	470.81±33.12		17	158.44±8.23	
GCF	Female	35	249.12±22.01	0.24	23	179.61±4.77	0.02*
	Male	50	315.29±44.10		17	206.32±9.58	

*Significant differences at p≤0.05

Table 5. Comparison of MCP-1 levels in clinical samples based on sex

MCP-1	Sex	Patient n=85	Mean± SEM	P-value	Control n=40	Mean± SEM	P-value
Serum	Female	35	299.62±11.37	0.80	23	163.03±22.30	0.654
	Male	50	295.45±12.14		17	178.45±25.69	
GCF	Female	35	272.27±21.21	0.48	23	171.51±19.21	0.388
	Male	50	293.14±19.49		17	147.09±19.55	

*Significant differences at p≤0.05

Table 6. Comparison of MIP-1α levels in clinical samples based on sex

MIP-1α	Sex	Patient n=85	Mean± SEM	P-value	Control n=40	Mean± SEM	P-value
Serum	Female	35	103.86±10.48	0.60	23	69.39±7.69	0.799
	Male	50	96.60±8.96		17	72.02±5.93	
GCF	Female	35	94.65±9.21	0.67	23	67.19±8.31	0.681
	Male	50	99.69±7.33		17	72.47±9.69	

*Significant differences at p≤0.05

The same investigation was found in the comparison of both serum and GCF MIP-1α levels in both males and females in the patient group. Also, there is no significance in the serum and GCF MIP-1 levels in control groups. Findings have indicated the weak effect of sex on MIP-1 production and the higher levels may relate to other factors (Table 6).

Discussion

In this study, we looked at the levels of MIP-1α, MCP-1and IL-34 in the serum and GCF, and their relation with age and sex. Our findings contribute to our understanding of the immunological mechanisms underlying periodontitis and highlight the importance of considering age and sex as factors affecting disease susceptibility. Further research is warranted to explore the implications of these findings in the development of personalized treatment strategies for periodontitis. In the recent past, there has been an indication that IL-34 impacts immune cells and takes a role in immunological-inflammatory responses, affecting the genesis and development of several disorders. Increased IL-34 production either promotes inflammatory cascades or modifies immunological tolerance, depending on the clinical state. As a result, IL-34 may be employed as a predictive biomarker for a variety of conditions, including cancer, transplant rejection, infections, inflammatory illnesses, and autoimmune disorders (Ge and Huang, 2019). Aging alters immunological and inflammatory responses, resulting in the loss of periodontal tissue in older persons (Wadia, 2020). Additionally, the higher frequency of periodontal destruction in older adults may be due to uncontrolled periodontal disease building up over time, instead of that age is a factor in the development of periodontal disease (Genco, 2020). The aging process may generate a considerable drop in chemotaxis, motility, and periodontal ligament cell proliferation rates (Groessner *et al*., 1992). Periodontal ligament cells in elderly individuals demonstrated decreased levels of chemotaxis and growth compared to periodontal ligaments in young individuals (Nishimura *et al*., 1997), also, the decline in bone production can be caused by decreasing precursors of osteoblast spreading or reduced production and release of essential bone proteins (Abiko *et al*., 1998). MCP-1 was found to be age-related, its levels tended to rise with middle age, peaking in their fifties. Females had higher serum levels of IL-34, MCP-1 andMIP-1α than men. Therefore, the

study of sex biology as a moderating factor of innate and adaptive responses may result in a variety of disease susceptibility; the sex may be attributed to hormones production, environmental and genetic factors, which can result in a variety of outcomes (Klein and Flanagan, 2016). Hormones have the potential effect of changing immunological components and responses, such as the presentation of antigens and expression, cytokine generation, apoptotic factor expression, and death of cells. Steffens *et al*. (2015). In particular showed that Progesterone has increased the production of the inflammatory mediator prostaglandin E2 and the accumulation of polymorphonuclear leukocytes in the gingival sulcus, as well as inhibited the production of interleukin 6 by human gingival fibroblasts, while high testosterone related with severity of periodontitis. The cases discussed by Yu *et al*. (2008) demonstrated how the same hormone exhibits an exaggerated inflammatory response to the plaque at various ages and stages. *In vitro* and *in vivo* investigations (Dumas *et al*., 1995; Abiko *et al*., 1998) have demonstrated that aging causes functional and structural changes in fibroblasts, Gingival fibroblasts (GFs) are frequently impacted by oral microbes and their products like lipopolysaccharides (LPS) present in the cell walls. The LPS induces GF to release many pro-inflammatory mediators, involving prostaglandin E2 (PGE2), plasminogen activator (PA), and interleukin (IL)-1 (Abiko *et al*., 1998). The impact of inflammatory chemicals on GF and PLFs in the periodontal ligament may be a major factor in the extent of periodontal infection.

Conclusion

The current study concluded that age and gender factors might influence the progression of periodontitis either directly or indirectly. Additionally, the elderly and gender conditions may alter the immune response and immune mediators such as proinflammatory interleukins due to hormonal or genetic factors. The limited investigations in this field required more, extensive, and time-consuming searches to determine the reliable interaction of these factors with periodontitis.

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