



Assessment of the Level of Interleukin-12 in Gingival Crevicular Fluid of a Group of Patients with Aggressive Periodontitis and a Group of Healthy Subjects

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Abstract

Objective Aggressive periodontitis (AgP) is a type of periodontal disease that is relatively prevalent among Sudanese population. The disease generally affects younger individuals and might lead to tooth loss if undetected early, leading to costly and long periodontal treatment. Until today, no reliable detection tool is present, so diagnosis is confirmed only after periodontal tissue loss has already occurred. Interleukin-12 (IL-12) has both proinflammatory and immune-regulatory effects and it has been implicated in the pathogenesis of other inflammatory diseases such as rheumatoid arthritis. However, it was not studied extensively in Sudanese population. Therefore, the aim of this study was to measure and compare the level of IL-12 in the gingival crevicular fluid (GCF) of patients with AgP and healthy subjects without periodontitis.

Materials and Methods In this study, 30 patients with AgP and 30 healthy subjects were recruited. The periodontal parameters included bleeding on probing (BOP), periodontal pocket depth (PPD), and clinical attachment level (CAL). GCF levels of IL-12 were measured.

Results A total of 60 participants were enrolled in this study with female predominance of 83% and males comprising 17%. The results of this study showed slight elevation in the level of IL-12 in the GCF in AgP group with a mean value of (60.7) and a mean value of (52.7) in the healthy subjects group; however, the difference was not statistically significant (p -value = 0.120). Also, no statistically significant correlation was found between the level of this interleukin and periodontal parameters with slight elevation in AgP group. The p -value for BOP, PPD, and CAL was 0.369, 0.985, and 0.797, respectively.

Conclusion The slight increase in the level of IL-12 in GCF of AgP patient and slight elevation in sites with attachment loss suggest a possible role of this cytokine in the pathogenesis of AgP. More studies are required to determine the exact role of this cytokine in AgP.

Keywords

- ▶ aggressive periodontitis
- ▶ gingival crevicular fluid
- ▶ Interleukin-12

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Introduction

Periodontal diseases are a group of disorders with different etiologies and clinical manifestations. They include periodontitis, which is a chronic multifactorial inflammatory disease associated with dysbiotic plaque biofilms and characterized by progressive destruction of the tooth-supporting apparatus. Its primary features include the loss of periodontal tissue support, manifested through clinical attachment loss (CAL) and radiographically assessed alveolar bone loss, presence of periodontal pocketing, and gingival bleeding.¹

Aggressive periodontitis (AgP) is a type of periodontal disease that is characterized by a rapid rate of disease progression, absence of any systemic involvement, and familial aggregation of cases. There is usually an inconsistency between the number of local factors and the periodontal destruction; these risk factors are only detected in a subset of AgP cases.² The prevalence of AgP ranges from 0.5 to 2.5% in different populations.³

Many studies have underscored the importance of the immunoinflammatory response to bacterial infection in the pathogenesis of periodontitis and tissue damage.⁴

Cytokines play a vital role in the direction of inflammatory responses toward either protective or destructive processes.⁵ While the balance between pro- and anti-inflammatory cytokines may protect the periodontal tissue from destruction, an imbalance may result in disease progression.⁶

Interleukin-12 (IL-12) is a cytokine with both proinflammatory and immunoregulatory activity having a major role in the initiation and enhancement of gingival inflammation.⁷ Additionally, IL-12 has been implicated in the pathogenesis of several diseases such as psoriasis,⁷ rheumatoid arthritis,⁸ and periodontitis.⁹

Despite numerous advances in the understanding of the pathogenesis of chronic inflammatory diseases, AgP is still only diagnosed once connective tissue and bone destruction have occurred.¹⁰ Treatment of AgP is time consuming and of high cost; therefore, its prevention, early detection, and management are factors that, if effectively addressed, are likely to yield considerable healthcare benefit.¹¹

Although clinical and radiographic examinations are essential to assess tissue destruction, they may be limited or not sufficient enough to identify certain sites of active disease or the degree of host susceptibility to future disease.¹²

This in turn emphasizes the importance of characterizing biomarker profiles that might functionally interrelate in AgP, which would be suitable to help the development of future novel diagnostic tools that would strongly assist current clinical and radiographic diagnostic approaches.

Currently, there are many different components in gingival crevicular fluid (GCF) that have been investigated as diagnostic and prognostic markers of periodontal disease progression involving inflammatory mediators, markers of oxidative stress, host-derived enzymes, tissue-breakdown products, and mediators of bone homeostasis.¹³

The level of IL-12 in GCF has not been thoroughly investigated, especially in Sudan; therefore, shedding light on this issue is worthy and has promising value.

Based on the aforementioned points, the present study will assess the level of IL-12 among a group of Sudanese patients with AgP.

Materials and Methods

The present study was performed on patients attending the Periodontology Department at Khartoum Dental Teaching Hospital, Sudan. Cases satisfying the eligibility criteria were asked to participate in the study after reviewing and signing an informed written consent. The participants were divided into two groups:

Group A: Patients with AgP (localized and generalized type).

Group B: Healthy subjects.

A nonprobability sampling technique was adopted where a total number of 60 subjects were recruited from dental clinics of Khartoum Dental Teaching Hospital.

Inclusion Criteria

- Subjects included in the study were those who were diagnosed with AgP in accordance with clinical criteria agreed upon by the consensus at the Workshop of Periodontics in 1999.¹⁴
- Subjects who had at least two sites with ≥ 5 mm pocket depth (PD), with ≥ 2 mm interproximal clinical, and radiographic bone loss on first molars and/or incisors and no more than two other teeth diagnosed as having localized AgP. However, subjects who had attachment loss of ≥ 5 mm, PDs ≥ 5 mm, and radiographic bone loss and at least three affected teeth other than first molars and incisors were diagnosed as having generalized AgP.

Smokers, those with systemic illnesses or disorders that might influence periodontal health or medicines, and people who had recently had periodontal treatment or antibiotics, were all excluded from the study.

Data Collection Tools and Techniques

- Demographic data included age, gender, in addition to medical history, which were recorded for each subject.
- A calibrated examiner (Sidahmed M) recorded all clinical periodontal indices: plaque index,^{15,16} gingival index,¹⁷ PD, and CAL for each patient, after the collection of GCF.
- Measurements for those indices were recorded from four sites per tooth.
- University of Michigan "O" probe with William's markings was used to perform the periodontal examination.
- The bone loss estimation was radiographically assessed for each patient to determine the severity and pattern of alveolar bone loss.

GCF Sampling

- Following clinical examination, periodontal sites for GCF collection were isolated with cotton rolls to prevent saliva contamination and air-dried gently with removal of supragingival plaque. For AgP patients, samples were collected from a diseased or affected site (PD \geq 5 mm with concomitant presence of BOP, CAL \geq 2 mm, and radiographically detected bone loss). All the selected sites of healthy subjects were periodontally healthy.
- GCF samples were collected from each site with a sterile absorbent paper strip gently inserted into the sites for 30 seconds and then removed and placed immediately into a cryo vial and put into the cryo box. Samples were then transported under cold temperature to the Institute of Endemic Diseases Laboratory where they were stored at -70 degrees Celsius until analysis was undertaken.

Measurement of IL-12

A quantitative sandwich enzyme-linked immunosorbent assay (ELISA) technique was employed using Human IL-12 ELISA MAX Deluxe Set by BioLegend (San Diego, CA) (has a detection rate of 7 pg/mL of GCF).

The following procedure for the quantitative sandwich ELISA technique was performed:

- On day 1:
 - 100 μ L of diluted capture antibody solution was added to each well. The plate was sealed and incubated overnight between 2°C and 8°C
- On day 2:
 - Plates were washed four times with an addition of 200 μ L 1X Assay Diluent A to each well, then plates were sealed and incubated at room temperature for 1 hour with shaking on a plate shaker. All subsequent incubations with shaking were performed similarly.
 - Plates were washed four times and 100 μ L diluted standards and samples were added to the appropriate wells. Plates were sealed and incubated at room temperature for 2 hours with shaking.
 - Plates were washed four times with 100 μ L diluted detection antibody solution into each well. Plates were sealed and incubated at room temperature for 1 hour with shaking.
 - Plates were washed four times with 100 μ L diluted Avidin-HRP solution added to each well. Plates were sealed and incubated at room temperature for 30 minutes with shaking.
 - Plates were washed five times with soaking for 30 seconds to 1 minute per wash; 100 μ L of freshly mixed tetramethylbenzidine (TMB) substrate solution was added to each well and incubated in the dark for 15 minutes.
 - 100 μ L of stop solution was added to each well and absorbance was read at 450 nm and 570 nm within 15 minutes.

Statistical Analysis

- Statistical Package for the Social Sciences software computer program version 23.0.0.0 was used.
- Means and standard deviations of parameters were calculated. Student's *t*-test or Mann-Whitney U test was used to compare clinical and demographic parameters and biomarker levels among groups.
- Spearman correlation was performed to evaluate associations between clinical parameters and IL-12 levels.

Ethical Consideration

- Ethical clearance was obtained from the University of Khartoum Research Ethics Board and a written ethical clearance was also obtained from Khartoum State Ministry of Health.
- Before performing the periodontal examination or obtaining GCF samples, a written informed consent was obtained after explaining the nature and purpose of the study, declaring that participation is voluntary, and that refusal will not affect a patient's right to receive treatment. Patients were informed that they may withdraw their participation, even during the data collection procedure, without suffering any penalty of loss of privilege.

Results

A total number of 60 participants were recruited (30 cases diagnosed with AgP and 30 healthy controls) for this study. The AgP group included 7 (23.3%) males and 23 (76.7%) females; the mean age of AgP patients' group was 24.2 ± 3.3 years. Out of the 30 AgP patients, 19 (63.3%) had generalized AgP, while 11 (36.7%) had localized AgP. The healthy subjects group included 27 (90%) females and 3 (10%) males with a mean age of 25.1 ± 4.6 , as shown in **Fig. 1**.

Periodontal parameters show that the AgP patients' group had bleeding on probing of $67.5 \pm 31.2\%$ compared with $9.5 \pm 2\%$ for healthy subjects. The AgP group had a mean PPD of 5.8 ± 0.8 mm and mean CAL of 6.3 ± 1.2 mm (**Table 1**).

GCF levels of IL-12 were detected using ELISA that showed a mean concentration of 52.7 and 60.7 ng/mL for healthy subjects and AgP patients' group, respectively. An

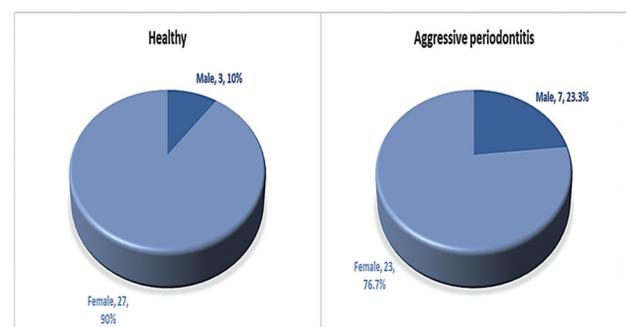


Fig. 1 Frequency of distribution of the aggressive periodontitis and healthy groups by gender.

Table 1 Measurement values of periodontal parameters among cases and control groups

	Healthy	Aggressive periodontitis
BOP (%)	9.5 ± 2	67.5 ± 31.2
PPD	–	5.8 ± 0.8
CAL	–	6.3 ± 1.2

Abbreviations: BOP, bleeding on probing; CAL, clinical attachment level; PPD, periodontal pocket depth.

Table 2 Mean values of IL-12 concentration in the GCF of cases with AgP and healthy subjects

	n	Mean	SD	p-Value ^a
Healthy	30	52.7	18.3	0.120
Aggressive periodontitis	30	60.7	21.2	

Abbreviations: AgP, aggressive periodontitis; GCF, gingival crevicular fluid; IL-12, interleukin-12; SD, standard deviation.

Independent sample's *t*-test was performed.

^ap-Value is not statistically significant.

independent samples' *t*-test was used to compare the mean of IL-12 level in AgP group and healthy subjects; the *p*-value was 0.120 that was found not statistically significant (► **Table 2**).

The independent samples' *t*-test was also performed to compare the concentration of IL-12 in localized versus generalized AgP patients, with a mean of 55.9 and 63.5, respectively, with a *p*-value calculated at 0.347, which was not statistically significant (► **Table 3**).

Pearson correlation was performed to correlate the concentration of IL-12 with periodontal parameters; no statistically significant difference was found with the level of IL-12 and degree of CAL and PPD. This finding may be explained by the different disease statuses during time of sample collection.

The difference in means was also demonstrated according to gender (► **Table 4**). The three males in the control group had a mean of IL-12 of 51.4 compared with seven males in the AgP group with a mean of 58.6. No statistically significant difference was found between the two groups when an independent samples' *t*-test was performed (*p*-value=0.442). On the other hand, there were 27 healthy females with a mean of IL-12 concentration of 52.8 compared with 23

Table 3 Comparison of mean of IL-12 according to type of aggressive periodontitis

	n	Mean	SD	p-Value
Generalized aggressive periodontitis	19	63.5	21.2	0.347
Localized aggressive periodontitis	11	55.9	21.1	

Abbreviations: IL-12, interleukin-12; SD, standard deviation.

Note: Independent samples' *t*-test performed.

Table 4 Correlation between GCF level of IL-12 and periodontal parameters

		BOP	PPD	CAL
Healthy	Pearson correlation	0.038		
	<i>p</i> -Value	0.841		
Aggressive periodontitis	Pearson correlation	–0.17	–0.003	0.049
	<i>p</i> -Value	0.369	0.985	0.797

Abbreviations: BOP, bleeding on probing; CAL, clinical attachment level; GCF, gingival crevicular fluid; IL-12, interleukin-12; PPD, periodontal pocket depth.

females in the AgP group with a mean of 61.4, with a nonstatistically significant difference as well (*p*-value=0.159).

Discussion

Despite advances in the diagnosis of periodontal diseases in general, AgP remains to be only diagnosed after CAL has already occurred. Interest in GCF biomarker profiles as a future diagnostic tool is on the rise to possibly help in early detection and diagnosis of this condition.¹⁸

The present analytical cross-sectional study was aimed at evaluating and comparing the gingival crevicular fluid level of proinflammatory cytokine, IL-12, among a group of Sudanese patients diagnosed with AgP and a group of healthy subjects. A total number of 60 participants were enrolled, with 30 patients with AgP and 30 healthy subjects. The periodontal parameters measured included BOP, PPD, and CAL.

The results of this current study revealed that the GCF level of IL-12 was increased slightly in AgP patients' group in comparison with the healthy subjects group; however, the difference was not statistically significant. This is in agreement with a study also conducted in Sudan, by Zein Elabdeen HR et al, where multiple cytokines were measured in the GCF revealing that the level of IL-12 in AgP patients' group was enhanced but that the level difference was not statistically significant when compared with healthy subjects.⁵

This may be explained in part by the disease state of AgP patients (period of either activation or quiescence) during which the GCF samples were collected. In addition, this may be due to the immune system attempting to locally decrease the inflammatory response and augment molecules involved in the healing process.⁵ It should be taken into consideration that different immune responses might be triggered by different dysbiotic microbial communities.^{13,18} Factors relating to the GCF analytical method must be considered as well.¹⁹ Contradicting results were found in a study by Branco-de-Almeida et al²⁰ in which a highly significant role of IL-12 was found in the GCF of localized AgP patients, suggesting a localized immune response to periodontal pathogens responsible for localized AgP. Another study found the level of IL-12 to be increased with an increase in the inflammation of periodontal tissues of chronic and AgP patients.²¹

Similar results were also found by Sánchez-Hernández et al.²² It should also be noted that the aforementioned studies measured the level of IL-12 in serum, which could be affected by many factors^{23,24} and multiple studies did not find a correlation between concentrations of different cytokines in serum and GCF.^{25,26}

When IL-12 levels were compared between localized and generalized AgP patients, the difference was not statistically significant; levels were slightly increased in generalized AgP patients, yet this difference could be attributed to the fact that there was a higher number of patients with generalized AgP over those with localized AgP. More studies should be conducted with focus on the ratio of generalized AgP to localized AgP patients.

Conclusions

The results of this study demonstrated that the concentration of IL-12 in the GCF was slightly higher in AgP patients' group, although the difference was not statistically different. No significant correlation was found between level of IL-12 in the GCF with periodontal parameters (BOP, PPD, CAL) or with age and gender.

Strengths and Limitations of the Study

The strength of this study lies in the use of GCF as a valid, noninvasive, diagnostic tool and in that the level of IL-12 in the GCF has not been previously investigated in general nor among the Sudanese population specifically. The limitations of this study include the small sample size, which consequently did not allow for results to be generalized to a broader community. A study with a larger sample size, utilizing the new classification of periodontal and peri-implant diseases and conditions, should be conducted to draw more definite conclusions. The uncontrolled design and clear dominance of female patients may have also affected the results of this study.

Conflict of Interest

None declared.

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