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Association analysis of the *IL-1* gene cluster polymorphisms with aggressive and chronic periodontitis in the Algerian population

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ABSTRACT

Objective: There is strong evidence that genetic as well as environmental factors affect the development of periodontitis. Various studies suggest that genetic polymorphisms of the interleukin-1 (IL-1) genes are associated with an increased risk of developing the pathogenesis. The aim of the present study was to investigate the possible relationship between two polymorphisms of IL-1 gene cluster IL-1B (C + 3954T) (rs1143634) and IL-1A (C − 889T) (rs1800587) SNPs and the aggressive and chronic periodontitis risk in a case control study in Algerian population.
Methods: 279 subjects were recruited and received a periodontal examination: 128 healthy controls and 151 cases. From cases, 91 patients were having a chronic disease whereas 60 subjects with aggressive form. All these subjects were genotyped for IL-1A (C − 889T) and IL-1B (C + 3954T) polymorphisms using TaqMan real time PCR technology. Frequencies of IL-1 alleles, genotypes and the haplotypes were also examined.
Results: Significant differences were found in the carriage rate of both minor alleles of the IL-1A (C − 889T) and IL-1B (C + 3954T) polymorphisms of aggressive periodontitis cases compared with healthy controls (OR [95%CI] = 1.61 [1.03–2.49], $p = 0.03$), (OR [95%CI] = 1.69 [1.09–2.63], $p = 0.01$), respectively. The result did not reach significance with the chronic form.
Conclusion: The studied polymorphisms of the IL-1 genes appear to be associated with susceptibility to aggressive periodontitis (AgP) in the Algerian population.

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1. Introduction

It is well established that genetic and environmental factors affect the severity and lifetime risk of developing periodontal disease. Furthermore, it is recognized that microbial factors cannot be solely responsible for periodontitis. Although the host's responses to bacterial challenge in periodontal tissues vary depending on the individual immune genetic heritage; so the genetic factors explain the clinical variability in periodontitis. The evidence for a genetic susceptibility to periodontitis comes from twins[1,2] and association studies.[3,4] However, the genetic basis has not been clearly defined. Since the attachment loss observed in patients with periodontitis, has been shown to be induced by Gram-negative anaerobic bacteria mediated by inflammatory activation, many studies have suggested that some polymorphisms of candidate genes related to inflammatory response may influence the susceptibility to periodontitis. Furthermore, it has been hypothesized that periodontitis is linked to an increased or unregulated production of inflammatory cytokines. The Interleukin-1 (*IL-1*) cytokine genes are among the candidate genes involved in regulation of the inflammatory immune response and bone resorption.[5] They are among the most frequently studied genes involved in periodontitis predisposition.[6,7,8] *IL-1* is a bone resorption stimulating cytokine implicated in the development of periodontal lesions. It is also considered one of the most active stimulators of osteoclastic activity.[9] Recent studies suggest that the quantitative production of this mediator depends on a specific allelic combination in its gene cluster.[10] The *IL-1* gene cluster is localized on the chromosome 2 and many polymorphisms have been identified in *IL-1A*, *IL-1B*, and *IL-1RN* genes. *IL-1A* and *IL-1B* are in close proximity and encode respectively IL-1a and IL-1b proteins. Furthermore, *IL1B* activates the expression of a large panel of genes encoding key proteins of the inflammatory response,[11] and an exacerbated secretion of IL-1b has very harmful consequences for the organism.[12] *IL-1A* (C − 889T) (rs1800587) and *IL-1B* (C + 3954T) (rs1143634) polymorphisms have been most frequently reported to be associated with the risk of periodontitis in many population based studies. These polymorphisms are located within the regulatory regions of the genes, with a potential functional importance by modulation of the IL-1 protein production,[13] and they have been related with the development and the severity of autoimmune and infectious diseases.[14] According to the latest classification of Armitage,[15] periodontitis manifests itself in two clinical forms in otherwise healthy subjects: chronic periodontitis (CP) and aggressive periodontitis (AgP). The chronic periodontitis trait is the most common, with a prevalence of 10–15%,[16] and the aggressive periodontitis trait has a prevalence of 1.29%.[17] Two previous studies have reported the evidence of genetic predisposition to the aggressive periodontitis in the Algerian population through two approaches: family segregation analysis and quantitative genetics studies.[18,19] Nevertheless, there is no data relating the implication of *IL-1A* (C − 889T) and *IL-1B* (C + 3954T) polymorphisms to periodontitis in this population. In this context, the purpose of the present study is to investigate the association of these polymorphisms of the IL-1 genes with aggressive and chronic periodontitis risk in Algerian population.

2. Material and methods

2.1. Population study

The study's objectives and procedures were approved by the ethics committee at the National Evaluation and Planning Committee of the Algerian university Research. This case-control study included study population of 279 unrelated subjects of Algerian origin (over 16 years old) and consisted of 151 periodontitis cases (60 AgP; 91 CP) and 128 healthy controls. All participants signed a consent form after being informed of the nature of study. The subjects were recruited from four periodontal services of university hospitals: University hospital of Oran, University hospital of Sidi belabess, University hospital of Tlemcen and Setif. The clinical periodontal examination was performed by periodontics specialists. Clinical pocket depth (PD) and clinical attachment level (CAL) were measured at six sites of each tooth, the proximal plaque index (PI) and the gingival index (GI) were determined; the alveolar bone loss proximal cross was evaluated by a panoramic and/or a series of plain radiographs. The measurement was performed by the evaluation of clinical and radiographic parameters to detect the presence of alveolar bone loss, and to confirm the presence or absence of periodontitis in all participants. The subjects were asked to complete a questionnaire of personal information, dental hygiene, familial aggregation and medical history. The sample was divided into three groups: control subjects without a periodontitis, subjects with aggressive periodontitis and subjects with chronic periodontitis.

The inclusion criteria required that all subjects must be in good general health, did not have any manifestation of systemic diseases or medical disorders (diabetes mellitus, immunological disorders, hepatitis, HIV infection, rheumatic diseases, and cardiovascular involvement), pregnancy, genetic disorders, endodontic lesions, gingivitis and necrotizing periodontal diseases. Moreover the subjects who had a recent history of systemic antibiotic or anti-inflammatory treatment within the previous 3 months or any other regular medication were also excluded from this study. Only clear diagnostic cases with chronic or aggressive periodontitis individuals, and the healthy controls that did not have a self/family history of periodontal disease (based on clinical and radiographic examination) were included in this study. Any suspicion in the clinical classification was excluded from the study.

The patients arranged as chronic or aggressive periodontitis were diagnosed according to the criteria of the classification system of the American Academy of Periodontology established in 1999 at the International Workshop for Classification of Periodontal Diseases and Conditions.[20] Diagnosis of aggressive periodontitis is made on clinical, radiographic and historical findings which show very rapid evolution of osteolysis and rapid clinical attachment loss (proven by radiographs obtained a few years apart. When this was not possible, a young age was used as a sign of rapid progression in patients $\leq$35 at the time of the initial diagnosis), usually affecting persons under 30 years of age, but patients may be older, and possible familial aggregation of disease. The severity of attachment loss was inconsistent with the amount

of mineralized plaque. The diagnosis of generalized aggressive periodontitis is based on clinical grounds: minimal accumulation of mineralized plaque. They had clinical attachment loss in at least 30% of the teeth, at least three affected teeth other than first molars or incisors with a generalized interproximal probing pocket depth, bone loss in vertical and horizontal affected sites (are detectable in radiography). The diagnosis of localized aggressive periodontitis is based on the following criteria: interproximal attachment loss and interproximal probing pocket depth for permanent teeth, of which at least one was a first molars or incisors only. A positive family history confirm the diagnostic of the aggressive form but patients showing clear clinical signs of aggressive periodontitis even without a reported positive family history were still included. Chronic periodontitis is characterized with the presence of local factors such as plaque and calculus. At least bone destruction of three teeth in different quadrants, had an interproximal profound probing pocket depth or clinical attachment loss detectable in the X-ray. Localized chronic periodontitis was diagnostic when 30% or less of sites affected and generalized chronic periodontitis is described as more than 30% of sites affected. Control subjects without any periodontal disease must have: a probing depth $\leq$3.5 mm, had no teeth with abnormal attachment or bone loss was present at any site in clinical examination and radiographs. 5 to 10 ml whole blood samples were collected from each participant in EDTA tubes.

2.2. DNA extraction

Genomic DNA was isolated from peripheral blood leukocytes by means of the Stratagen kit (Agilent Technologies, USA). The DNA samples were stored at −20 °C in TE buffer (10 mM tris, 1 Mm EDTA) until required. The concentration and the purity of DNA were estimated by measuring the absorbance at wavelengths 260 nm and 280 nm. DNA integrity was checked and quantified using agarose gel electrophoresis.

2.3. Genotyping

All samples were genotyped on TaqMan real time PCR (Applied Biosystems (ABI), Foster City, CA, USA). The test TaqMan PCR reaction was performed in 20 μl mixture containing 1× of TaqMan Assay, TaqMan Genotyping MasterMix and DNA per well, negative control was included in all plaques (well without DNA). The essay includes two locus-specific PCR primers that flank the SNP of interest (F forward, and R reverse) and two allele-specific oligonucleotide TaqMan fluorescent probes (VIC and FAM). Context Sequence [VIC/FAM] used for *IL-1A* (C − 889T) (rs1800587) polymorphism gene is GATTTTTACATATGAGCCTTCAATG **[A/G]** TGTTGCCTG GTTACTATTATTAAAG. In each part Context Sequence [VIC/FAM] used for *IL-1B* (C + 3954T) (rs1143634) polymorphism gene is **CATAAGCCTCGTTATCCCATGTGTC** **[G/A]** AAGAAGA-TAGGTTCTGAAATGTGGA.

2.4. Statistical analysis

The Hardy–Weinberg equilibrium was evaluated using Pearson's χ^2 test for controls with degree of freedom. The association between *IL-1A* (C − 889T) and *IL-1B* (C + 3954T) polymorphisms and periodontitis risk were calculated as odds ratio (OR) with 95% confidence intervals (CI). The fisher's exact test was used. The differences were considered statistically significant when $p < 0.05$. Additionally, haplotypes distribution analyses were estimated by using the software THESIAS version 3.1. All statistical analyses were performed by EpiInfo computer software version 7.

3. Results

3.1. Patients and controls characteristics

279 subjects were enrolled in this case-control study, 151 patients were diagnosed with Aggressive (AgP) or chronic periodontitis (CP) and 128 subjects were diagnosed as periodontally healthy. All demographic and clinical data were summarized in Table 1. There are very few smokers in our sample. The average age is 25.9 years for aggressive patients, and for chronic periodontitis there is 37.36 years. The majority of subjects are females. The clinical parameters (the plaque index (PI), clinical attachment level (CAL), the probing depth (PD) and the gingival Index, (GI) are higher in the periodontitis patient groups than in healthy control group.

3.2. Distribution analysis of IL-1A and IL-1B frequencies between cases and controls

All samples were analyzed for polymorphisms of the *IL-1A* (C − 889T) and *IL-1B* (C + 3954T). The genotype distributions of these polymorphisms were consistent with the assumption of Hardy–Weinberg equilibrium ($p > 0.05$, $\chi^2 < 5.99$). Our results indicated a signficant differences in the genotype distributions of *IL-1B* (C + 3954T) but, no signficant differences in the genotype distributions of *IL-1A* (C − 889T); neither was observed any statistical variation in the allele frequencies of the studied polymorphisms in all cases and controls (Table 2).

3.3. Frequencies of IL-1A (C − 889T) genotypes and alleles

Cases were stratified according to the chronic or aggressive form and we found that the distribution of the *IL-1A* (C − 889T)

Table 1 – Clinical characteristics of periodontitis (cases) and control subjects.

Characteristics	Cases $n = 151$		Controls $n = 128$
	PgA $n = 60$	PC $n = 91$	
PI (mean ± s.d)	1.89 ± 0.67	2.29 ± 0.72	0.35 ± 0.08
CAL (mean ± s.d)	2.27 ± 0.70	1.81 ± 0.68	–
PD (mean ± s.d)	6.75 ± 1.35	3.96 ± 0.64	1.49 ± 0.5
GI (mean ± s.d)	1.93 ± 0.52	2.11 ± 0.50	0.026 ± 0.08
Age (mean ± s.d) years	25.94 ± 5.03	37.36 ± 8.82	26,69 ± 7,52
Smokers (%)	00 (00.00%)	5 (5.49%)	02 (1.56%)
No smokers (%)	60 (100%)	86 (94.50%)	126 (98.43%)
Gender male/female	13/47	17/74	50/78

Plaque index, PI; clinical attachment level, CAL; Probing depth, PD; Gingival Index, GI; standard deviation, s.d.

Table 2 – Distribution of genotypes and alleles frequencies of IL-1A − 899 and IL-1B + 3954 in all cases and healthy controls.

	Cases		Controls	
	n	%	*n*	%
IL1A − 889 Genotypes				
CC	55	(36.42%)	53	(41.40%)
CT	70	(46.35%)	58	(45.31%)
TT	26	(17.21%)	17	(13.28%)
Total	151		128	
Alleles				
C	180	(59.60%)	164	(64.06%)
T	122	(40.39%)	92	(35.93%)
IL-1B + 3954 Genotypes				
CC	77	(50.99%)	53	(41.40%)
CT	51	(33.77%)[a]	61	(47.65%)
TT	23	(15.23%)	14	(10.93%)
Total	151		128	
Alleles				
C	205	(67.88%)	167	(65.23%)
T	97	(32.11%)	89	(34.76%)

[a] $p = 0.03$, OR = 0.57[0.34–0.95].

genotypes observed was significantly different between AgP and controls (OR [95%CI] = 2.62 [1.11–6.20], $p = 0.02$) which was confirmed by a difference in allele distribution (OR [95%CI] = 1.61 [1.03–2.49], $p = 0.03$) (Table 3). Thus, the overall distribution of the *IL-1A* (C − 889T) genotypes was found to be significantly different between generalized aggressive periodontitis (GAgP) cases and controls (OR [95%CI] = 3.37 [1.29–8.78], $p = 0.01$) and we also observed a significant difference for the allelic distribution (OR [95%CI] = 1.87 [1.13–3.09], $p = 0.01$) (Table 4). Concerning CP group, the homozygosis for the C allele at position −889 was noted in 39.56% compared to 41.26% of healthy subjects and homozygous for T allele in 10.98% of CP while 13.49% of healthy subjects, but these differences failed to reach statistical significance ($p = 0.9$) (Table 3). Also the overall distribution of genotype and allele frequencies were not significantly different between chronic periodontitis (localized and generalized) and controls ($p = 0.9$).

3.4. *Frequencies of IL-1B (C + 3954T) genotypes and alleles*

In the subgroup analyses by disease category an association with *IL-1B* (C + 3954T) polymorphism alleles was detected between the AgP cases and controls (OR [95%CI] = 1.69 [1.09–2.63], $p = 0.01$). When looking at the different disease entities: localized aggressive periodontitis (LAgP), generalized aggressive periodontitis (GAgP) and CP, a statistically significant association was detected between LAgP and healthy controls (OR [95%CI] = 0.22 [0.05–0.65], $p = 0.002$) (Table 4).

No statistical differences were observed between chronic periodontitis cases localized and generalized and controls alleles ($p > 0.05$), but the overall distribution of genotype frequencies was different ($p = 0.03$). The homozygosis for the C allele was noted in 51.64% of chronic periodontitis compared to 41.40% of healthy subjects, and homozygous of T allele was noted in 17.58% of chronic periodontitis compared to 10.93% of healthy subjects, but these differences failed to reach significance (Table 3).

Table 3 – Distribution of genotypes and alleles frequencies of IL-1A-899 and IL-1B + 3954 in aggressive and chronic cases and healthy controls.

	AgP		CP		Controls	
	n	%	*n*	%	*n*	%
IL1A − 889 Genotypes						
CC	19	(37.58%)	36	(39.56%)	53	(41.40%)
CT	25	(45.39%)	45	(49.45%)	58	(45.31%)
TT	16	(17.02%)[a]	10	(10.98%)	17	(13.28%)
Total	60		91		128	
Alleles						
C	63	(52.50%)	117	(64.28%)	164	(64.06%)
T	57	(47.50%)[b]	65	(35.71%)	92	(35.93%)
IL-1B + 3954 Genotypes						
CC	30	(50.00%)	47	(51.64%)	53	(41.40%)
CT	23	(38.33%)	28	(30.76%)[c]	61	(47.65%)
TT	7	(11.66%)	16	(17.58%)	14	(10.93%)
Total	60		91		128	
Alleles						
C	63	(52.50%)	122	(67.77%)	167	(65.23%)
T	57	(47.50%)[d]	60	(33.33%)	89	(34.76%)

[a] $p = 0.02$; OR: 2.62[1.11–6.20].
[b] $p = 0.03$; OR: 1.61[1.03–2.50].
[c] $p = 0.02$; OR: 0.51[0.28–0.93].
[d] $p = 0.01$; OR: 1.69[1.09–2.63].

Table 4 – Distribution of genotypes and alleles frequencies of IL-1A − 899 and IL-1B + 3954 in localized and generalized aggressive periodontitis patients and healthy controls.

	GAgP		LAgP		Controls	
	n	%	*n*	%	*n*	%
IL1A − 889 Genotypes						
CC	12	(29.26%)	7	(36.84%)	53	(41.40%)
CT	16	(39.02%)	9	(47.36%)	58	(45.31%)
TT	13	(31.70%)[a]	3	(15.78%)	17	(13.28%)
Total	41		19		128	
Alleles						
	40	(48.78%)	23	(60.52%)	164	(64.06%)
T	42	(51.21%)[b]	15	(39.47%)	92	(35.93%)
IL1B + 3954 Genotypes						
CC	15	(36.58%)	15	(78.94%)	53	(41.40%)
CT	19	(46.34%)	4	(21.05%)[c]	61	(47.65%)
TT	7	(17.07%)	0	(0.0%)	14	(10.93%)
Total	41		19		128	
Alleles						
C	49	(59.75%)	34	(89.47%)	167	(65.23%)
T	33	(40.24%)	4	(10.52%)[d]	89	(34.76%)

[a] $p = 0.01$; OR: 3.37 [1.29–8.78].
[b] $p = 0.01$; OR: 1.87 [1.13–3.09].
[c] $p = 0.01$; OR 0.23 [0.05–0.79] (Fisher's exact test).
[d] $p = 0.002$; OR 0.22 [0.05–0.65] (Fisher's exact test).

3.5. *Composite genotypes and haplotypes study*

The most frequent *IL-1A* and *IL-1B* composite genotype was the homozygous for the allele 2 of *IL-1A* and allele 1 of *IL-1B*, (2-2/1-1). It was more frequent in cases (33.11%) than in controls (32.81%). The prevalence of the positive composite genotype (at least one allele 2 is present at each locus) was higher in cases (35.09%) than controls (46.09%), but the difference was not significant ($p = 0.06$), (Table 5). Concerning the haplotypes analysis shown in Table 6, no statistical differences were found in our sample ($p > 0.05$).

4. Discussion

It has been suggested that polymorphisms of some cytokines have a direct influence on the inflammatory diseases. Therefore, polymorphisms of the *IL-1* genes have been implicated in the pathogenesis of periodontal diseases in view of a many proinflammatory characteristics of this cytokine.[21] IL-1a is the membrane form while IL-1b is the secreted form; both show high levels in gingival crevicular fluid in periodontitis patients.[22,23] Kornman et al. reported an

Table 5 – Distribution of the IL-1A − 889 and IL-1B + 3954 composite genotypes.

	Cases		Controls	
Composite genotypes	*n* = 151	%	*n* = 128	%
1-1/1-1	4	02.64	1	0.78
1-1/2-2	13	08.60	9	07.03
2-2/1-1	50	33.11	42	32.81
1-2/1-1	23	15.23	10	07.81
1-1/1-2	8	05.29	7	05.46
1-2/2-2	10	06.62	4	03.12
2-2/1-2	4	02.64	10	07.81
1-2/1-2	38	25.15	44	34.37
2-2/2-2	1	00.66	1	00.78
Positive genotype	53	35.09	59	46.09

$p = 0.06$; 1: major allele; 2: minor allele.

association between the *IL-1* polymorphisms and adult periodontitis,[3] and several other studies have reported a confirmation of this hypothesis;[5,24] whereas others have rejected this relationship.[8,25] In the present study, we tested the allelic association of the *IL-1* gene cluster polymorphisms *IL-1A* (C − 889T) and *IL-1B* (C + 3954T) between AgP, CP and healthy controls subjects in Algerian population. As various studies we found that periodontitis disease is usually more common in females than males.[26,27,28] However, several other studies have noted different gender distributions.[29,30] The fact that there is a majority of women may be due in part to hormonal exchange during the menstrual cycle and pregnancy which may consequently worsen the clinical course of this disease.[26] On the other hand, the recruitment of subjects in this study was done at random, yet we recall that our sample is composed mainly of female non-tobacco consumers, so this can be explained by the socio-cultural factor in our population. As consequence, we have not sought a correlation between smoking and periodontitis. For patient's age parameter, it is in accordance with the latest American Academy of Periodontology criteria.[15] Aggressive periodontitis usually affecting persons under 30 years of age but patients may be older, in fact we note in our study 5 patients older than 40 years of age. Chronic periodontitis as occurring mostly in adults, but it can be seen in younger people in fact we note in this study 11 patients with chronic periodontitis under than 20 years of age.

Our results of the association between the *IL-1A* (C − 889T) and periodontitis are in agreement with Lopez et al. and Kornman et al. studies, in which the minor allele 2 of *IL-1A* is often more frequent in cases than in control group.[3,31] As our results, many authors found a higher risk of severe periodontitis in carriers of the allele 2 of the *IL-1A*.[3,32] Regarding the role of *IL-1* in the pathogenesis of periodontitis, several studies

Table 6 – Study association of haplotypes.

	Cases (*n* = 151)	Controls (*n* = 128)	*p*-Value	Odds ratio IC 95%
Haplotypes				
Haplotype TC	21	10	0.10	1.9 [0.80–4.21]
Haplotype TT	40	36	0.95	1.08 [0.60–1.84]
Haplotype CC	82	73	0.64	1.11 [0.69–1.79]
Haplotype CT	8	9	0.54	1.35 [0.50–3.61]

have shown increased production of IL-1a by circulating monocytes in patients with periodontitis. It has been reported that allele 2 of *IL-1A* is associated with an almost 4-fold increase in IL-1a protein levels.[33] Indeed, Kiani et al. found in patients with advanced periodontitis a strong but not significant trend towards increased IL-1a production by peripheral blood polymorphonuclear cells,[34] while no association was found between the allele 2 of the *IL-1A* and periodontitis in other studies on Caucasian[6,35] and Japanese populations.[36]

In the present study, no association of *IL-1A* gene polymorphism was detected for the chronic form of periodontitis, such the result in Chinese,[37] and in Jordanian populations.[38] In contrast to other studies performed in the Caucasian population.[3,39] It should be possible to explain this variance results by ethnic specificity of each population and inter-individual variation in cytokine production, we suggest that further studies should be based to confirm or refute these results.

Through bone resorption and its proinflammatory properties, *IL-1B* has been considered to have an effect in the pathogenesis of periodontal disease; as consequence more importance has been given to it. It has been found in higher concentrations at periodontal disease sites and several studies have reported increased levels of IL-1b in the gingival crevicular fluid and gingival tissues of patients with periodontitis.[40] However, in view of the etiologic complexity of periodontitis, the role of *IL-1B* is not yet exactly clear. Our results support a positive association between AgP and allele 2 of the *IL-1B* (C + 3954T) polymorphism. This result is in agreement with studies realized in Italian and Chilean populations.[41,42] Also, Diehl et al. and Parkhill et al. identified the *IL-1B* polymorphism as implicated in periodontitis,[43,44] too. Indeed, the genotype 2-2 of *IL-1B* (C + 3954T) polymorphism was associated with an increase in IL-1b production. Pociot et al. found that monocytes from patients carrying the allele 2 of the *IL-1B* gene at position +3954 produced a greater amount of IL-1b than patients without this allele.[45] So, the allele T appears to be related to the severity of periodontal disease.[24] On the other hand, Gore et al. found that neutrophils from patients with advanced periodontitis and carrying *IL-1B* (C + 3954T) allele 2 produced increased IL-1b levels compared to patients without this allele but the differences were not significant.[22] In contrast to other studies, Scapoli et al. conclude no association between AgP and *IL1B* gene was found in Italian Caucasian population,[46] and a study of Galbraith et al. found no significant relationship between the genotype status and the level of cytokine production.[47] While Santtila et al. found that allele 2 of the *IL-1B* polymorphism was associated with decreased IL-1b secretion.[48] Thus the association between *IL-1B* polymorphism and levels of IL-1b is not fully clarified.

In the present study, LAgP patients have a higher prevalence of allele 1 for *IL-1B* (C + 3954T), and the homozygous for this allele is the most frequent genotype in our sample. This result agrees with findings of other studies.[31] Walker et al. found that allele 1 for *IL-1B* is shown in all African-American LAgP patients;[49] Gonzales et al. compared *IL-1A* (C + 4845T) and *IL-1B* (C + 3954T) genotypes in two populations (European-Caucasians and central American-Hispanic) with AgP, for both polymorphisms, allele 1 was the most common allele, but these results were not statically significant.[50] Also Pociot et al. reported allele 2 as a rare polymorphism of the *IL1B* gene and he suggested that this allele was responsible for an increase in IL-1b production in LAgP patients,[45] while other studies reported no association between LAgP and *IL-1B* allele 1 polymorphism.[43] Our results show a significant protective effect of the allele 2 (OR = 0.22). This is contrary to results found in the Turkish LAgP patients which found that susceptibility to LAgP is increased by homozygosis of *IL-1B* (C + 3954T) allele 1.[26] These conflicting results may suggest the presence of several genetic profiles for different forms of periodontitis in each separate population or they may be due to the diversity of detection methods and inter-individual variation in cytokine production, and we propose another future study that should clarify these assumptions.

However, we haven't found any association between *IL1B* (C + 3954T) polymorphism and GAgP and this finding is consistent with the results reported in European Caucasian and Japanese populations.[6,36] On the other hand, there were no significant differences in the distribution of *IL-1B* (C + 3954T) among CP and healthy controls in the studied population, in contrast to studies that have previously reported this polymorphism to be associated with the severity of CP,[51] and other conducted in Caucasian and Brazilian populations.[3,52]

The severe form of CP was reported to be strongly associated with the presence of allele 2 of both *IL-1B* (C + 3954T) and *IL-1A* (C − 889T) (composite genotype). However in our population we did not find a statistically significant association between periodontitis and the composite genotype neither with the chronic form of periodontitis. This might have resulted from differences in ethnic background and disease. We propose to increase the sample size to confirm our findings.

It has been reported that the association between positive genotype and periodontitis is on the assumption that these polymorphisms can modulate the secretion of *IL-1* but this relationship has not been completely demonstrated. Indeed, no significant differences were found in IL-1b production from monocytes obtained from positive-genotype and negative-genotype patients.[53] Also Engebretson et al. found elevated levels of IL-1b in the gingival crevicular fluid in shallow sites in patients carrying the positive genotype, but no statistical differences were found for deeper pockets.[54] In the present study, 35.09% of patients with periodontitis were identified as carriers of positive genotype versus 64.90% of patients carrying negative genotype but this result did not reach significance $p = 0.06$.

5. Conclusion

This is the first study to explore the association of the *IL-1* gene and periodontitis in the Algerian population where allele and haplotype frequencies were established. We were interested in two clinical forms of periodontitis, chronic and aggressive. These two forms were studied and compared to healthy controls from the same population. Furthermore, the feature of this study is that subjects in our sample were selected from

a homogeneous population with similar ethnic and socioeconomic status. It is possible that the contribution of *IL-1* genes was more involved in determining the genetic susceptibility for AgP compared to CP in the present study. It can be concluded that *IL1* gene polymorphisms contribute to susceptibility to this disease, *IL1* genotype appears to be an important risk factor for AgP in this population. Therefore, it will be necessary to explore other genes that are involved in bone resorption and periodontal tissue inflammation (like those involved in bone metabolism and vitamin D receptor), and it will be necessary to raise the size of our sample. This is the only study conducted in the Algerian population. Thus, we proposed to perform other studies where the number of males and the smoking factor must be more representative to confirm or affirm our results in Algerian patients with periodontitis disease.

Conflict of interest statement

The authors report no conflicts of interest related to this study.

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