Objectives Periodontitis is a pathological condition of the oral cavity, originating from multiple factors, including microbial, environmental and genetic factors. The vulnerability to several pathologies has been studied with the relationship to genetic polymorphisms, and one of the most prominent is the single nucleotide polymorphisms throughout the genome. The study aimed to find out the association of single nucleotide polymorphism (SNP) of interleukin-1β +3954 gene with chronic periodontitis (CP) in Pakistan

Materials and Methods This case–control study was conducted at Dow University of Health Sciences. DNA was extracted from the blood and amplified by using conventional polymerase chain reaction of respective genes followed by sequencing. Mann–Whitney test accessed the difference of clinical parameters between cases and controls, and Fisher’s exact test was applied to access the association of alleles between subjects. Data entered and analyzed using SPSS 21.

Results Significant differences were observed in clinical parameters in cases and controls ($p<0.001$). In the IL-1β +3954 gene, T alleles were significantly higher in cases as compared with controls ($p<0.001$). Genotype CC was significantly dominant in the controls and genotype CT and TT in patients (Chi-square = 19.83, $p<0.001$).

Conclusion Within the study’s limits, IL-1β +3954 gene polymorphism is associated with periodontitis and is expected to be among the several causes of respective pathology in Pakistan’s population.
This pathological condition triggers with microbial colonization and biofilm formation; however, disease advancement occurs when the host–immune system effectively responds against pathogens. Progression of the periodontal disease elicits adaptive immune mechanism and releases a complex set of pro- and anti-inflammatory cytokines. Production of the altered amounts of these cytokines governs inflammatory lesions, resulting in the damage of periodontal tissue attachment and respective bone loss.

Interleukins are a particular subclass of cytokines directly involved in immune and inflammatory responses of several connective tissues of the body, including teeth and supporting structures. Several cytokines or proteins, including tumor necrosis factor, interleukin 8 (IL-8), interleukin (IL-1), interleukin (IL-6), interleukin 10 (IL-10), osteocalcin, and many others have been reported to have an association with periodontal disease progression.

These cytokines have also been proposed as potential diagnostic or predictive biomarkers for periodontitis. Among individuals affected with chronic periodontal disease worldwide, a variable degree of different cytokines’ production has been observed, and studies have been conducted to reduce their salivary concentration. This variation is mostly allied with the genetic polymorphisms in either interleukin genes and/or in their regulatory factors that vary among different races, communities, and ethnicities. One of the several interleukins associated with CP is IL-1 (IL-1α and IL-1β). The IL-1β gene regulates the transcription, synthesis, and functions of downstream pro-inflammatory cytokines IL-1β. IL-1β cytokine is a potent stimulator of osteoclastic activity and indirectly affects the severity of periodontitis in terms of alveolar bone loss. Elevated levels of IL-1β cytokine have been reported in patients affected with periodontitis than in controls. Moreover, the significant role of SNP of the IL-1β +3954 gene, located on chromosome 2q13, has been documented in several studies conducted in various populations and their respective subgroups in periodontitis.

However, data from different populations, including Pakistan, is still not available. Appreciating this gap, we performed this study. Our study was designed to illuminate the role of SNP on IL-1β +3954 with chronic periodontitis (CP) in Pakistan’s population and to determine whether the respective polymorphism is a risk marker for periodontal disease.

**Materials and Methods**

**Selection of Participants**

This case–control study was performed at Dow University of Health Sciences, Karachi after the institutional review board of Dow University of Health Sciences’ approval, and informed written consent was obtained from all the participants before their partaking in the study. A total of 119 participants between 18 to 50 years were enrolled for the purpose; the case group comprises 61 periodontitis patients who visited the Dental OPD of Dow International Dental College and Dr Ishrat ul Ebad Institute of Oral and Health Sciences. The control group were the staff, students, instructors, and teachers from different Dow University institutes, showing no clinical and radiographic signs of periodontal disease and no history of oral and dental treatment in the last 3 months. Smokers, expecting and nursing mothers, immunosuppressed people, and entities with any other systemic illness were excluded from the study.

**Clinical Examination**

A complete intraoral examination was conducted for all recruited participants by using a periodontal probe (UNC 15, Hu-Freidy’s, United States) by the principal investigator. Demographic, medical, and dental history was recorded before the clinical assessment. Oral hygiene was recorded by using a simplified oral hygiene index. Bleeding on probing, plaque index (PI), gingival index, probing pocket depth (PPD), and clinical attachment loss (CAL) were measured by following standard protocols at six sites of every tooth except third molars. These sites include the distobuccal, buccal, mesiobuccal, distolingual, lingual, and mesiolingual.

**Sample Collection and DNA Extraction**

After scrutinizing CP patients and controls, venous blood (1–3 mL) was collected in an EDTA tube (BD Vacutainer) and stored at 4°C for DNA extraction by using PureLink Genomic DNA Mini Kit (Thermo Fisher Scientific-K182001). The collected DNA was confirmed by agarose gel electrophoresis (1% Agarose, 30 minutes, 120v). The extracted DNA samples’ concentrations and purity were measured at 260 nm by using Colibri Microwolume Spectrometer (TiterTek-Berthold, Berthold Detection Systems GmbH, Bleichstrasse, and Pforzheim, Germany).

**Amplification and Genotyping**

For the polymerase chain reaction (PCR), the respective primers were designed from the Gene sequence obtained from National Center for Biotechnology Information. The primers used for the amplification of IL-1β +3954 are Forward: 5’ - CTC AGG TGT CCT CGA AGA AATCAA - 3’ Reverse 5’ - GCT TTT TTG CTG TGA GTC CCG - 3’ and to amplify the selected fragments of DNA, conventional PCR was performed. For each 50 µL reaction, 4 µL (~200 ng) DNA sample, 25 µL of master mix (Thermo Scientific DreamTaq PCR Master Mix (2×), Catalog Number K1071) and 2 µL (0.2 µM) of each reverse and forward primer were added. The PCR program was set as initial denaturation was at 94°C for 5 minutes, followed by 35 denaturation cycles at 94°C for 45 seconds, 30 seconds annealing, and 30 seconds of extension at 72°C. The annealing for IL-1β +3954 was conducted at 57°C, and a final extension was performed at 72°C for 10 minutes. The amplified DNA was confirmed by agarose gel electrophoresis (1% Agarose in 1 X TBE run for 100 V –20 minutes), using 50 bp DNA ladder (Gen script). Amplicons purification and the sequencing were performed commercially by Macrogen, Inc., Korea (www.macrogen.com), using the respective forward primers. For nucleotide alignment, MEGA 7 was used. Genotypes were determined by electropherogram, as reported previously. The Hardy–Weinberg equilibrium was determined by using HW Calculator.
Statistical Analysis

The Mann–Whitney test was employed to identify the difference of clinical parameters between cases and controls. The association of allele and genotypes with subjects status was assessed through Fisher’s exact test and Chi-square test of independence. Values <0.05 were considered to indicate statistical significance. SPSS 21 was used for the entry and processing of the data.

Results

The patients mean age group was 33.31 ± 8.27 years, and in controls, it was 31.53 ±5.88 years. The difference in age between both groups is not significant (p = 0.431). The total number of males was 78, and 41 females were recruited for the study; among patients, 42 (68.9%) were males and 19 (31.1%) were females, and 36 (62.01%) males and 22 (37.99%) females were among the controls. The mean CAL, PPD, PI, tooth loss, and number of mobile teeth were significantly higher in patients as compared with the controls (p < 0.001; ►Table 1).

Sequences were visually observed on electropherograms obtained from DNA sequencing. Single peaks represented the homozygous genotype (►Fig. 1A and 1C), whereas double peaks were characterized as heterozygous genotype (►Fig. 1B). Controls display homozygosity for the C allele, and it is the proposed wild type in our population for the position +3954 in IL-1β +3954 gene (►Fig. 1A; as displayed by the single peak observed at the respective position). Patients with mild-to-severe infections were heterozygous for the above-said positions as displayed by the double peaks (►Fig. 1B), whereas CP patients mostly displayed homozygosity for the T allele at +3954 in the IL-1β gene (►Fig. 1C).

Data analysis for IL-1β +3954 showed a high prevalence of C allele in controls, that is, 57.14% (n = 100) and in patients it was reported as 42.8% (n = 75). In periodontitis patients, the T allele prevalence was reported higher; it was 74.6% (n = 47); however, in controls, T alleles frequency was 25.39% (n = 16). It was found that T alleles were significantly higher in patients as compared with controls (Chi-square = 18.68, p ≤ 0.001; ►Table 2).

The frequency was determined as the total percentage of all the subjects recruited (n = 119); it was expressed that the homozygous CC of IL-1β +3954 was the most frequent genotype in cases and controls comprises 52.9% (n = 63) in the population. The second most common genotype was CT, observed in 41.17% (n = 49) of the total. The homozygous genotype TT was present in 5.88% (n = 7) samples.

For IL-1β +3954, the frequencies of genotype in the controls group, out of total subjects recruited (n = 119), were found to be 66.7% (n = 42) for genotype CC, 32.7% (n = 16) for the heterozygous genotype CT and 0% (n = 0) for TT (►Table 2). Within the controls (n = 58), the IL-1β +3954 percentage of genotype CC, CT, and TT were reported 72.41, 27.58, and 0.00%, respectively (►Table 2).

Among the periodontitis patients (n = 61), the recorded genotypes were 33.3% (n = 21) for CC, for heterozygous CT it was found to be 66.3% (n = 33), and for TT it was observed 100% (n = 7). Within the patient’s group, the percentage of genotype

Table 1  Demographic and clinical parameters of patients and controls

| Variable               | Controls   | Patient    | p-Value |
|------------------------|------------|------------|---------|
| Mean age (y)           | 31.53 ± 5.88 | 33.31 ± 8.27 | p=0.431 |
| Gender                 | Total      |            |
| Male                   | 36 (62.1%) | 42 (68.9%) | 78      |
| Female                 | 22 (37.9%) | 19 (31.1%) | 41      |
| Total                  | 58         | 61         | 119     |
| CAL (mm)               | 0.086 ± 0.28 | 4.02 ± 1.67  | <0.001  |
| PPD                    | 1.53 ± 0.73 | 3.55 ± 1.25   | <0.001  |
| BOP (%)                | 16.46 ± 12.07 | 32.83 ± 18.08 | <0.001  |
| PI                     | 0.75 ± 0.67 | 1.97 ± 0.46   | <0.001  |
| Tooth loss             | 0.10 ± 0.35 | 1.34 ± 1.84   | <0.001  |
| Mobility               | 0.18 ± 0.47 | 2.55 ± 3.40   | <0.001  |

Abbreviations: BOP, bleeding on probing; CAL, clinical attachment loss; GI, gingival index; PI, plaque index; PPD, probing pocket depth.
Note: Clinical parameters are given as means ± standard deviation. The p-values were calculated by using Mann–Whitney test. The participant’s count was expressed in percentage (%).
CC, CT, and TT was reported 34.42, 54.09, and 11.47%, respectively (∼Table 2). The TT genotype was only found in patients, and the difference between the controls and diseased group was found to be significant; Pearson’s Chi-square = 19.83, \( \chi^2 < 0.001 \) as analyzed by Fisher’s exact test where probability value (p-values) < 0.05 was considered significant (∼Table 2).

The Hardy–Weinberg equilibrium analysis of the IL-1β +3954 genotype distribution among different groups shows that the probability of differences between controls and periodontitis patients does not exist in the population (Chi-square = 0.397, \( \chi^2 = 0.52 \) with 1 degree of freedom; ∼Table 3).

**Discussion**

Several studies have reported that genotypic polymorphisms associated with the variation in phenotypes, such as expression of the disease severity and its onset. A similar direction of study is being conducted for a better understanding of CP. The frequency of many genetic alleles varies among different populations, and several studies have found contradictory results among different studied populations. Considering the high incidence ratio of CP in Pakistan’s population, it was crucial to conduct a similar study to correlate an association between gene polymorphism and CP.

In the current study, we found that the homozygous CC genotype of IL-1β +3954 was highly prevalent among the controls. However, Homozygous TT and heterozygous CT genotype predominantly present in cases (∼Table 2). Similar findings have been reported in previous studies.22-24 A meta-analysis also suggested that the TT genotype at IL-1β +3954 may be associated with an increased CP risk.25 However, contrary to our findings, few previous studies have demonstrated conflicting results with no association between SNP at +3954 in the IL-1β gene and CP.4,26,27 The variance in this association could be due to the diverse ethnicity of the targeted populations.

Limited sample size and multiple ethnicities are major limitations. However, to the best of our knowledge, this is the first study investigating the association of SNP of any gene, specifically IL-1β +3954, in Pakistan’s population with periodontitis or any other dental disease. Our research’s findings and results will be served as a valuable guide and representation of Pakistan’s population for future studies and may be useful in detecting periodontitis-susceptible individuals in the future.

**Conclusion**

In this study, the association between periodontitis and single nucleotide polymorphisms in IL-1β +3954 gene was investigated. In IL-1β +3954, the mutated allele T was responsible, and the TT genotype showed the highest prevalence in diseased individuals. An increased number of periodontitis patients were also reported in the CT genotype, and the CC genotype was predominant among the controls in IL-1β gene.

**Table 2** Distribution of allele and genotype in controls and periodontitis patients

| Gene          | Allele | Controls | Patients | Total | OR (95% CI)     | Chi-square, \( p \)-Value |
|---------------|--------|----------|----------|-------|-----------------|---------------------------|
| IL-1β +3954   |        |          |          |       |                 |                           |
| Allele        | C      | 100 (57.14) | 75 (42.8) | 175 (100) | 0.255 (0.135–0.482) | 18.68, \( p < 0.001 \) |
|               | T      | 16 (25.39)  | 47 (74.60) | 63 (100)  |                 |                           |
|               | Total  | 116       | 122       | 238     |                 |                           |
| Genotype      | CC     | 42 (66.7)  | 21 (33.3) | 63 (100)  |                 | 19.83, \( p < 0.001 \) |
|               | CT     | 16 (32.7)  | 33 (66.3) | 49 (100)  |                 |                           |
|               | TT     | 0 (0.0)    | 7 (100)   | 7 (100)   |                 |                           |

Abbreviations: CI, confidence interval; OR, odd ratio.

**Table 3** Hardy–Weinberg equilibrium for genotypes of IL-1β +3954 and IL-10 −819

| Genotype   | Expected | Observed | Chi-square, \( p \)-Value |
|------------|----------|----------|---------------------------|
| IL-1β +3954|          |          |                           |
| CC         | 64.3     | 63       | Chi-square = 0.397, \( p = 0.52^* \) |
| CT         | 46.3     | 49       |                           |
| TT         | 8.3      | 7        |                           |

*With 1 degree of freedom.
Our study concludes that the single nucleotide polymorphism of IL-1β +3954 gene could be a risk factor for periodontitis in Pakistan's population.

**Funding**
None.

**Conflict of Interest**
None declared.

**Acknowledgments**
The authors would like to acknowledge Ms. Perbhat Ali, Ms. Anum Razzak, and Ms. Sabahat Aziz from Dow University of Health Sciences for their time and efforts during the completion of this research.

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