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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

Background: Aggressive periodontitis 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. Cytokines play a major role in the initiation and also progression of this disease. Interleukin 12 has both pro-inflammatory 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 Interleukin 12 in the gingival crevicular fluid of patients with aggressive periodontitis, healthy subjects and to correlate its level with periodontal parameters to hopefully help in the development of future detection tools.

Methods: In this study, 30 patients with aggressive periodontitis and 30 healthy subjects were recruited. The periodontal parameters included; bleeding on probing (BOP), periodontal pocket depth (PPD), clinical attachment level (CAL). Gingival crevicular fluid (GCF) levels of interleukin 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 interleukin-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 bleeding on probing, pocket depth and clinical attachment loss=(0.369), (0.985), (0.797) respectively.

Conclusion: The slight increase in the level of interleukin 12 in GCF of aggressive periodontitis patient and slight elevation in sites with attachment loss suggests a possible role of this cytokine in the pathogenesis of aggressive periodontitis. More studies are required to determine the exact role of this cytokine in aggressive periodontitis.

Key words: Aggressive periodontitis, IL-12, Gingival crevicular fluid

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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].

AgP is a type of periodontal disease that is characterized by rapid rate of disease progression, absence of any systemic involvement, and familial aggregation of cases. There is usually an inconsistency between the amount 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%.7 in different populations [3].

Many studies have underscored the importance of the Immuno-inflammatory response to bacterial infection in the pathogenesis of periodontitis and tissue damage [4].

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

IL-12 is a cytokine with both pro inflammatory and Immuno-regulatory activity and has 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, aggressive periodontitis is still only diagnosed once connective tissue and bone destruction has occurred [10] keeping in mind that Aggressive periodontitis is time consuming plus of high cost of treatment and therefore it's prevention, early detection and management are issues which, 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 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 the 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 interleukin-12 in GCF has not been thoroughly investigated specially in Sudan, therefore, shedding a light on this issue is worthy and has a promising value.

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

MATERIAL AND METHODS

The present study was carried out on patients attending the Periodontology department at Khartoum Dental Teaching Hospital. 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.

Non-probability 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 aggressive periodontitis in accordance with the clinical criteria agreed by the consensus at the Workshop of Periodontics in 1999 [14].

Subjects who had at least two sites with ≥ 5 mm pocket depth with ≥ 2 mm interproximal clinical and radiographic bone loss on first molars and/or incisors and no more than two other teeth were diagnosed as having localized aggressive periodontitis. Whereas subjects who had attachment loss of ≥ 5 mm and pocket depths ≥ 5 mm and radiographic bone loss and at least three affected teeth other than first molars and incisors were diagnosed as having generalized aggressive periodontitis.

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, and in addition to medical history were recorded for each subject. A calibrated examiner (Sidahmed M) recorded all clinical periodontal indices; plaque index (PI) [15,16], gingival index (GI) [17], pocket depth (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 ≥ 5 mm with concomitant presence of BOP, CAL ≥ 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 absorbing paper strip gently inserted into the sites for 30 seconds then removed and placed immediately into cryo vial and put into the cryo box then transported in cold temperature to the Institute of Endemic Diseases laboratory where it was stored at -70 degrees Celsius until analysis was undertaken.

Measurement of IL12

A quantitative sandwich ELISA technique was employed using Human IL-12 ELISA MAX™ Deluxe Set by Bio

Legend (has a detection rate of seven pg/ml of GCF).

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

On day one
100 µl 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 two
Plates were washed four times with addition of 200 µl 1XAssay Diluent A to each well, plate were sealed and incubated at room temperature for one hour with shaking on a plate shaker. All subsequent incubations with shaking were performed similarly.

Plates were washed four times and 100 µl diluted standards and samples were added to the appropriate wells. Plates were sealed and incubated at room temperature for two hours with shaking.

Plates were washed four times with 100 µl diluted Detection Antibody Solution to each well. Plates were sealed and incubated at room temperature for one hour with shaking,

Plates were washed four times with 100 µl diluted Avidin-HRP solution added to each well. Plates were sealed and incubated at room temperature for 30 minutes with shaking.

Plates were washed 5 times with soaking for 30 seconds to one minute per wash, 100 µl of freshly mixed TMB Substrate Solution to each well and incubated in the dark for 15 minutes.

100 µl of stop solution was added to each well. Read absorbance at 450 nm and 570 nm within 15 minutes.

Statistical analysis
Statistical Package for the Social Sciences (SPSS) software computer program version 23.0.0.0 was used.

Means and standard deviations of parameters were calculated. Students- t-test or Mann Whitney test was used to compare clinical and demographic parameters and biomarker levels among groups.

Spearman correlations were 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 (KSMOH).

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 patient's right to receive treatment. Patients were informed that they may withdraw their participation even during 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 aggressive periodontitis while 11 (36.7) had localized aggressive periodontitis. The healthy subjects group included 27 (90%) females and 3 (10%) males with a mean age of 25.1 ± 4.6.

Periodontal parameters show that the AgP patients' group had bleeding on probing of 67.5 ± 31.2% compared with 9.5 ± 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 which showed a mean concentration of (52.7) ng/ml and (60.7) ng/ml for healthy subjects and AgP patients group respectively. 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) and was found not statistically significant (Table 2).

Independent samples' T test was also performed to compare the concentration of IL-12 in localized vs. generalized aggressive periodontitis 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 may be explained by the different disease status 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 7 males in the AgP group with a mean of (58.6). No statistically significant difference was found between the two groups when independent samples' T test was

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

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

	N	Mean	SD	P value
Healthy	30	52.7	18.3	0.12
Aggressive periodontitis	30	60.7	21.2	

Independent sample's T test was performed. *P value is not statistically significant.

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	
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		
	P value	0.841		
Aggressive periodontitis	Pearson Correlation	-0.17	-0.003	0.049
	P value	0.369	0.985	0.797

performed (P value=.442). On the other hand, there were 27 healthy females with a mean of IL-12 concentration of (52.8) compared with 23 females in the AgP group with a mean of (61.4) with a non-statistically significant difference as well (P value= 0.159).

DISCUSSION

Despite advances in the diagnosis of periodontal diseases in general, aggressive periodontitis remains to be only diagnosed after clinical attachment loss 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].

This present analytical cross-sectional study was aimed at evaluating and comparing the gingival crevicular fluid level of pro-inflammatory cytokine interleukin 12 among a group of Sudanese patients diagnosed with aggressive periodontitis and a group of healthy subjects. A total number of 60 participants were enrolled; 30 patients with aggressive periodontitis 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 healthy subjects group, however, the difference was not statistically significant which is in agreement with a study conducted in Sudan by Hager et al, where multiple cytokines were measures in the GCF, it was shown that the level of IL-12 in AgP patients group was enhanced but that the level difference was not statistically significant when compared to healthy subjects [5].

This maybe 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 also be considered that different immune responses might be triggered by different dysbiotic microbial communities [13,18]. Factors relating to GCF analytical method must be considered as well [19]. Contradicting results were found in a study by Branco et al. [20] whose results indicated a highly significant role of IL-12 in the GCF of Localized aggressive periodontitis patients, suggesting a localized immune response to periodontal pathogens responsible for localized AgP. Another study found the level of interleukin- 12 to be increased with increase in the inflammation of periodontal tissues of chronic and aggressive periodontitis patients [21].

Similar results were also found by Sanchez et al. [22] It should be taken into consideration that the aforementioned studies measured the level of IL-12 in serum, which might be affected by many factors [23,24] and multiple studies found no correlation when comparing between concentrations of different cytokines in serum and GCF [25].

When IL-12 levels were compared between localized and generalized AgP patients, the difference was not statistically significant, although the levels were slightly increased in generalized AgP patients, this difference could be attributed to the fact that the number of patients with generalized aggressive periodontitis outweighed that of those with localized aggressive periodontitis. More studies should be conducted with regard to the ration of generalized AgP to localized AgP patients included.

CONCLUSIONS

The results of this study demonstrate that 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 using GCF as a valid non-invasive diagnostic tool and the fact that the level of IL-12 in the GFC has not been investigated thoroughly in general and among Sudanese population in specific. The limitations of this study include the small sample size and consequently the results cannot be generalized to the broader community based on this study alone. A study with a larger sample utilizing the new classification of periodontal and peri-implant diseases and conditions should be conducted to draw definite conclusions. The

uncontrolled design and clear dominance of female patients might affect the results of the study.

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