Short Communication

The Assessment of IL-4 and IgG1 Levels in Patients with Rheumatoid Arthritis and Periodontitis: A Serological Study in Hilla City, Iraq

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Abstract

Background: Periodontal disease, which has a complex etiology, is prevalent and influenced by several factors, including microorganism invasion, host health, and external environmental factors, contributing to its development. It is unknown whether rheumatoid arthritis (RA) and periodontitis are related. **Objectives:** The current study aims to assess the relationship between periodontitis and RA by measuring the concentrations of interleukin 4 (IL-4) and immunoglobulin G1 (IgG1) in patients' serum and quantifying *Porphyromonas gingivalis* in subgingival plaque samples. **Materials and Methods:** The study comprised patients with periodontitis, RA, and healthy controls. The levels of IL-4 and IgG1 in the serum samples were measured using an enzyme-linked immunosorbent assay. The identification of subgingival plaque bacteria is made by using the polymerase chain reaction technique. **Results:** The results showed the levels of IgG1 were high in healthy people compared to those with periodontitis and those with periodontitis and arthritis, where the levels in healthy people were 2.3 ± 0.28, 2.06 ± 0.55, 2.05 ± 2.34, 1.82 ± 0.77 in the age groups 41:50, 20:30, 51:60, 31:40, respectively. It was found that there are significant differences between the values of IgG1 and IL-4 in patients with periodontitis at $P \leq 0.05$ which is considered a differential sign. **Conclusion:** The concentrations of IL-4 and IgG1 are decreased in patients (periodontitis and periodontitis with rheumatoid arthritis) compared to their concentrations in healthy people. Thus, we indicate IL-4 was related to reducing the severity of periodontitis disease and RA.

Keywords: Inflammatory cytokines, periodontitis, *Porphyromonas gingivalis*, rheumatoid arthritis

Introduction

Periodontitis is among the most well-known chronic inflammatory nontransmittable infections.[1] It is caused by *P*orphyromonas *gingivalis*, a Gram-negative, committed anaerobic bacterium that lives in the mouth.[2] Periodontitis is an inflammatory disease of dental supporting tissues, including gingiva, periodontal ligament, and bone. It has been indicated as one cause of rheumatoid arthritis (RA).[3] RA is the type of arthritis that is most well-known.[4] Genetic and environmental factors impact a chronic B-cell inflammatory autoimmune disease with rheumatic symptoms, RA. Clinical research has demonstrated a connection between periodontal disease (PD) and RA.[5] Those who have RA, compared to healthy controls, are more likely to have severe periodontitis or missing teeth.[6] Contrarily, those with PD were found to be more vulnerable to RA than those in good condition.[7] Additionally, it has been demonstrated that the nonsurgical treatment of PD improves rheumatic problems.[8]

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Materials and Methods

Samples collection

This study was conducted in the College of Dentistry, University of Babylon, and Merjan Teaching Hospital, Rheumatology Unit Babylon (from December 2022 to February 2023). Patients with periodontitis and RA (80

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patients, male and female) with age range 20–60 years. Patients were grouped into two subdivisions (each 40), including 40 patients with periodontitis and 40 with periodontitis and RA. The control group included 40 healthy people (males and females). All subjects in this study had been assigned an agreement to participate in this study, depending on the clinical and serological parameters according to the 2010 ACR/EULAR criteria.

This study involved 40 patients with RA and 40 patients with severe chronic periodontitis who had probing depths (PD) >5 mm, with clinical attachment loss of ≥3 mm, as well as 40 individuals who had healthy periodontal tissues. Clinical periodontal parameters were recorded before beginning periodontal therapy, including PD, clinical attachment loss, plaque index, and bleeding on probing (BOP). Blood samples were taken on the same day of clinical examination. The samples were stored at -20°C until laboratory analysis. Enzyme-linked immune sorbent assay (ELISA) was used to measure the levels of interleukin 4 (IL-4) and immunoglobulin G1 (IgG1).

A total of 360 subgingival plaque samples were collected, and three samples were collected from every subject. The sample collection takes place at two locations for each periodontitis patient. The sample collection was made from the teeth with clinical attachment loss (CAL) ≥ 3mm. The test sites were gently cleaned from supra gingival plaque and were air-dried and maintained dry using cotton rolls, and then sterile paper points size 30 were used to collect the subgingival plaque sample. Any paper point contaminated with blood was thrown away. The paper points from every sampling site were put immediately into a microfuge tube with 1ml of phosphate-buffered saline and stored at -20°C.[9] Polymerase chain reaction (PCR) was used to detect the *P. gingivalis* species. The specific primers sequence of *P. gingivalis* is explained in Table 1.

Blood sample

A 3 mL of blood was put in a gel tube and centrifuged at 4000 rpm for five minutes to separate serum and then frozen at -20°C to measure the amounts of the cytokines levels; IL-4 and IgG1.[11]

Enzyme-linked immunosorbent assay

ELISA kit is used to measure IL-4 and IgG1 concentration in serum quantitatively. The kit was provided by Bioassay Technology Laboratory (Nanhu Dist, Jiaxing, Zhejiang, China).

Ethical approval

The study was conducted following the ethical principles in the Declaration of Helsinki. It was carried out with patients' verbal and analytical approval before the sample was taken. The study protocol, subject information, and consent form were reviewed and approved by a local ethics committee according to document number 82, including the number and date on January 25, 2022, to get this approval.

Results

Clinical periodontal parameters such as the plaque score (PS), BOP, pocket depth (PD), and CAL were measured as part of the intraoral examination. Six locations were used to evaluate each tooth's PS, BOP, PD, and CAL.

The Kruskal–Wallis test was used for the data analysis. Table 2 shows that there was a significant difference ($P < 0.05$) between the H, periodontitis, and periodontitis with rheumatoid groups in all clinical parameters (PS, BOP, PD, and CAL). The significant difference was mainly between the healthy and the groups (*P* and PR).

PCR should allow prompt and accurate identification of bacteria. The principle of the method is simple when a pure PCR product of the 16S gene is obtained, sequenced, and aligned against a bacterial DNA database. The bacterium can be identified.[12] In the current study, *P. gingivalis* was diagnosed by PCR as it was isolated from a swab of patients with periodontitis and RA. Figure 2 shows five bands representing five isolates of *P. gingivalis*. Figure 1

Table 1: Specific primers sequence of *P. gingivalis*[10]

Primer name		Sequence 5′-3	Product size (bp)
GIN	F	5′AGG CAG CTT GCC ATA CTG CG-3′	404
	R	5′ACT GTT AGC AAC TAC CGA TGT-3′	

Table 2: Clinical periodontal parameters

Clinical parameters	Healthy ($n = 40$)	Periodontitis ($n = 40$)	Periodontitis and rheumatoid ($n = 40$)	*P*-value between and within groups
PS (%)	7.945 ± 2.194	35.965 ± 8.482	42.052 ± 12.278	0.000*
BOP (%)	5.655 ± 1.280	33.222 ± 8.083	33.337 ± 8.752	0.000*
PD (mm)	1.332 ± 0.489	3.425 ± 0.863	3.503 ± 0.788	0.000*
CAL (mm)	0.000 ± 0.000	2.917 ± 1.074	2.904 ± 1.107	0.000*

CAL = clinical attachment loss, PD = pocket depth, BOP = bleeding on probe, PS = plaque score

*$P < 0.05$: a significant difference. Kruskal–Wallis test

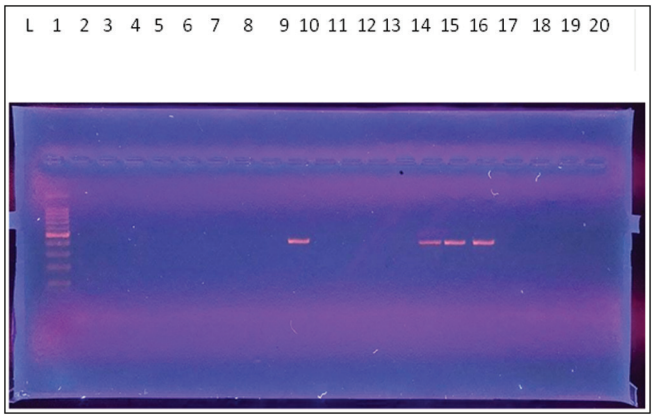

Figure 1: Agarose gel electrophoresis (ladder = 100–1000), (pb404). Agarose—1%, volt—80, mA—20, time—80 min of extracted DNA of *P. gingivalis*

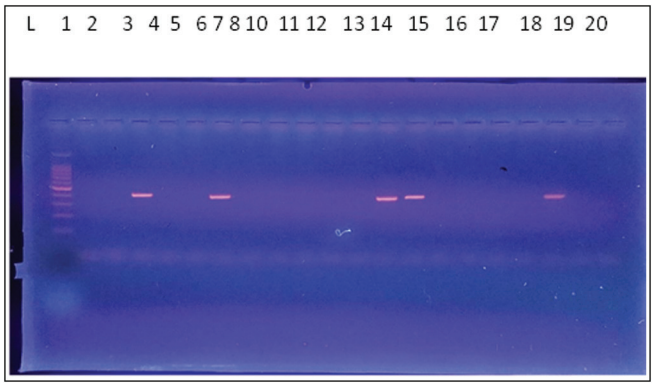

Figure 2: Agarose gel electrophoresis (ladder = 100–1000), (pb404). Agarose—1%, volt—80, mA—20, time—80 min of extracted DNA of *P. gingivalis*

shows that isolates 9, 14, 15, 16 showed positive results for *P. gingivalis*, whereas 12, 13, 17, 18, 19, 20 isolates showed negative results for *P. gngivalis* for group periodontitis.

In Figure 2, isolates 3, 6, 13, 14, and 18 showed positive results for *P. gingivalis*, whereas 1, 2, 4, 5, 7, 8, 9, 10, 11, 14, 15, 16, 17, 19, 20 isolates are showed negative results *gingivalis* for the group (healthy).

In Figure 3, isolates 5 and 6 showed positive results for *P. gingivalis*, whereas 1, 2, 3, 4, 7, 8, 9, 10, and 11 isolates showed negative effects for *P. gngivalis* for groups (periodontitis and rheumatoid).

ELISA was used to measure the levels of IL-4 and IgG1 concentration in the serum of the samples for age groups 20:30, 31:40, 41:50, and 51:60 of people with gingivitis and those with gingivitis and arthritis together, in addition to healthy people [Table 3].

Discussion

Periodontitis and RA are inflammatory immune illnesses where leukocyte permeation and cytokines stimulate

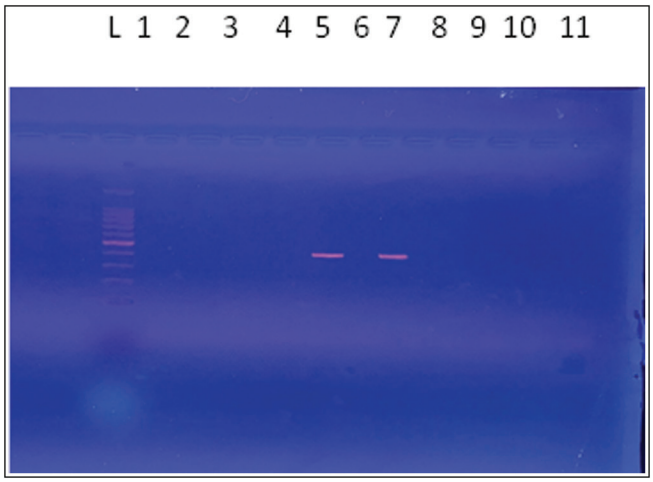

Figure 3: Agarose gel electrophoresis (ladder = 100–1000), (pb404). Agarose—1%, volt—80, mA—20, time—80 min of extracted DNA of *P. gingivalis*

alveolar bone loss, synovitis, and joint demolition interleukin-4, produced from T-helper 2 cells, is a crucial cytokine for the growth and proliferation of B lymphocytes. IL-4 has an anti-inflammatory effect owing to its efficient inhibition of the production of proinflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), IL-1α, IL-1β, IL-6, and IL-8 by monocytes/macrophages.[13] The IL-4 and IgG1 samples obtained from patients were primarily low or undetectable. They remained, on average, in two groups (periodontitis and RA) on the same level to some extent. Still, the level of IL4 and IgG1 samples obtained from the control was higher than the patients with periodontitis and RA.

In the current study, the results showed that the levels of IgG1 were high in healthy people compared to those with periodontitis and those with periodontitis and arthritis, where the levels in healthy people it is 2.46 ± 0.22, 2.32 ± 0.3 in the age groups 31:40, 51:60, respectively. At the same time, the levels of IgG1 reached 2.27 ± 0.09, 2.25 ± 0.31 in age groups 41:50 and 20:30, respectively. In the patients with periodontitis, the age group 41:50 for people with periodontitis showed the highest level of IgG1, reaching 2.18 ± 0.55, whereas the lowest level of IgG1 was for the age group 51:60, reaching 1.77 ± 0.77. In comparison, the levels of IgG1 reached 1.98 ± 0.64, 1.85 ± 0.85 in the age group 31:40 and 20:30, respectively. In patients with rheumatoid and periodontitis, IgG1 levels reached 2.28 ± 0.13 in the 31:40 age group, while the lowest level of IgG1 in the age group 20:30, 41:50, 51:60 reached 2.19 ± 0.23, 2.61 ± 0.19, 2.268 ± 0.33 respectively. This is consistent with previous studies showing that IgG1 concentrations were decreased in patients with arthritis.[14]

The results in the present study showed that the levels of IL4 were high in healthy people compared to those with periodontitis and those with periodontitis and arthritis, where the levels in healthy people were 2.3 ± 0.28,

Table 3: The levels of IL-4and IgG1 in healthy, periodontitis, rheumatoid, and periodontitis group

Age					Periodontitis			
		IgG1				IL-4		
	20:30	31:40	41:50	51:60	20:30	31:40	41:50	51:60
Mean ± SD*	1.85	1.98	2.18	1.77	0.86	0.95	0.89	0.87
	0.85	0.64	0.55	0.77	0.22	0.56	0.29	0.28
Age					Rheumatoid arthritis and periodontitis			
		IgG1				IL-4		
	20:30	31:40	41:50	51:60	20:30	31:40	41:50	51:60
Mean ± SD*	2.19	2.28	2.261	2.268	0.70	0.73	0.82	0.77
	0.23	0.13	0.19	0.33	0.09	0.17	0.32	0.20
Age					Control			
			IgG1				IL-4	
	20:30	31:40	41:50	51:60	20:30	31:40	41:50	51:60
Mean ± SD*	2.25	2.46	2.27	2.32	2.06	1.82	2.3	2.05
	0.31	0.22	0.09	0.3	0.55	0.77	0.28	0.34

*(SD+: high; −:low) L.S.D. = 0.789 at $P \leq 0.05$

2.06 ± 0.55, 2.05 ± 2.34, 1.82 ± 0.77 in the age groups 41:50, 20:30, 51:60, 31:40, respectively. In contrast between periodontitis patients and patients with periodontitis and arthritis together, where the highest levels of IL4 were 0.95 ± 0.56 in periodontitis patients within the age group 31:40, followed by the age group 41:50 51:60, 20:30 reached 0.89 ± 0.29, 0.87 ± 0.28, 0.86 ± 0.22, respectively. The results showed that the levels of IL4 were the lowest in people with periodontitis and arthritis together compared to healthy people. For those with periodontitis, it reached 0.70 ± 0.09, 0.73 ± 0.17, 0.77 ± 0.20, 0.82 ± 0.32 in the age groups 20:30, 31:40, 51:60, and 41:50, respectively [Table 3]. This is consistent with the results of previous studies, which showed that IL-4 concentrations decreased in patients with periodontitis as the disease progressed. It is proposed that IL-4 may play a significant role in the remission or improvement of PD.[15,16] Statistical analysis revealed that there were significant differences below the level of significance $P \leq 0.05$ between IgG1 and IL-4 values in all patients with periodontitis and the value of L.S.D. about 0.789 compared to those with periodontitis and arthritis and healthy people, as no significant differences appeared between different age groups. There is a weak correlation between IgG1 and IL-4 in the periodontitis group, about 0.3904, while in the rheumatoid and periodontitis group, there is an intermediate correlation of about 0.5586. While in the case of healthy people, there is an inverse correlation between IgG1 and IL-4, about -0.8179. This means that the higher the IgG1, the lower the level of IL-4 in the blood of healthy people [Table 3].

Conclusion

This study showed a relationship between periodontitis and arthritis, as the concentration of IL4 and IgG1 are decreased in patients compared to the concentration of cytokines in healthy people. This evidenced the cytokines' role in reducing the disease's severity and arrival in the last stages. However, more research is needed to find out whether this association between periodontitis and arthritis affects the teeth and joints and the role of bacteria in the mouth in stimulating autoimmunity and thus determining the best treatment for inflammatory diseases.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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