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Genetic Association Of Interleukin 1B (*rs1143643*) Gene Polymorphism with Apical Periodontitis In South Indian Population - A Pilot Study

Research Article

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Abstract

Aim: To demonstrate the association between IL 1- β (*rs1143643*) gene polymorphism with periapical periodontitis in South Indian population - A case control pilot study
Objective: To determine the genotype frequency of IL 1- beta gene polymorphism in patients with deep carious lesions with (cases) or without periapical lesions (control). To derive a statistical association between the gene polymorphism with periapical periodontitis.
Need for Study: The present study is first of its kind which is directed towards finding an association between IL 1- beta gene polymorphism with periapical periodontitis in the south Indian population. If found to be significant, the SNP can be used as a possible genetic marker to determine the susceptibility to periapical periodontitis.
Materials and Methods: A case-control association study was performed to assess the association of IL 1- beta (*rs1143643*) polymorphisms in individuals having deep caries with and without apical periodontitis. Cases were defined as subjects with deep caries only (control n=50) and deep caries with apical periodontitis (cases n=50). Genomic DNA was extracted from the collected salivary swab samples. ARMS PCR was carried out to genotype the SNP of IL 1- beta gene. Two allele specific forward primers and one common reverse primer was used for every sample and two PCR reactions were carried out. Genotypes were identified by electrophoresis and amplification of alleles with both primers were designated as heterozygous.
Conclusion: There was no significant association between IL 1- beta gene polymorphism with periapical periodontitis in the south Indian population. Gene expression closely resembled East Asian population. IL 1- beta (*rs1143643*) polymorphism should be analyzed with a larger sample size to suggest its role as a significant marker associated with apical periodontitis in the South Indian population.

Keywords: Apical Periodontitis; ARMS-PCR; Cytokines; Deep Caries; IL 1-β (*rs1143643*); Periapical Lesion; SNP.

Introduction

Apical periodontitis is a multifactorial process characterized by the dynamic encounter between microbial factors and host defense. The size and characterisation of bone loss depends on the microbial load, defense mechanism of the host and a balance between pro and anti- inflammatory mediators. Cytokines play a pivotal role in inflammatory and immune responses of bone. The commonly identified cytokines associated with pulpal/periapical diseases include tumor necrosis factor (TNF) and interleukins (IL). The proinflammatory cytokines such as Interleukins (IL-1) are responsible for modulating the host responses to microbial infection and induce extracellular metabolism and bone resorption [1]. IL-1a and IL-1b are key cytokines demonstrated in apical periodontitis lesions and are mediators of inflammation and bone resorption. Pro inflammatory cytokines such as interleukins are

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frequently isolated from periapical lesions [2, 3].

Pulpal diseases are dynamic and progress to periapical diseases if left untreated [4]. During a microbial challenge, some individuals may respond with an exaggerated immunoinflammatory response which is explained by the concepts of genetic polymorphism and biological modifiers [5]. Genetic polymorphisms lead to altered expression of genes and are capable of generating a deficiency in the immune status of individuals. This could explain the reason for individuals with specific genotypes being more susceptible to disease or could present an increase in severity of disease progression. Polymorphisms in IL1B, IL6, and IL8 have been extensively associated with pathogenesis of apical periodontitis [6].

Apical periodontitis is primarily of microbial origin but is influenced by the systemic status, immune condition and certain genetic polymorphism that is responsible for variations in host defense to infection. Genetic association studies have been performed in endodontic research evaluating the influence of genetic polymorphism of gene expression on the development of apical periodontitis.

Our study focused on single nucleotide polymorphisms, also known as SNP. These genetic polymorphisms are the most common form of DNA sequence variation, and account for more than 90% of all variations present in the human genome. Studies identifying genetic polymorphisms with pathogenesis of apical periodontitis can serve to manufacture chair side kits for identifying the inflammatory markers associated with the increased risk population. Analyzing the association between genetic polymorphism and phenotypes associated with apical periodontitis can serve as a predictor for progression of the disease as well as prognosis of treatment [7].

These types of studies have provided new etiologic perspectives for apical periodontitis pathogenesis, with a greater focus on host response. Studying the association of SNP in some cytokine genes with treatment outcome would be clinically translatable if IL-1β might also play a role in the initiation and up-regulation of the inflammatory response in apical periodontitis by increasing the levels of inflammatory mediators promoting bone resorption [8]. Previously our team has a rich experience in working on various research projects across multiple disciplines [9-23]. Now the growing trend in this area motivated us to pursue this project.

Materials And Methods

A pilot study was undertaken with a total sample size of 100 subjects comprising 50 patients with deep dental caries with periapical lesion (cases) and 50 patients with deep dental caries without periapical lesion (control). This was checked by intra oral periapical radiographs taken using paralleling technique. Patients in the age group of 18 - 60 years of age were selected for the study. Careful medical history was taken to include only individuals with a non contributory medical history. Cases with periapical abscess with pus discharge and sinus tract [24] and patients with diabetes, endocrine disorders, inflammatory diseases or under systemic antibiotics or hormonal therapy for the past 6 months were excluded from the study [25].

Each subject was evaluated by two calibrated examiners for clinical/radiographic signs and symptoms of deep caries with and without periapical lesion and classified accordingly (case/control). The periapical status was assessed using the periapical index [26]. To collect a sample for DNA analysis, the inside of the cheek was scraped with sterile swab [27]. Genomic DNA was obtained from these swab samples using the Purelink DNA mini Kit, Invitrogen. The expected yield of DNA was an approximate concentration of 50µg/µL. The ideal storage temperature for DNA was minus 20°C until genotyping was performed. Figure 1 shows the schematic representation of the methodology employed in the present study. The ARMS (Amplification Refractory Mutation System) PCR was carried out to genotype the SNP of IL 1-β (rs1143643) gene. A total of three primers were used, with two forward and one common reverse primer, where each of the forward primers was specific to a particular allele. Therefore for every DNA sample, two PCR reactions were carried out, each containing one of the allele specific forward primer (F1; F2) and the common reverse primer. The genotypes were directly identified by electrophoresing the products on a 1-1.5 % agarose gel. Amplicons observed with both the primers (F1 and F2) were designated as heterozygous, whereas amplicons with just one set of primer (F1+R/F2+R) is designated as either homozygous wild-type (GG) or homozygous mutant (AA) (Fig. 2). The PCR reaction conditions are as follows: initial denaturation at 94°C for 4 mins, denaturation at 94°C for 45 secs, annealing at 58°C for 45 secs, extension at 72°C for 45 secs, for 35 cycles followed by a final extension at 72°C for 4 mins [28]. The comparison of allele frequencies between different ethnic groups was performed from the data obtained from Ensembl genome browser (https://asia.ensembl.org/Homo_sapiens/Gene/Summary? db=core; g=ENSG00000125538;r=2:112829751-112836816) (Fig. 3).

Statistical analysis

χ^2 analysis was used to test for deviation of genotype distribution from Hardy-Weinberg equilibrium and to determine whether any significant differences existed in allele or genotype frequencies between cases and controls groups. The association between genotypes and risk of apical periodontitis was analysed by calculating odds ratio (OR) at 95% confidence interval (95% CI). Statistical tests along with the logistic regression analysis were performed using the SPSS software 14.0 version (SPSS Inc., Chicago, Illinois, USA). The value of $p< 0.05$ was considered to have statistical significance.

Results

The genotype of interleukin 1β (*rs1143643*) as designated using ARMS PCR is shown in figure 2. The allele frequency for G and A allele was found to be 0.57 and 0.43 for cases, 0.52 and 0.48 for controls respectively (Table 1). The G allele is the ancestral allele and A is the variant allele. Both the case and the control groups were in agreement with Hardy-Weinberg equilibrium. The comparison of allele and genotype frequencies were found to be insignificant between the two groups with a p value of 0.673 as assessed by Chi-square test at 2 degrees of freedom. Comparison of allele frequencies between different populations revealed that the allele frequencies observed in the present study population was similar to that observed with the East Asian population.

Table 1. Genotype frequencies of IL-1 beta (*rs1143643*) gene polymorphism among the cases and controls* For departure from Hardy-Weinberg equilibrium (HWE), chi square with one degree of freedom. The genotype frequency of cases and controls do not differ significantly χ^2 df (P = 0.673).

GROUPS	GG	GA	AA	G	A	HWE (p value)*
Case (N=50)	16 (32%)	25 (50%)	9 18%)	0.57	0.43	0.887
Control (N=50)	12 (24%)	28 (56%)	10 (20%)	0.52	0.48	0.389

Figure 1. Methodology flow chart.

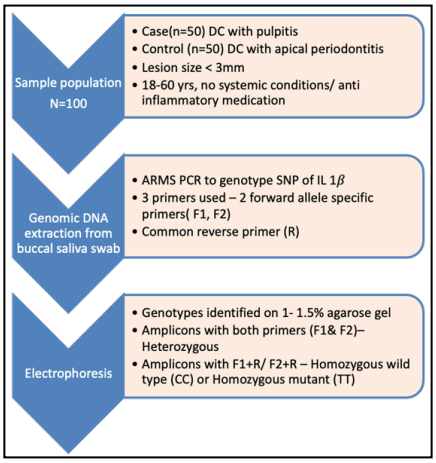


Figure 2. G/A polymorphism (*rs1143643*) of IL-1 beta gene: Allele specific PCR amplification (202 bp) demonstrating the genotypes [Lane 5: M = 100 bp DNA marker] Lane 1 and 2 - same sample amplified by both the sets of primers, amplification seen only with G allele specific primer (Lane 1), hence GG homozygous; Lane 3 and 4 - same sample amplified by both the sets of primers, amplification seen only with A allele specific primer (Lane 4), hence AA homozygous; Lane 6: Negative control, Lane 7 and 8 – sample amplified with both sets of primers, hence GA heterozygous.

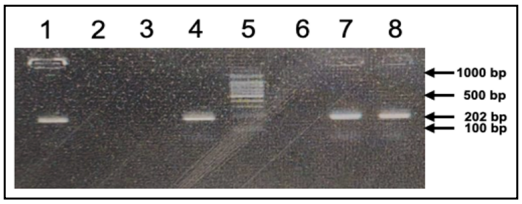
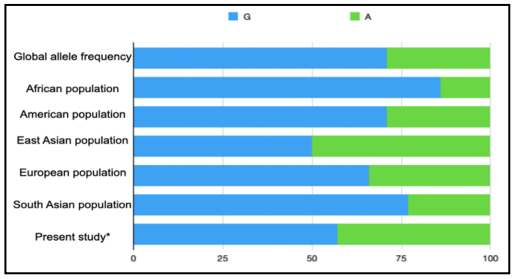


Figure 3. Comparison of allele frequencies of IL-1 beta (*rs1143643*) gene polymorphism among different populations with the present study group.



Discussion

Our institution is passionate about high quality evidence based research and has excelled in various fields [13, 29-38]. Polymorphism in cytokine genes may be considered as a risk factor for the individual's increased susceptibility to apical tissue destruction. A noninvasive tool such as saliva diagnostic test to analyze the level of periapical inflammation could be predictive and useful as an adjunct to radiographic diagnosis. Understanding the level of immune response can provide definitive reasons for varying rates of progression in deep dentinal caries.

Studies have isolated IL- 1β more frequently from symptomatic cases associated with periapicallesions [39, 40]. Few studies concluded that there was no significant difference between symptomatic and asymptomatic cases of apical periodontitis with interleukin levels [3, 41]. Kornan et al has studied a strong association between composite genotypes (allele 2 of IL-1α and IL-1β) and severe periodontitis in adults [42]. Morsaniet al identifies the significant association between the genetic polymorphisms (*rs1143634*) and apical periodontitis [1]. Dill et al studied genetic polymorphism in IL1B (*rs1143643*) showed allelic and genotypic association with rapidly progressing apical periodontitis [43].

The levels of IL-1β was significantly higher in radicular cysts compared with periapical granulomas. Symptomatic lesions are associated with increased levels of IL-1β and IL-6 that infers the active progression of disease process [44].

Menezes-Silva et alinvestigated the influence of polymorphisms in matrix metalloproteinase (MMP) genes to an individual's increased susceptibility to apical tissue destruction in response to deep carious lesions [45]. Yang et al observed increased expression of cytokines IL-1α and IL-1β in periapical granulomas from primary teeth and addressed cytokines to be a major contributing factor for progression of periapical lesion [46].

Certain hypotheses remain as an unquenched thirst and one among them is the understanding of human genetics, their immune response to various pathogens encountered. In order to fully understand genetic factors contributing towards initiation and progression of apical periodontitis, association study designs with an increased sample size, twin studies, segregation studies and linkage analysis can help in defining the genetic markers significantly promoting the inflammatory process. Limitations of the study include a small sample size, as this was just a pilot study that cannot replicate the gene expression patterns of the entire South Indian population.

Acknowledgement & Declaration

We would like to acknowledge my mentors and guide for helping me in performing the clinical study and better understanding of the subject. We declare that an informed consent was obtained from the patients before sample collection.

Conclusion

Polymorphisms in cytokine genes may contribute to an individual's increased susceptibility to apical tissue destruction in response to deep carious lesions. There was no significant association between IL 1- beta gene polymorphism with periapical periodontitis in the South Indian population. Gene expression closely resembled East Asian population. IL 1- beta (rs1143643) polymorphism should be analyzed with a larger sample size to suggest a role as a significant marker associated with apical periodontitis in the South Indian population.

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