Original Article

Assessment Of The Levels Of Interleukin-4 In The Gingival Crevicular Fluid Of A Group Of Patients With Chronic Periodontitis And A Group Of Non-Periodontitis Subjects

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Abstract

The symptoms of periodontitis include inflammation of the tissues that support teeth. Numerous investigations have discovered a connection between rheumatoid arthritis, low levels of anti-inflammatory cytokines, and periodontal disease. This study sought to determine whether there were any correlations between the levels of Interleukin-4 in gingival crevicular fluid from patients with and without chronic periodontitis and indices of disease severity. **Material and Methods:** An ELISA test was performed on gingival crevicular fluid samples collected from 88 subjects with chronic periodontitis (44) and non-periodontitis (44) for the determination of IL-4 levels. **Results:** In the non-periodontitis group, the mean IL-4 concentration was highest (0.3± 0.006 pg/mL), and the chronic periodontitis group had the lowest mean IL-4 concentration (0.02 ± 0.027pg/mL). **Conclusion:** In patients with periodontitis, IL-4 concentrations decreased as the disease progressed. It is proposed that IL-4 may play a significant role in the remission of or improvement of periodontal disease.

Keywords: Chronic periodontitis, non-periodontitis, IL-4

Introduction

Generally, the interaction between dental plaque and immune system components leads to periodontitis (1). Increased cellular and humoral immune responses are among the many immunological characteristics of periodontitis (2). It is essential for immunocompetent cells to develop, grow, proliferate, and differentiate using the cytokines produced by multiple types of eukaryotic cells (2). The lymphocyte, specifically the Th-1 and Th-2, is an example of immunocompetent cell that produces a group of cytokines required for B cell responses and tissue homeostasis (3). Any disruption in the homeostasis sequence will result in the emergence of immunological diseases such as periodontitis (2,4). Periodontitis is a multifactorial disease caused primarily by microbial biofilm containing various

microorganism colonies. Clinical signs of periodontitis include deeper probing, a higher plaque index, a lower clinical attachment level, bleeding on probing, and bone loss. It is believed that a complex interplay of genetic, environmental, and bacterial variables, with bacterial and host factors playing a crucial role, results in periodontitis. When these two factors are out of balance, the transition from health to inflammatory disease is completely different. The imbalance manifests itself as an increased plaque biofilm, decreased host resistance, and increased bacterial virulence (5). It is believed that periodontitis, which afflicts 10% to 15% of the world's population, is the main factor behind adult tooth loss. (6). The last ten years have seen several researchers have identified altered cytokine production in periodontitis and evaluated its role in the progression of disease. Numerous studies have established that IL-4 reduction in diseased periodontal tissues correlates with periodontal disease activity. The reduction in IL-4 led to the hypothesis that periodontal disease may be triggered by reduced IL-4 (7,8).

Interleukin-4 plays an anti-inflammatory role by being a growth and survival factor for lymphocytes. It is considered to play a prominent role in parasitic infections, allergic reactions, and autoimmune diseases and has protective effect in rheumatoid arthritis and diabetes (9). IL-4 demonstrates anti-inflammatory properties owing to its ability to effectively cease monocytes and macrophages from the formation of proinflammatory cytokines such as TNF-a, IL-1a, IL-1b, IL-6, and IL-8. Aside from this, it effectively prevents the production of pro-inflammatory mediators. The factors include reactive oxygen species (ROS), reactive nitrogen species (RNS). It also includes prostaglandins in monocytes and macrophages. Anti-inflammatory properties are possessed by IL-4 receptor antagonists, which are produced in response to IL-4 (10).

Extensive evidence suggests that IL-4 plays a role in a variety of systemic diseases; however, there is limited evidence that IL-4 also plays a role in the onset and development of periodontitis (11,12).

Data from studies on rheumatoid arthritis suggest that IL-4 may be important in the downregulation of inflammatory processes underlying RA pathogenesis and may beneficially control the disease's progression (9).

Given that these processes also take place in chronic periodontal inflammation, it would be beneficial to compare the levels of IL-4 in healthy individuals and those with periodontitis to determine if this cytokine is involved in the development of periodontal inflammation.

Despite contradicting results (7, 13) recent researches indicate a connection between periodontitis and high IL-4 levels. (14,15).

These data disparities might be accounted for by changes in IL-4 production rates between populations, which might be influenced by IL-4 genetic variants linked to either susceptibility to or protection against chronic periodontitis (16,17).

A study conducted among the Sudanese population found that genetic factors may have an impact on the IL-4 systems and that IL-4 is a likely candidate gene for susceptibility to periodontal disease. Particularly in the Sudanese population, the levels of interleukin-4 in GCF have not been sufficiently investigated or studied (18). These facts, along with the fact that the cytokine perspective on periodontal disease and its connective systemic conditions offers broad and promising perspectives on future developments of more accurate methods of diagnosis and more effective therapeutic protocols, have led to the current study's goal of determining the level of IL-4 in a group of Sudanese patients with chronic periodontitis.

Materials and Methods

Study Population: From the periodontology department's patient database at the Faculty of Dentistry at the University of Khartoum, 44 patients with chronic periodontitis CP (N = 44) were selected. For inclusion in the International Workshop for a Classification of Periodontal Diseases and Conditions for Chronic Periodontitis, consent was required to collect samples for immunological testing, excellent general health, and a diagnosis of chronic periodontitis (19). Smoking, diabetes mellitus, malignant diseases, immunodeficiency, and history of cardiovascular abnormalities were among the exclusion factors (such as coronary artery disease or hypertension). The comparison group (N = 44 non-periodontitis patients) was chosen concurrently with the patients and balanced in terms of age, sex, and smoking habits. Each non-periodontitis individual consented to undergo immunological testing and had at least 20 healthy

teeth. The exclusion criteria for the patients with periodontitis were the same. Using a University of Michigan O probe with William's marks, measurements of probing depth (PD) and clinical attachment loss (CAL) were taken from six locations on each tooth as part of a full periodontal examination. Individuals in the non-periodontitis group lacked either a history of gingivitis or clinical evidence of periodontitis (no PPD >4 mm and no clinical attachment loss around any tooth). The Ethical Committees of the Faculty of Dentistry at the University of Khartoum approved this study. The study was conducted in accordance with the Helsinki Declaration, with written informed consent provided by all participants.

Choosing a site and collecting GCFs

One examiner conducted all clinical periodontal examinations and selected the sampling sites (M.M). Using a Michigan O probe marked with William's marks, locations with >2 mm CAL were found in patients with chronic periodontitis. The sites with the largest clinical indications of inflammation, highest CAL, and deepest probing were chosen as the sample sites. Following gentle drying with a blast of air, supragingival plaque was removed without contacting the marginal gingiva. A perio-strip was used to gently push the marginal gingiva for 30 s at the gingival sulcus entrance in order to collect the gingival crevicular fluid for testing purposes to avoid saliva contamination.

By placing the perio-strip at the sulcus entrance for 30 s in the non-periodontitis group, gingival crevicular fluid was collected from numerous locations with no inflammatory symptoms. During the course of the experiment, gingival crevicular fluid samples were immediately collected from both groups, transferred to airtight plastic containers, and stored at 70°C until analysis.

Interleukin-4 assay

Following the manufacturer's instructions, we estimated the concentration of cytokines in the samples from both groups using the ELISA Max Deluxe Set Human IL-4 (catalogue no:430304, USA). A capture of antibody solution was placed on the plate on the first day and incubated overnight between 2 °C and 8 °C.

The plate was then cleaned four times the following day by adding 200ul of 1X Assay Diluent A to each well. The plate was sealed and shaken on a plate shaker for an hour at room temperature (500rpm). Following a fourth round of washing the plate with wash buffer, 100 µL diluted standards and samples were added to the appropriate wells. As soon as the plate had been resealed, it was incubated for two hours at room temperature while shaking the plate repeatedly for a further two hours after resealing. The plate was washed four times, and each well received 100 µL of a diluted Detection Antibody solution. The plate was then sealed and incubated for an hour at room temperature with shaking. To incubate the plate for 30 min at room temperature, the plate was rinsed four more times, and 100 µL of avidin-HRP solution was added to each well. After addition, the plate was sealed, and incubated for 30 min at room temperature. The dish was rinsed five more times, soaking for 30–120 s each time. Each well received 100 µL of freshly mixed TMB Substrate Solution and was then incubated for 15 min in the dark. Following the addition of 100µL Stop Solution to each well, the plate was immediately read using a spectrophotometric reader. Within 15 min, the absorbance was measured at 450 and 570 nm. Subtracting the absorbance at 570nm from the absorbance at 450nm will yield the absorbance at 570nm. A standard curve derived from known concentrations of the relevant cytokine was used to estimate the quantity of IL-4 in the samples within 15 minutes using spectrophotometer measurements at wavelengths of 450 nm and 570 nm. Analyzing absorbance at 450 nm led to the determination of absorbance at 570 nm. The ELISA MAX Deluxe Set kit has a detection sensitivity for IL-4 is 2 pg/mL.

Statistical Analysis

- The data were analyzed with SPSS 25.0.0.0 software (Statistical Package for Social Sciences). Data are presented as mean ± SD. IL-4 levels between the two groups were compared using the Mann-Whitney test, which is a widely used statistical technique.
- Comparing IL-4 levels in a chronic periodontitis group with Clinical Attachment Loss (CAL), Pocket Depth (PD), and Pocket Height (PH) was done using Spearman's rank correlation.

Results

There were 88 participants in this study, 44 of whom had chronic periodontitis, and 44 did not. The demographic characteristics of the enrolled participants are presented in Table 1. With a mean age of 40.9 ±11.7 years, the chronic periodontitis group included 17 (38.6%) males and 27 (61.4%), females, whereas the non-periodontitis group included 20 males (45.5%) and 24 (54.5%) females.

In both the periodontitis and non-periodontitis groups, clinical parameters (PD and CAL) and IL-4 levels were evaluated in GCF. Table 1 summarizes the periodontal measures of the participants enrolled in this study. When the two groups were compared, the data showed statistically significant differences in all periodontal parameters tested (p=0.001). According to the degree of clinical attachment loss, the mean values in the periodontitis group suggested moderate to severe periodontal tissue destruction.
The mean IL-4 levels in the GCF samples of the patients and the comparative groups are presented in Table 3. IL-4 was detected in both groups; however, the concentration levels in the GCF samples were higher in the non-periodontitis subjects compared with the periodontitis patients, showing statistically significant differences (p=0.001). Table 3 provides a summary of the statistical analyses of both groups.
Analysis was performed to determine the correlation between IL-4 levels and clinical parameters (PD and CAL). When CAL and IL-4 were correlated, a significant inverse relationship between the two was identified (R=0.343, p0.02); Similarly, a significant inverse relationship between chronic periodontitis and PD was identified. (R= 0.197, p= 0.01) (table 4).

Discussion

Bacterial biofilms cause chronic and complex inflammatory diseases, and host mediator production is crucial to disease progression in periodontitis. It has been reported that inflammation of the periodontium is associated with the production of a variety of pro-inflammatory and anti-inflammatory cytokines. The anti-inflammatory properties of IL-4 have been linked to the etiology of periodontal disease, offering evidence that it plays a crucial role in its emergence (7). When the immune profile changes from TH1 to TH2, inflammation usually resolves and B cells begin producing antibodies. For TH1 responses to transform into TH2 responses, IL-4 is required (20).

Recent research indicates that the cytokine profile inside the gingiva and the activation of B cells by TH2 cells at periodontitis sites are both faulty (21). One of the mechanisms underlying these activities is the production of specific cytokines and interactions within the gingival cytokine network. Additionally, some studies have reported lower IL-4 concentrations in areas with chronic periodontitis (22,23).

A total of 88 participants participated in this study. Several previous studies have used smaller sample sizes, but this study used a significantly larger sample size (7,8). A significant difference (higher mean and SD) in the rate of bleeding was seen between the chronic periodontitis group and the non-periodontitis group when testing for probing depth, pocket depth, and clinical attachment loss. It is possible to explain this correlation by demonstrating a link between inflammation and periodontitis clinical parameters, including bleeding and deep probing. Several studies have been conducted on the effects of bleeding, including studies conducted by Caton et al. and Davenport et al., which demonstrated that bleeding is indicative of histological inflammatory changes occurring within tissues. There are

several factors which may be indicative of a future attachment loss, such as bleeding on probing, pus production, and increasing the depth of the probing (24-26).

The study found that participants without periodontitis had significantly higher IL-4 levels. Together, our findings support previous research in that TH1 immunological profiles within inflamed gingiva fail to change to TH2 immunological profiles (27). The negative relationship between the concentration of IL-4 and the levels of CAL and PD levels supports this conclusion. Due to its comparatively low concentration in periodontitis patients compared to non-periodontitis patients, IL-4 may have prevented the transition from a TH1 to a TH2 immunological profile (28). To support these hypotheses, a long-term investigation of the Sudanese population is required.

Researchers have found that patients with chronic periodontitis have lower levels of interleukin-4 in their gingival crevicular fluid than healthy individuals. These findings suggest that, although the reason for this localized decline has not yet been identified, IL-4 may play a protective role in periodontal tissues by providing them with a strong immune response (29). We confirmed the findings of Yamamoto et al., who observed that topical application of recombinant IL-4 may reduce periodontal inflammation (30). This could be a non-invasive option for the treatment of chronic periodontitis. The potential of this field should be explored in future studies.

Conclusion

The results of the current study showed that IL-4 levels were higher in the non-periodontitis group than in the chronic periodontitis group. Therefore, it is possible that IL-4 may be able to be used as a marker for remission or improvement of periodontal disease.

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Table 1 displays the demographic characteristics of chronic periodontitis and non-periodontitis groups

		Non-periodontitis group	Chronic periodontitis group
Gender	Male	20 (45.5%)	17 (38.6%)
	Female	24 (54.5%)	27 (61.4%)
Age in years	Mean ± SD	40.9 ± 11.7	39.4 ± 11.9

Table 2: Comparison between Chronic periodontitis group and non-periodontitis group in Clinical Parameters

	Non-periodontitis group		Chronic periodontitis group		P value
	Mean	**SD**	**Mean**	**SD**	
CAL	0.00	0.00	6.64	1.82	0.001**
PPD	0.00	0.00	4.43	1.73	0.001**
BOP	46.43	21.01	53.91	15.45	0.05*

Table 3: Levels of IL-4 in patients with chronic periodontitis and non-periodontitis subjects. IL-4 levels were evaluated by enzyme-linked immunosorbent assay (ELISA). Data are presented as the mean ± standard deviation.

	Healthy group		Chronic periodontitis group		P value
	Mean	**SD**	**Mean**	**SD**	
IL-4	0.3	0.006	0.02	0.027	0.001**

Table 4: Correlation between IL-4 and periodontal parameters (CAL &PD) in chronic periodontitis group assessed by the Pearson correlation coefficient (r) and associated probability (p).

		IL-4	**CAL**	**PPD**
IL-4	Spearman's Correlation Coefficient		- 0.343	- 0.194
	P value		0.023*	0.207
CAL	Spearman's Correlation Coefficient	- 0.343		0.866
	P value	0.023*		0.001**
PPD	Spearman's Correlation Coefficient	- 0.194	0.866	
	P value	0.207	0.001**	

***Correlation is significant at the 0.05 level. **Correlation is significant at the 0.01 level.**