LIBYAN JOURNAL OF MEDICINE
2023, VOL. 18, 2225252
https://doi.org/10.1080/19932820.2023.2225252

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ORIGINAL ARTICLE

Screening of interleukin 17F gene polymorphisms and eight subgingival pathogens in chronic periodontitis in Libyan patients

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ABSTRACT

Background: Chronic periodontitis (CP) is triggered by periodontal pathogens influenced by genetic and environmental factors. Recent studies have suggested that anti-inflammatory cytokines such as interleukin 17 (IL−17) play a prominent role in the pathogenesis of CP.

Aim: This study aimed to investigate the association between eight sub-gingival pathogens and interleukin 17F (IL−17F) gene single nucleotide polymorphisms with CP among Libyans.

Materials and Methods: A case–control study was conducted on 100 individuals between the ages of 25–65 years. Species-specific 16S rRNA primers for each pathogen were used in a multiplex PCR reaction to detect sub-gingival pathogens from a paper point sample. DNA was also extracted from buccal swab samples and IL−17F polymorphisms were detected by Sanger sequencing.

Results: A highly significant association between the seven sub-gingival pathogens and CP, (p-value 0.0001) and a high prevalence of *P. intermedia* (100%), *T. forsythia* (96%), *T. denticola* and *E. corrodens* (92%), *P. gingivalis* (82%), *C. rectus* (74%), *P. nigrescens* (72%), *A. actinomvcetcmcomitans* (40%) were found in the case group compared with control group. A novel variant in the c. *34 G>A in IL−17F gene caused a change in glutamic amino acid to lysine amino acid, position on chromosome number (6) in the third exon, mRNA/genomic position 597, found in 14.6% of CP patients (p-value = 0.010) while the IL−17F (rs763780) SNP showed no association with CP (p-value = 0.334).

Conclusion: P. intermedia appear as keystone pathogen for CP in the Libyan population. A novel variant in the IL−7F gene may be related to the severity of CP.

ARTICLE HISTORY
Received 24 January 2023
Accepted 11 June 2023

KEYWORDS
chronic periodontitis; sub-gingival pathogens; novel SNP; IL-17F; polymorphism

1. Introduction

Chronic periodontitis (CP) is an inflammatory disease characterized by the irreversible destruction of the periodontium [1]. It is one of the dominant poly-microbial infections and considered the second most common disease worldwide after dental decay [1]. Socransky et al. in 1998 proposed that oral diseases could be better understood by focusing on the complexes of organisms rather than on individual pathogens. They identified five complexes of bacteria that were repeatedly found together in periodontitis [2]. Most of the pathogenic complex, including *P. gingivalis*, *T. denticola*, and *T. forsythia* termed the red complex, was strongly associated with CP. They are also often associated with each other and with diseased sites and may inhibit innate host defence functions [2,3]. Other recognized pathogens are called the orange complex, including *P. intermedia*, one preceding colonization and proliferation, *P. nigrescns*, and *C. rectus which* increase the depth of periodontal pockets [3]. Also, there is a green complex including *A. actinomycetemcomitans* and *E. corrodens*, which are often considered important in periodontal disease [4]. Understanding of the etiology and pathogenicity of CP has increased significantly with extensive analysis of the dental plaque associated with either clinically healthy or diseased sites. A microbial shift has been noted in the oral cavity from mostly Gram-positive in the healthy periodontium to mostly Gram-negative in the diseased site [5]. The subgingival microbial profile associated with CP has been reported as being significantly different by ethnicity and geographical location [2,6]. However, T cells have been recognized to play an important role in the pathogenesis of periodontitis, T helper 1 (Th1), T helper 2 (Th2), and T helper 17 (Th17) cytokines can be detected in the gingival tissues of patients with periodontitis [7]. T helper 17 cells have a protective role in host defence in the clearance of bacterial and fungal infections, but they have also been implicated in playing a pathogenic role in chronic inflammatory conditions [8].

IL−17 is a pro-inflammatory cytokine secreted by activated T cells after transformation to T helper 17

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cells. The IL−17 family contains six members IL−17 (A, B, C, D, E, F) and five receptors, IL−17 R (A, B, C, D) and SEF [7]. IL−17F has a role in the regulation of inflammation, osteoclast activity, amplification of the pro-inflammatory response, and promotion of autoimmunity [9]. IL−17F has been recognized to play an important role in the pathogenesis of periodontitis and can be detected in the gingival tissues, crevicular gingival fluid (CGF), saliva, and plasma of patients with periodontitis. Imbalances in cytokine production may affect the induction of bone resorption in periodontal disease [8,10].

IL−17F has mainly a protective role in oral squamous cell carcinoma, previous studies investigated the expression of IL−17F in oral cancers and reported a protective role of IL−17F in oral cancer while one suggested a protumorigenic effect for IL−17F [11–14].

Single nucleotide polymorphisms (SNP) is a variation at a single position in a DNA sequence, that replaced with any of the other three kinds of nucleotides (Adenine, Guanine, Cytosine, Thymine) that occurs at a specific position in the genome, where each variation is present to some significant degree within a population with a frequency above 1% [15].

Most genetic research in periodontitis has focused on gene polymorphisms that play a role in immune regulation or metabolism, such as cytokines, cell-surface receptors, chemokines, enzymes, and factors related to antigen recognition [16].

IL17F (rs763780) has a diverse spectrum of effects depending on the target organ, it is clear that IL17F (rs763780) is an important locus for effects related to the human immune system, previous studies on the IL17F (rs763780) variant in asthmatic patients showed that this polymorphism is protective against asthma and characterized the function of this mutant at the molecular level, other study showed that the IL17F (rs763780) variant in risk locus for psoriasis [17,18].

The present preliminary study aimed to investigate eight sub-gingival pathogens in CP patients and examine the association between IL-17F (rs763780) gene polymorphisms and the susceptibility to CP in the Libyan population.

2. Materials and methods

2.1. Study population

This case-control study consisted of 100 Libyan adults (50 females and 50 male). Fifty healthy controls (HC) and 50 CP patients whose ages ranged between 25 - 65 years were included. Libyan adults were randomly selected from volunteers who live in different geographical places in Libya, divided into three geographic regions (West, East, and South). Patients enrolled signed an informed consent form. Ethical approval for this study was obtained from the Bioethics Committee at Biotechnology Research Center (BEC. BTRC0 5–2018). Questionnaires in Appendix 1 and 2 were filled, and oral examinations carried out in the Periodontology Department of Faculty of Dentistry, University of Tripoli, in addition to other public and private dental clinics, the study was conducted in the laboratories of Genetic Engineering Department at Libyan Biotechnology Research Center in Tripoli-Libya.

Inclusion criteria for the current study were; absence of systemic diseases such as (i.e. immunologic and autoimmune disorders, diabetes mellitus, rheumatoid arthritis, inflammatory bowel diseases, and psoriasis), patients who had not received any periodontal treatment during the last six months and non-smoker patient. Patients not meeting these criteria were excluded.

2.2. Clinical examination

The periodontal status of each participant was examined based on Periodontal Disease Index (PDI). By using University of Michigan '0' probe with William's markings Probing depth (PD), clinical attachment loss (CAL), and Bleeding on probing (BOP) were measured for each tooth at six sites (mesiovestibular, vestibular, distovestibular, mesiolingual, lingual, and distolingual).). Radiographic examination was performed by using full-mouth multiple of periapical radiographs to determine the amount of bone loss around the teeth [19].

Accordingly, the participants were diagnosed either CP patients or HC group. The CP group included all individuals who had at least three sites in each tooth with PD >3 mm, CAL >3 mm, and more than 30% of BOP [20,21].

2.3. Sample collection

2.3.1. Sub-gingival sample (paper point) for detection of periodontal pathogens

Paper point (META BIOMED, China) size 30 were sterilized by autoclave (121°C, 15 psi, 15 min), then gently inserted in the bottom of the sulcus for 10 seconds, as shown in Figure 1. The samples were placed in a sterile 1.5 ml microcentrifuge (Eppendorf) tube containing phosphate buffer saline (PBS) and transported in ice containers.

2.4. PCR detection of periodontal pathogens

Bacterial DNA was extracted using a modified protocol according to Ashimoto et al. [14] Species specific primers were used in 16S rRNA-based multiplex PCR assay (QIAGEN®, Germany) [22]. The specificity of primer sets was assessed by blasting their sequences against reference oral bacterial 16S rRNA gene

sequences in Human Oral Microbiome Database (HOMD). A total volume of 50 µl containing 2× master mix 25 µl, 10× primer mix 2 µm each primer 5 µl, RNase-free water 15 µl, and template DNA, 5 µl was used. Thermocycling program was initial denaturation at 95°C for 15 min, followed by 38 cycles of denaturation at 95°C for 30 sec, primer annealing at 57°C for 90 sec and extension at 72°C for 90 sec, and a final extension at 72°C for 10 min. The PCR products were fractionated in a 1.5% agarose gel electrophoresis in Tris-Borate EDTA buffer.

The gel was visualized in a UV trans-illuminator (Vilber Lourmat UV transilluminator) to examine the presence of PCR fragments at the expected length by comparison with a 100 bp DNA ladder (metabion International AG, Germany). To achieve the most accurate multiplexing results, primers were evaluated individually before the multiplexing. Sterile paper point size 30 mm was added as a negative control in all PCR reaction.

2.5. Buccal swab sample for IL17 F gene detection

Samples were collected by gently rubbing the inside of the mouth using a non-invasive cotton-tipped buccal swab on both sides of the buccal mucosa, for 10 seconds.

2.6. Genotype determination

Isohelix DNA Isolation Kits were used for extraction of human genomic DNA. A nanodrop lite spectrophotometer was used to measure concentration and purity of extracted DNA. Agarose gel electrophoresis was used to detect the integrity of the DNA.

IL– 17F (rs763780) gene was performed by polymerase chain reaction (PCR), using specific primers described by Zacarias et al. The specificity of primers was checked by BLAST [23].

The PCR reaction mix contained; 1× GoTaq™ Reaction, Buffer 10 µl of 5× Buffer (with 1.5 mM MgCl2), 1 µl of dNTPs (0.2 mM each dNTP), with 1.5 µl each of forward and reverse primers (0.3 µM), 1.25 µl of GoTaq™ DNA Polymerase, 2 µl of templates DNA was added and finally the total volume of 50ul was made up using nuclease-free water.

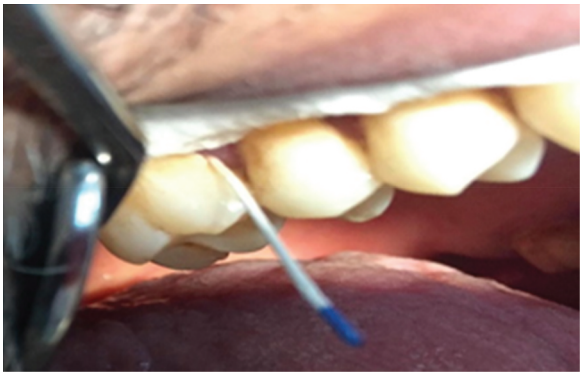

Figure 1. Method of paper point sample collection in CP patient.

To analyze the successful run of the PCR program and to detect PCR product of the correct length agarose gel electrophoresis was used, PCR products were purified by using PureLink® PCR Purification Kit (Invitrogen) to remove excess salts, PCR primers, and dNTPs. Post-Purification Agarose Gel Electrophoresis was run to obtain purified PCR product.

The purified PCR products were cycle sequenced to detect the presence of IL−17F (rs763780) SNP in the DNA fragment. Sanger sequencing method was applied and manufacturer's protocol was followed using the BigDye® Terminator v3.1 Cycle Sequencing kit (Applied Biosystems). Data analysis output were imported to the Sequencher software version 5.1.

2.7. Statistical analysis

All statistical analyses were performed using the SPSS version 25 statistical software package (SPSS Inc., Chicago, Illinois, USA). The Mann – Whitney U test was used to identify the differences in clinical periodontal parameters (PD, CAL, BOP) between CP patients and HC group. A Chi-square test was used to determine the association between eight sub-gingival pathogens and CP and also, to determine the association between IL−17F gene polymorphism and CP, a *p*-value of less than 0.05 was considered statistically significant for all analyses.

3. Results

The study included 28 females (56%) and 22 males (44%) in CP group. 22 females (44%), and 28 males (56%) in HC group. The geographical distribution in CP and HC groups among Libyans; as summarized in Table 1.

Statistically significant association between CP and age ($P = 0.014$) was found. Whereas no statistically significant associations were observed between CP and gender ($P = 0.230$), the distribution of the participants according to age groups and gender as summarized in Table 2.

The clinical parameters (PDI, BOP, CAL, PD) of CP and HC groups were comparable in mean and standard deviation (Table 3). The association was significantly high (p-value = 0.0001) association between clinical parameters (PDI, BOP, CAL, PD) in the two study groups.

Highly significant association was found between the eight sub-gingival pathogens and CP (p-value = 0.0001), except *A. actinomvcetcmcomitans* (p-value =

0.007) as shown in Table 4. The results of agarose gel electrophoresis of (red, orange green) complex in CP group were shown in Figures 2, 3, 4.

In total, 28 bacterial combinations were tested for the CP group by using the Chi-square test. Statistically significant odds ratios ($p < 0.05$) were obtained for 18 of the 28 bacterial combinations. Significantly positive association was obtained in 9 of the 18 with a high odds ratio for any two species; *P. gingivalis/ T. denticola, T. forsythia* and *P. intermedia* at (OR = 4.28, 8.78, 8.78).

A. actinomycetemcomitans/E. corrodens, P. nigrescens, P. intermedia, and *C. rectus* (OR = 8.5, 13.5, 28.5, 28.5). Another high ratio between *C. rectus/P. intermedia* (OR = 12.64), *P. nigrescens/ P. intermedia* (OR = 13.61), as shown in Table 5.

Genotype analysis in mRNA/genomic position 597 a novel variant c. *34 G>A in IL−17F was found in 14.6% of CP patients. The substitution of a single nucleotide causes a change from glutamic amino acid to lysine amino acid. This change is found within the sequence AGAAGCTGTAGAAATGCCACT on chromosome number (6), in the third exon of IL−17F. Figure 5. This novel variant has not been described in any database (NCBI dbSNP, ClinVar, ExAc, 1000 genome) nor reported in other studies. The analysis of genotypic and allelic frequencies of the novel variant c. *34 G>A in IL−17F showed a significant relation between novel genetic variant c. *34 G>A and CP (p-value = 0.010). In addition, A allele considered as minor allele frequency (MAF), the distribution of novel genetic variant c. *34 G>A in CP was only in severe cases (PDI score 6) as summarized in Tables 6, 7).

The analysis of genotypic and allelic frequencies for mRNA/genomic position 553, as shown in Figure 6, the analyzed polymorphisms showed that there is no association between IL−17 F (rs763780) and CP (p-value = 0.334) as summarized in (Table 8 ,9).

Table 1. Geographical distribution of CP and HC groups.

Geographical reign	CP N (%)	HC N (%)	Total 100
West	22 (44)	30 (60)	52
South	17 (34)	8 (16)	25
East	11 (22)	12 (24)	23

4. Discussion

CP has a multifactorial etiology, stemming from the development of biofilm on the tooth surface and gingiva to induce a gingival inflammation, continued along with the supporting tissues of the teeth leading to progressive attachment loss, pocket formation and bone loss, and eventually tooth loss [2,24].

The present study was investigated the association between eight sub-gingival pathogens and CP and among the Libyan population. Little information has been published regarding the periodontal conditions

Table 2. Association between chronic periodontitis and both age and gender.

Parameter	Distribution	CP	HC	p-value
Age	25–35	14	26	0.014
	36–45	15	15	
	46–55	16	9	
	56–65	5	0	
Gender	Male	22	28	0.230
	Female	28	22	

Table 3. Analysis of clinical parameters by mean and standard deviation.

HC (Mean ± SD, n = 50)	CP (Mean ± SD, n = 50)	Clinical parameters
67.0 ± 1.90	5.40 ± 0.49	PDI
7.34 ± 12.15	38.58 ± 11.85	BOP
1.90 ± 0.67	8.46 ± 1.47	CAL
1.90 ± 0.67	6.08 ± 0.98	PD

Table 4. Prevalence and distribution of the 8 sub-gingival pathogens in CP and HC groups.

Sub-gingival pathogens	CP n = 50%)	HC n = 50%)	p-value
P. intermedia	50 (100)	20 (40)	0.0001
T. forsythia	48 (96)	3 (6)	0.0001
T. denticola	46 (92)	10 (20)	0.0001
E. corrodens	46 (92)	10 (20)	0.0001
P. gingivalis	41 (82)	6 (12)	0.0001
C. rectus	37 (74)	16 (32)	0.0001
P. nigrescens	36 (72)	15 (30)	0.0001
A. actinomycetemcomitans	20 (40)	8 (16)	0.007

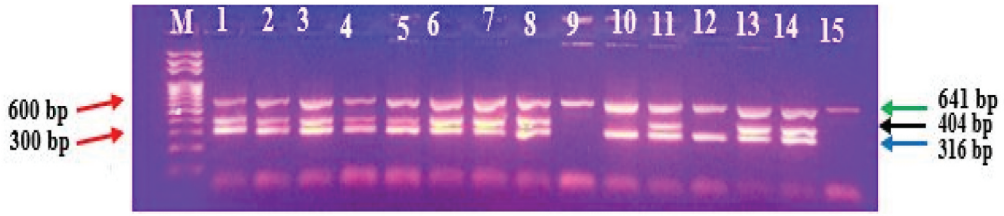

Figure 2. Multiplex bands of specific 16S gene regions in red complex pathogens for CP group.

Lanes 1–15 amplified target DNA *for T. denticola* at sizes 316 bp, *P. gingivalis* at sizes 404 bp, and *T. forsythia* at size 641 bp. M represents the 100 bp DNA ladder. Red arrows represent the corresponding sizes in the ladder for approximate estimation of PCR amplicon size. Green arrow represent the expected amplicon size of 641 bp, black arrow represents the expected amplicon size of 404 bp and blue arrow represents the expected amplicon size of 316 bp.

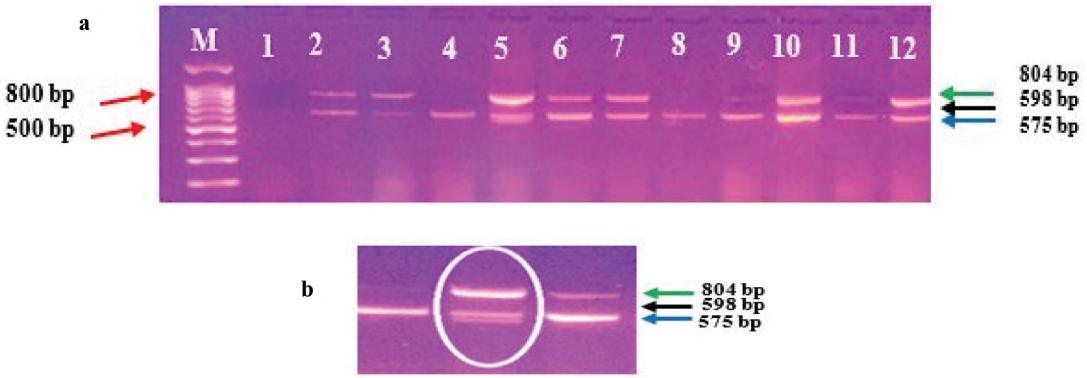

Figure 3. Multiplex bands of specific 16S RNA gene regions in orange complex pathogens for CP group.

A. Lanes 1–12 show amplified target DNA for *P. intermedia* at sizes 575 bp, *C. rectus* at size 598 bp, and *P. nigrescens* at size 804 bp. M represents 100 bp DNA Ladder. Red arrows represent the corresponding sizes in the ladder for approximate estimation of PCR amplicon size. Green arrow represents the expected amplicon size of 804 bp, black arrow represents the expected amplicon size of 598 bp, and blue arrow represents the expected amplicon size of 575 bp.

B. Magnification of sample number 5.

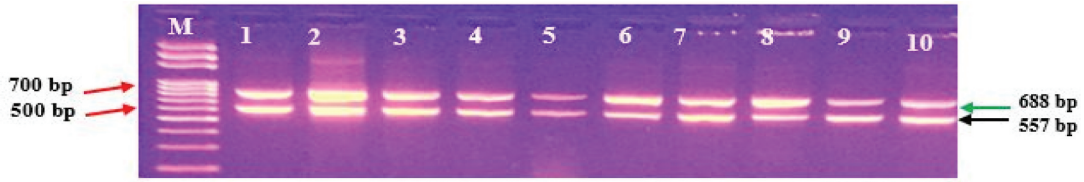

Figure 4. Multiplex bands of specific 16S gene regions in green complex pathogens for CP group.

Lanes 1–10 show amplified target DNA for *A.actinomycetemcomitans* at sizes 557, *E. corrodens* at size 688 bp. *M* = 100 bp DNA Ladder red arrows represent sizes in the ladder for approximate estimation of PCR amplicon size green arrow represents the expected amplicon size of 688 bp and black arrow represents the expected amplicon size of 557 bp.

*Agarose gel electrophoresis of PCR products revealed a single band at the predicted size.

in the Libyan population and their effects on periodontal health.

Previously a cross-sectional survey was conducted among the young adults in Sebha city, south of Libya and 40.63% of patients were detected with pockets depth (4–5 mm) and 4.06% were detected with pockets depth (>5 mm), only 4.7% of young adults in Sebha had healthy periodontium [25]. CP was initiated through the colonization and invasion of sub-gingival pathogens or their products into the periodontal tissues leading, directly or indirectly, to degradation of the periodontium resulting in tissue destruction [26]. The clinical impact of sub-gingival periodontal pathogens extends beyond the oral cavity. Strong associations have been found between periodontal pathogens and several systemic conditions including cardiovascular disease, preterm birth, and preterm low birth weight [27,28], in addition to autoimmune diseases such as diabetes mellitus and rheumatoid arthritis [29,30].

The eight sub-gingival pathogens exhibited a significant relationship with PD, CAL and BOP were detected more frequently in deeper periodontal pockets (≥5 mm) in CP patients which agrees with Socransky and Haffaiee [2]. *T. forsythia* could be useful as an indicator bacterium of periodontal destruction in its early phase [29].

Table 5. Odds ratio (95% confidence intervals) of associations among species tested from ($N = 50$) chronic periodontitis subjects.

Sub-gingival pathogens	*P.gingivalis*	*T. denticola*	*T.forsythia*	*A. actinomycetemcomitans*	*P. nigrescens*	*C. rectus*	*P. intermedia*	*E. corrodens*
P. gingivalis								
T. denticola	4.28* (0.87–21.06)							
T. forsythia	8.78* (1.06–72.52)	1.91 (0.33–10.97)						
A. actinomycetemcomitans	0.14* (0.05–0.36)	0.06* (0.01–0.19)	0.03* (0.006–0.13)					
P. nigrescens	0.47 (0.17–1.25)	0.24* (0.07–0.83)	0.11* (0.04–0.52)	13.5* (2.81–64.67)				
C. rectus	0.78 (0.27–2.19)	0.24* 0.07–0.83)	0.14* (0.02–0.67)	28.5* (3.52–230.1)	1.01 (0.38–2.62)			
P. intermedia	8.78* (1.09–74.29)	3.91 (0.42–36.37)	1.95 (0.17–22.79)	28.5* (3.52–230.1)	13.61* (1.69–109.10)	12.64* (1.57–101.7)		
E. corrodens	2.78* (0.70–11.04)	0.91 0.21–0.91)	0.45 (0.08–2.62)	8.5* (2.20–32.83)	6.61* (1.39–31.27)	2.89 (0.86–9.76)	0.23 (0.025–2.17)	

* Refers to significant p-value.

Global differences in prevalence of sub-gingival pathogens in patients with CP visualized in different populations in Mediterranean countries (Spain, Italy, Turkey, and Morocco), west Asia (Iran, Yemen), East Asia (China, Japan), South Asia (India), South America (Brazil), Western Europe (Switzerland), and Middle Africa (Congo) [31,32]. The differences in prevalence rates are not caused by geography solely, as well as differences among different ethnic or racial groups, variety of microbial identification methods, including culture [33,34].

In the current study, a significantly positive association was obtained in 9 of the 18 with a high odds ratio for any two species of 8 sub-gingival pathogens. The relationship between the red complex bacteria showed a positive association between (*P. gingivalis/T. denticola*) and (*P. gingivalis/T. forsythia*) (OR = 4.28, 8.78). The relationship between the orange complex bacteria (*P. intermedia, P. nigrescens* and *C. rectus*) showed remarkable positive association between *C. rectus/ P. intermedia* (OR = 12.64), *P. nigrescens/ P. intermedia* (OR = 13.61). Additionally, there was an obvious association between red and orange complexes bacteria and there was a strong positive association between *P. gingivalis* and *P. intermedia* (OR = 8.78).

The relationship between the two green complex bacteria (*A. actinomycetemcomitans* and *E. corrodens*) showed a strong positive association (OR = 8.5), also, the relationship between the green and orange complex bacteria showed a very strong positive association between *A. actinomycetemcomitans/P. nigrescens, P. intermedia* and*C. rectus* (OR = 13.5, 28.5, 28.5).

Comparing our results to Ashimoto et al., similar positive results were obtained but with lower OR values, they were obtained between T. *denticola/ P. gingivalis*(OR = 3.44), *P. gingivalis/P. intermedia* (*OR*= 5.85), *P. intermedia/C. rectus* (OR = 3.33), *A. actinomycetemcomitans/P. nigrescens*(OR = 3.57) [22].

While comparing to Mahalakshmi *et al.*, similar positive results but with lower OR values, they were obtained between, *A. actinomvcetcmcomitans/E. corrodens(OR = 4.54), A. actinomvcetcmcomitans/P. nigrescens (OR = 4.73), P. intermedia/C. rectus*(*OR = 3.05*) [35].

High odds ratio between eight sub-gingival pathogens may indicate a symbiotic relationship in periodontal pockets. On the other hand, some pathogens may colonize together in periodontal pocket since they both produce destructive disease without interacting with each other [22].

IL– 17 is a pro-inflammatory cytokine that stimulates T cells, fibroblasts and osteoclasts for bone resorption, and takes part in dendritic cell maturation. It produces and secretes a wide spectrum of

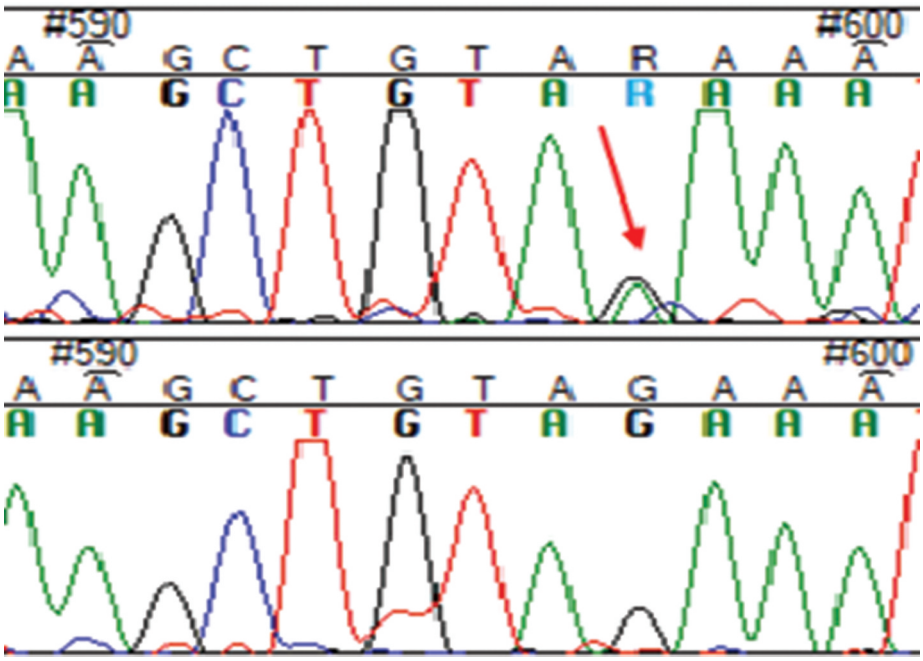

Figure 5. Chromatogram showing novel genetic variant c. *34 G>A in IL – 17 F gene (ambiguity code: R).

Table 6. Genotype frequency for novel genetic variant c. *34 G>A in IL – 17 F gene.

IL–17F Genotype	*CP N (%)*	*HC N (%)*	*MAF (CP)*	*p-value*
AA	0 (0)	0 (0)	A = 7.31	0.010
GG	35 (85.3)	8 (100)		
AG	6 (14.6)	0 (0)		
Total	41	8		

Table 7. Allele frequency for novel genetic variant c. *34 G>A in IL17F gene.

IL–17F Allele	*CP N (%)*	*HC N (%)*	*p-value*
G	76 (92.68)	16 (100)	0.010
A	6 (7.31)	0 (0)	
Total	82	16	

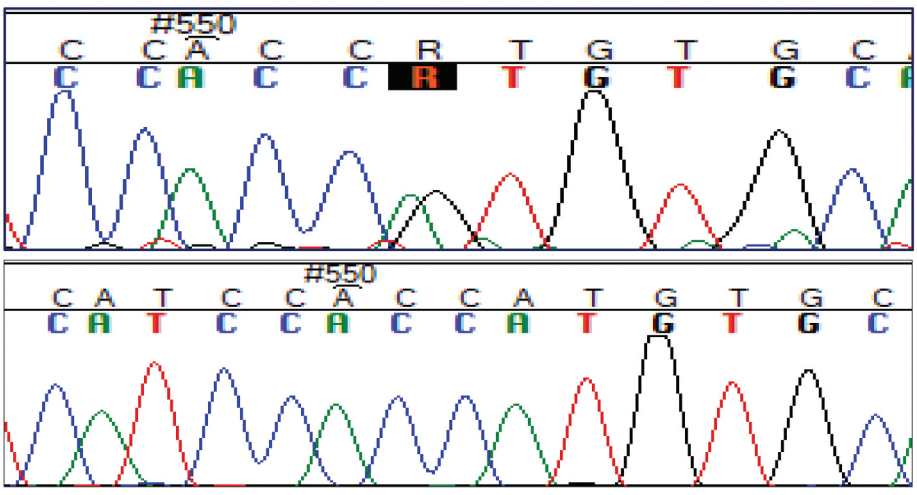

Figure 6. Chromatogram showing T7488C (rs763780) SNP in IL17F (ambiguity code: R).

Table 8. Genotype frequency for IL – 17F.

IL–17F Genotype	*CP N (%)*	*HC N (%)*	*MAF (CP)*	*p-value*
TT(A)	32 (78)	0(0)	C = 15.85	0.334
CC (G)	4(10)	8(100)		
TC (R)	5(12)	0(0)		
Total	41	8		

Table 9. Allele frequency for IL – 17F gene (rs763780).

IL–17F Allele	*CP N (%)*	*HC N (%)*	*p-value*
T	69 (84.14)	0 (0)	0.334
C	13 (15.85)	16 (100)	
Total	82	16	

inflammatory factors such as IFγ, tumour necrosis factor- α (TNF- α), IL– 6 and IL– 8.

IL– 17 levels in saliva, gingival crevicular fluid and plasma were observed to be significantly higher in periodontal disease [36–38].

Genetic studies of CP have focused on SNPs of genes encoding cytokines, the severity of CP may be affected by the genetic control of the cytokine function [39]. Genetic associations found in one population need not necessarily hold true in another population and vice versa, in Libya no reports, until now have investigated the association between IL-17F gene polymorphisms and CP.

A polymorphism is defined as a variant with a frequency above 1%. The term 'polymorphism' which has been used widely often leads to confusion because of incorrect assumptions of pathogenic and benign effects, respectively. According to the American College of Medical Genetics and Genomics standards and guidelines in 2015, the term polymorphism is replaced by 'variant' with the following modifiers: (i) pathogenic, (ii) likely pathogenic, (iii) uncertain significance, (iv) likely benign and (v) benign [40,41].

Several SNPs have been associated with the occurrence of CP, but the full range of genetic influence in outcomes remains to be determined. IL–17 gene polymorphisms may create phenotypic differences and allelic variants in the interleukin response which is important for an individual's susceptibility to CP, progression of the disease, or response to treatment [42]. IL–17F (rs763780, H161R) revealed that the His-to -Arg substitution at amino acid 161 in the 3rd exon of the IL–17F gene causes loss of the ability of IL–17F to induce expression of certain cytokines and chemokines [43]. IL– 17F (rs763780) gene polymorphism was found not associated with chronic periodontitis among different populations (Brazilian, Turkish and Indian), genotype and allele frequencies of IL–17F (rs763780) polymorphism were similar in both periodontitis and periodontally healthy individuals There was no relationship between the severity of the disease and genotype frequencies [23,43,44].

The present study agreed with previous studies among different populations such as Brazilian, Turkish and Indian, there was no association between IL– 17F (rs763780) gene polymorphism and CP among Libyan population (p-value = 0.334).

The study has a limited sample size; investigating a larger number of subjects in a larger geographic area would be more representative and informative.

5. Conclusion

Seven sub-gingival pathogens showed a strong association with CP in Libyan population. In Libya, the diagnosis of periodontal diseases is only based on the clinical examination. Analysis of the sub-gingival dental plaque in clinically diseased sites and determination of well-known subgingival pathogens in the periodontal pockets would allow the progression of the periodontal disease to be estimated. Accordingly, prediction of the disease progression would allow targeted preventive therapy. Investigation of the association between the sub-gingival pathogens and CP would develop a microbiological diagnostic method for CP to support clinical diagnosis.

The novel variant c. *34 G>A may be related to the severity of CP, more extensive genetic studies with a larger sample size are needed to clarify the association between novel variant c. *34 G>A and CP within the Libyan population. There was no association between IL – 17F (rs763780) gene polymorphism and CP among the Libyan population.

The use of the DNA sequencing method is generally preferred over other methods like restriction enzymes when screening the entire gene and allows the detection of more variants in IL–17F gene, especially when studying new populations.

In Libya, genetic studies regarding periodontitis are limited, the information contained within the human genome leads to better understanding mechanism and development of periodontitis; this information would be invaluable during screening and may provide potential therapeutic targets for periodontitis.

Acknowledgments

The authors would like to thank the staff of the Genetic Engineering Department at the Libyan Biotechnology Research Center in Tripoli, Libya for their valuable help and guidance.

Disclosure statement

No potential conflict of interest was reported by the authors.

The research team have submitted the sequence of IL −17 F novel SNP (c. *34 G>A) accession number OL632297 and sequence of IL−17 F (rs763780) accession number OL632296 in NCBI database.

Funding

The author(s) reported there is no funding associated with the work featured in this article.

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Appendix
Appendix 1 Questionnaire& consent form in Arabic language

الاستبيان

العمر ———— تاريخ الميلاد ———————– الاسم ثلاثي ————

مكان السكن–———————— الجنس ————————

المهنة ومكان العمل-————————رقم الحالة————فصيلة الدم ————————-

تاريخ التسجيل——— رقم الهاتف

خى العزيزة اخى العزيز: نشكركم خالص الشكر لاشتراكم معنا في هذه الدراسة والتي تهدف إلى تحديد عاقة تعدد أشكال النوكليوتيدات المفردة في جين انترلوكين 17(اف) بمرض التهاب اللثة المزمن وأكثر أنواع البكتيريا المصاحبة للمرض بين المرضى اليمنيين

الرجاء الإجابة على جميع هذه الأسئلة:

(1) ———— (هل تعاني من مرض السكري ؟ (نعم) (لا
(2) (هل تتناول أدوية للسكري؟ (انسولين) (اقراص).....
(3) ———— (هل تعاني من مرض ارتفاع ضغط الدم؟ ومنذ متى ؟ (نعم) (لا
(4) ———— (هل تعاني من الآم متكررة بالبطن؟ (نعم) (لا
(5) ———— (هل تعاني من امراض التهابات في الأمعاء ؟ (نعم) (لا
(6) ———— (هل تعاني من التهاب المفاصل (الروماتيزم) ؟ (نعم) (لا
(7) ———— (هل تعاني من داء الصدفية ؟ (نعم) (لا
(8) ———— (هل تعاني من امراض القلب/الجلطة؟ (نعم) (لا
(9) ———— (هل تناولت مضادات حيوية خلال 6 اشهر الماضية ؟ (نعم) (لا
(10) ———— (هل تناولت مضادات الالتهابات خلال 6 اشهر الماضية ؟ (نعم) (لا –
(11) (هل تعاني من امراض مناعية او اضطرابات المناعة الذاتية ؟ وما هي ؟ (نعم) (لا .....
(12) ———- (هل تناولت أدوية مثبطة للمناعة خلال 3 اشهر الماضية ؟ (نعم) (لا
(13) ———- (خاص بالسيدات: هل أنتي حامل او مرضعة ؟ (نعم) (لا

اقر أنا———————————————————- بأنني اسمح بأخذ العينة اللازمة لهذه الدراسة طالما أنها في إطار المتعارف عليه طبيا.

———————— التوقيع

Appendix 2
Patient Data sheet

PART 1: GENERAL DATA

Participant's name: _____ Date Examined: _____
Participant's Code: _____ Age: _____
Marital Status: □ S □ M □ W
Sex: □ Male □ Female
Education Attainment: □ High school or lower □ College or higher
Occupation: _____

PART 2: MEDICAL HISTORY

Smoking history: □ Smoker (_____ pack years) □ Non-smoker
Comorbidities:
□ Diabetes mellitus □ Inflammatory bowel diseases
□ Hypertension □ Rheumatoid arthritis □ Bronchial Asthma □ Autoimmune disease
□ Liver disease □ Renal disease
□ Cardiac disease □ Others (specify)
□ psoriasis

PART 3: CLINICAL DATA

BP: _____ Wt (kg): _____ Ht (cm): _____
Pertinent Physical Examination Findings:
_____

PART 4: PERIODONTAL EXAMINATION

Total Number of Teeth Present: _____
Total Number of Teeth Lost: _____
Periodontitis _____ Present _____ Absent
Severity of Periodontitis _____ Mild _____ Moderate _____ Severe
Other Findings: _____
Recommendation:_____