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Association of Interleukin-1 α gene polymorphisms in Sudanese patients with aggressive periodontitis

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The genetic factors as well as microbiological and other environmental factors play a role in the severity and lifetime risk of developing aggressive periodontitis. The aim of this present study was to evaluate the association between interleukin - 1 α (⁺⁴⁸⁴⁵) gene polymorphisms in Sudanese patients with aggressive periodontitis. We genotyped 30 aggressive periodontitis patients and 32 periodontal health participants for IL-1 α (⁺⁴⁸⁴⁵) using standard PCR amplification followed by restriction enzyme digestion and gel electrophoresis. Higher prevalence of heterozygous for IL-1 α (⁺⁴⁸⁴⁵) was found in localized aggressive patients (64.5%) when compared to controls (35.5%) ($P = .005$) in this Sudanese population study, while the prevalence of homozygous for IL-1 α (⁺⁴⁸⁴⁵) was higher in control groups (67.7%) than in localized aggressive patients (32.3%) ($p = .02$). Susceptibility to aggressive periodontitis is increased by heterozygous of IL-1 α (⁺⁴⁸⁴⁵). Moreover, IL-1 α (⁺⁴⁸⁴⁵) gene polymorphisms is an important risk factor of aggressive periodontitis in Sudanese population.

Keywords: aggressive, periodontitis, Interleukin-1 α , β , gene polymorphisms

INTRODUCTION

Aggressive Periodontitis (AP) is a type of periodontal diseases characterized by localized or generalized loss of alveolar bone, usually occurs in a younger age group (≥ 30 years) (Elamin et al., 2011)

A characteristic feature of the former is angular bone loss confined to the permanent first molars and/or incisors, while the latter shows bone loss around these teeth as well as other teeth. It has been suggested that the generalized form is an age-related extension of the localized.

Aggressive periodontitis, as the name is a type of periodontitis where there is rapid destruction of periodontal ligament and alveolar bone which occurs in the systemically healthy individuals of a younger age group. The disease is

generally found to have a racial and sex predilection, with blacks and female teenagers having higher risk for the disease compared to whites and males.

Over the years many definitions and classifications have been adopted for aggressive periodontitis. First described in 1923 as "diffuse atrophy of the alveolar bone", has undergone a series of terminology changes over the years to be finally named as "aggressive periodontitis" in 1999. Aggressive periodontitis was defined by the 1999 International Workshop for the Classification of Periodontal Diseases, according to three primary characteristics: absence of systemic conditions that might contribute to periodontal disease, rapid loss of clinical attachment and alveolar bone (Colin et al., 2000)

Etiology and risk factors of aggressive periodontitis:

Aggressive periodontitis is one of destructive periodontal diseases with multifactorial etiological factors, including host factors, microbiology and genetics.

Genetic Factors

Recently, studies of the risk of periodontitis have gained focus on the genes of immunoregulatory molecules, such as cytokines, chemokines, membrane surface receptors, and antigen recognition proteins. Cytokines such as interleukins (IL-1 α , IL-1 β , IL-6, and IL-10), surface receptors such as the Fc γ family (FCGRs), and (COX-2) and matrix metalloproteinase (MMP) are considered key factors in the progression of aggressive periodontitis recruitment (Komal et al.,2015)

Several genes have been found to be associated with Aggressive Periodontitis. The familial nature of Aggressive Periodontitis has led to speculation that a major gene defect is responsible for its transmission of the AGP .

In 1986, Boughman et al., reported that a major gene located on chromosome 4q was responsible for autosomal dominant transmission of Localized Aggressive Periodontitis in an extended family that also exhibited Dentinogenesis Imperfecta (Abdalla et al.,2017)

Other studies have been suggested that aggressive periodontitis is caused by mutations in multiple genes, combined with environmental effects. In the other hand, early studies suggested an X-linked mode of transmission of aggressive periodontitis, and subsequent studies support an autosomal mode of transmission (Alexandre et al.,2000).

Microbiologic Factors

In the 1999 international Workshop for Classification of periodontal Diseases and Conditions ,the microbial features of AGP were considered as a secondary feature for distinction between aggressive periodontitis and chronic periodontitis . The report suggested an elevated proportion of Aggregatibacter actinomycetemcomitans and some population of *Prophyromonas gingivalis* (Anne et al.,2007). Although other bacteria that have putatively been associated with aggressive periodontitis were proposed by the 1996 World Workshop in periodontics the bacterium Aggregatibacter actinomycetemcomitans is still strongly associated with aggressive forms of the

disease. Based on longitudinal studies, the presence of A. a is considered as a marker for progression of periodontal attachment loss (PAL), and breakdown of periodontal tissues around the teeth (Ashvini et al., 2018)

Seven serotypes (a–g) of A. a have been described. The prevalence of these serotypes is associated with a number of factors, such as demographic structure, ethnicity, and periodontal status of the carriers . Among the serotypes, a, b, and c are the most common. Serotype b is more pathogenic due to its higher capacity to produce leukotoxin, the main virulence factor of A. actinomycetemcomitans . The leukotoxin kills neutrophils and macrophages in cellular processes associated with the activation and release of proteases and interleukin-1 β (IL-1 β), respectively. These properties of the leukotoxin are believed to attenuate the host response and predispose to periodontal breakdown.

AM Elamin suggested that the prevalence of Aggregatibacter actinomycetemcomitans in Sudanese aggressive periodontitis patients is higher than in chronic periodontitis patients (Boughman et al., 1986)

Immunologic Factors

An important factor implicated in periodontal disease initiation is the host's immune response to bacteria. Individuals with localized aggressive periodontitis (LAGP) display a heightened immune response when whole blood is challenged with periodontopathic bacteria or their byproducts

Earlier work suggested that individuals with LgAP were characterized by an inherited defect of neutrophil function that led to a dampened immune response against the micro flora inhabiting the periodontal pocket. Specifically, when challenged with periodontal pathogens, the LAP neutrophils were reported to show impaired chemotaxis, phagocytosis, and killing of bacteria. Alternatively, it was also hypothesized that these impaired defense properties were in fact acquired defects caused by prolonged exposure to an inflammatory microenvironment.

Today, the understanding of neutrophil biology is that neutrophils arriving at a site with uncontrolled periodontal inflammation are primed to attack the invading pathogens by releasing lytic enzymes. These enzymes may, on the other hand, accelerate tissue destruction. It is hypothesized that the observed dampened neutrophil function in deep aggressive periodontitis lesions is due to the heavy commitment of primed neutrophils to debridement

so that less potency remains for chemotaxis and defense .

MATERIALS AND METHODS

The study participants interviewed by questionnaire .Age, gender, medical histories, and oral hygiene status had been record.Measurements were made at six sites per tooth (mesiobuccal, midbuccal, distobuccal, mesiopalat, midpalat, and distopalat) for the first incisor and first or second molars only , using a mouth mirror and a periodontal probe(University of Michigan-O probe) to assess indicators of periodontal health status.

Aggressive periodontitis in these participants was defined as subject with at least 4 teeth with interproximal sites showing \geq 3mm attachment loss and age between 10-30 years.

Five ml of venous blood was collected from each subject, immediately transferred into EDTA tubes (EDTA as anticoagulant) and stored at 4°C for DNA extraction.

Study area and study population:

This case control study was conducted during (June, July ,August 2016) in Khartoum Dental Teaching Hospital. The study participants were 62 subjects, 30 subjects of them diagnosed as Aggressive periodontitis for detection of interleukin-1 α and 32 subjects of participants as control.

Genetic analysis

DNA extraction:

DNA was extracted from blood samples using salting out method with Maxime PCR PreMix Kit (i-Taq):Product Catalog No.25025. Blood samples were taken by venipuncture from the arm vein of each subject and collected with EDTA as an anticoagulant . Genotyping was performed by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) technique using specific primers and enzymes (Table 1). The amplification condition of IL-1 α is described in Table 2. For PCR to occur, the product was visualized by staining with ethidium bromide using UV gel documentation system, using 2% agarose gel electrophoresis . PCR products of IL-1 α then were digested by restriction enzymes Taq 1 and Nco 1 (Fermentas, Germany). Cleaved DNA fragments were subjected to electrophoresis in 17% polyacrylamide gel for IL-1 β and in 2.5% agarose

gel for TNF- α . The final products were stained with silver nitrate (IL-1 β) and ethidium bromide .

Analysis of IL-1 α +4845 polymorphism

The primers 5' ATG GTT TTA GAA ATC ATC AAG CCT AGG GCA 3' and 5' AAT GAA AGG AGG GGA TGA CAG AAA TGT 3' were used. The 100 bp region of the IL-1 α +4845 gene was amplified for 45 cycles using the thermo cycler, with each cycle consisting of 94°C for 1 min, 94°C for 1 min, 56°C for 1 min, 72°C for 2 min, and 72°C for 5 min. DNA was digested with Fnu4HI restriction endonuclease (New England Biolabs, Beverly, MA, USA) at 37°C. The genotypes were expressed as high or low producers for the homozygous OR heterozygous

Statistical Analysis

Frequencies for patients and controls were compared by calculation of the odds ratio (OR) and 95% confidence intervals (95% CI). Genotypes were calculated for each individual, with those homozygous for the high producer allele classed as "high producer", heterozygous as "intermediate producer", and those homozygous for the low producer allele as "low producer" genotype, respectively. Genotype frequencies were then compared by control analysis. Ethical consideration of

This study was approved by the Ethical Committee of the Al-Neelain University and complies with the International Declaration of Helsinki.

RESULTS

Out of the ninety five of well diagnosed aggressive periodontitis Sudanese patients, thirty patients were interested to take part in this study eleven male and (19) females , from 15 to 25 years of age (mean age 19.33 \pm 2.5), after being consented. 32 Sudanese healthy volunteers were recruited for comparison seven male and fifteen female, from 18 to 30 years of age (mean age 23.25 \pm 4.0).

Demographic and clinical data are shown in table 1. Mean CAL in the LAgP group was significantly higher (8.11 \pm 2.27) than that in control groups (0.76 \pm 0.82). The frequency of ct, cc and tt genotypes in periodontitis group (n=30) were 15, 12 and 3, respectively and in control group (n=32) were 12, 10 and 0, respectively. statistically significant difference was found between the two groups (p=0.116). In periodontitis group and control group, the frequency of C and T alleles were 74, 42 and 88, 32, respectively.

Table 1: -Demographic and clinical data in case and control

	Agp	Controls
Male	11(36.7%)	15(46.9%)
Female	19(63.3%)	17(53.1%)
Mean age	15-25(19.33 \pm 2.5)	15-30(23.16 \pm 4.1)
CAL(mm)	8.11 \pm 2.27	0.76 \pm 0.82
PD(mm)	6.6 \pm 2.16	1.78 \pm 1.2
BOP(%)	84.5%	15.5%

Table 2: - Presence of IL-1 α +4845 polymorphisms and genotype distribution in the two study groups

Groups	Polymorphism genotype N (%)				
	(CT)	(CC)	(TT)	Herto	Homo
GAP	20(66.7%)	8(26.7%)	2(6.6%)	40(64.5%)	20(32.3%)
Periodontal health	12(37.5%)	20(62.5%)	0(%)	22(35.5%)	42(67.7%)

No statistically significant difference was found between the two groups ($p=0.114$, Table 2).

While the prevalence of heterozygous alleles for IL-1 α was higher in the control group (62.5%), the prevalence of IL-1 α homozygous allele was higher in localized aggressive periodontitis individuals (63.3%). This difference was statistically no significant ($\chi^2 = 22.51$, $P = 0.00$) (Table2).

DISCUSSION

The patient population of this study was selected from those referred for treatment at the Khartoum Dental Teaching Hospital during the period of the study. In the present study, we have evaluated 30 well diagnosed Sudanese aggressive periodontitis. The results of the present study, show that the overall male to female ratio, based on the population screened was 1:2. Other comparable studies corroborate the higher frequency of occurrence of aggressive periodontitis among females.

This result supports the evidence presented by (AshviniK .et al ., 2018) who reported 1:2male: Female ratio⁽⁹⁾ . Abdalla S A, and Abdalraouf A A in 2017 reported that males were slightly more affected than females (1:0.9) in Sudanese population (Boukourt KN, et al., 2015)

Moreover, a significant association between aggressive periodontitis and increased attachment loss (8.11 \pm 2.27), and probable pocket depth (6.6 \pm 2.16) was found in this study . This is observation agrees with other epidemiological studies (Elamin et al., 2018)

In this study, we evaluated the functional genetic polymorphisms of IL-1 α (+ 4845 C/T) for their association with aggressive periodontitis . Our study revealed significant positive correlation

between aggressive periodontitis and presence of IL-1 α (+4845) in this Sudanese population. This in agreement with previous studies of some African populations . (Boukourt KN et al.,) reported that there is significant association between IL-1 α and aggressive periodontitis in the Algerian population Karasneh et al., 2018)

A study done in Danish white adults population to evaluate the effect of the Genetic polymorphisms of cytokines with the susceptibility, severity, and clinical outcome of inflammatory diseases, such as aggressive periodontitis and chronic arthritis , the authors of this study suggested that IL-1 α genotypes were associated with cytokine levels in patients with aggressive periodontitis and chronic arthritis (Karimbux et al., 2012)

. Komal et al.,2015 in their study have reported that there is a positive association between aggressive periodontitis and the presence of the IL-1 α -889, allele 2 polymorphism in Indian patients (MaélsonKlever et al., 2017)

Karimbux et al.in their meta analysis concluded that IL-1 gene polymorphisms, most prominently IL-1 α (-889), IL-1 α (+4845) and IL-1 β (+3954), have been associated with periodontitis in whites (Michael,1996). Although our founding in this study supported by many previous study , some other studies disagree with this finding. Karasneh et al did not obtain any significant results for specific IL-1 genotypes and/or alleles to predict susceptibility to generalized aggressive periodontitis in a Jordanian population(Quappe et al., 2004)

A meta-analysis based on 19 case-control studies performed by (Wang . et al., 2014) suggested that interleukin-1 α -899 (+4845) C \rightarrow T polymorphism is not associated with aggressive

periodontitis susceptibility(Wang et al., 2014)

CONCLUSION

In conclusion, our data suggest that IL-1 α gene (+4548) may be associated with an increased risk of aggressive periodontitis in Sudanese population Nevertheless; the small group of participants in our study limited the investigation of cytokine gene polymorphisms in relation to the development of aggressive periodontitis . The findings of the present study highlight the need for more studies to elucidate the relative contributions of various factors in diseases with a multifactorial etiology where there is interplay among genetic factors.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

FA , and MO designed and performed the experiments and also wrote the manuscript and blood collection, and data analysis . AM performed DNA extraction , flow cytometry experiments,. WM and IM designed experiments and reviewed the manuscript. All authors read and approved the final version.

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