Association of BRAF gene polymorphism with susceptibility to chronic periodontitis- a case control study.

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Abstract

Periodontitis is a multifactorial disease of tooth supporting tissue that is caused by bacterial infection and increased immune response. T-cell proliferation plays an important role in host response to bacterial infection. BRAF is a positive regulator of T cell proliferation. The present study was aimed to evaluate the genetic association of BRAF gene polymorphism (rs10487888). A total of 100 subjects were recruited for this study, which included 50 CP and 50 healthy controls. Genomic DNA was extracted from the whole blood collected from the subjects. DNA was amplified using ARMS PCR technique. The genotype obtained based on the ARMS PCR pattern was recorded and used for statistical analysis. The distribution of genotypes and allele frequencies in the chronic periodontitis and control groups were compared using the chi-square test. The risk associated with individual alleles or genotypes was calculated as the Odds Ratio (OR) with 95% confidence intervals. Statistical significance in all tests was determined at $p < 0.05$. The C allele was found to be more predominant in the study population than T allele. The present study denotes that BRAF gene polymorphism is not associated with CP in the study group analyzed.

Keywords: Alleles, Chronic periodontitis, Polymorphism, BRAF

Accepted on November 16, 2021

Introduction

Periodontitis is a chronic inflammatory disease, influenced by multiple factors that caused destruction of the supporting tissues and resulted in tooth loss [1–4]. According to the World Health Organization report, severe periodontitis can lead to tooth loss in 5%-15% of the world population. Hence, it can be considered among the prevalent and important global health problems in terms of quality of life.

Periodontitis is initiated by microorganisms and perhaps viruses in the subgingival biofilm and further affected by lifestyle factors such as smoking, stress, diet and environment. It can also be affected by acquired systemic disease which reduces or hampers an optimal host response. Apart from this, some modifying disease genes can also be responsible for susceptibility to periodontitis.

Genetics is a non-modifiable risk factor which plays a role in determining the host susceptibility to periodontal destruction [5]. Humans share 99.9% of their genetic information. The 0.1% differs from one person to the other. There are a number of differences in the DNA sequences of two individuals and not all differences in the DNA sequences cause disease; such differences are known as polymorphism. Polymorphism differs from mutation in such a way that mutation causes the heritable alteration or change in the genetic material. A number of Single Nucleotide Polymorphisms like interleukin receptors, vitamin D receptor, matrix metalloproteinase receptors are determinants in disease susceptibility of genetically complex disease such as chronic periodontitis [6–9].

The RAF protein is made of three conserved regions: CR1, CR2 and CR3. CR1 and CR2 are situated in the N terminus. CR1 acts as the main binding domain for RAS; CR2 is the regulatory domain CR3 is situated in the C terminus and functions as the catalytic kinase domain. CR3 contains two regulatory regions [10] of the RAF family of protein kinases BRAF is the most frequently mutated and remains the most potent activator of MEK.

The RAF BRAF is encoded on chromosome 7q34. BRAF encodes a serine/threonine kinase that activates Mitogen-Activated Protein Kinase Pathway (MAPK). Signal pathways activate CRAF, and it inhibits BRAF activation by IL-1□. The activation results in MEK1/2 activation *via* Ser217/221 phosphorylation. By suppressing BRAF activation, the mechanical signals may likely alter a critical event important for the downstream IL-1□ signaling. This leads to the SOX-9, VEGF, and Myc upregulation responsible for cell proliferation in IL-1□ treated cells [11–13].

In addition to germline mutations, BRAF somatic mutations have been reported in Langerhans cell histiocytosis, erdheim-chester disease, and lung, colon, thyroid, and melanoma cancers, as well as in non-Hodgkin lymphoma.

To date more than thirty BRAF mutations have been identified, occurring in various frequencies. The most common is BRAF V600E Mutation (MT), which corresponds to a thymine to adenine transversion at position 1799, resulting in the substitution of valine by glutamate at position 600 of the protein. This lies within the activating segment of the kinase domain. It renders BRAF constitutionally active, increasing kinase activity relative to BRAF Wild Type (WT) by 10 times [14]. Because of this, co-mutations in the MAPK signalling cascade offer no selective advantages therefore BRAF

mutations are mutually exclusive with KRAS or NRAS mutations [15].

The V600E mutation accounts for more than 85% of BRAF mutations in melanoma, more than 50% of the mutations in non-small cell lung cancer and more than 95% of mutations in cholangiocarcinoma and hairy cell leukemia.

Literature evidence indicates that BRAF may be an important signaling molecule in T-cell mediated responses. These data elucidate that BRAF is a positive regulator of T-cell development [16]. Because T cells play an important role in the progression and control of periodontal disease [17], it is feasible that BRAF may be implicated in the pathogenesis of periodontitis.

Our team has extensive knowledge and research experience that has translate into high quality publications [18-**37**]. The aim of the present study was to study the association of BRAF gene Single Nucleotide Polymorphism (SNP) rs10487888 in chronic periodontitis patients.

Materials and Methods

A total of 100 individuals who reported to the department of periodontics were included in this cross sectional study. The sample size was calculated based on the previous study by Kaarthikeyan et al. [**38**] based on which sample size of 100 was derived keeping the power of the study as 80%. The subjects were divided into a CP group (n=50) and a control group (n=50) based on the clinical examination of probing pocket depth, clinical attachment loss and bleeding on probing. The CP group contained 50 patients (male-26; female-24) with the mean age of 39.02 ± 8.22.The CP patients were recruited based on the criteria of American Association of Periodontology (AAP)-1999**39** The control group contained 50 periodontally healthy subjects (male-26; female-24) with mean age of 41.34 ± 7.49.

Inclusion Criteria

Control group: Patients who are systemically healthy, healthy gingiva and gingivitis patients were included where the probing depth was < 3mm, CAL=0.

Test group: Patients who are systemically healthy, who had at least 20 remaining teeth, probing depth > 3 mm, CAL > 3mm.

Exclusion Criteria

Smokers, pregnant or lactating mothers, immune compromised individuals, subjects who underwent periodontal therapy within the past 6 months were excluded from this study.

The ethical clearance was obtained from the institutional review board and written informed consent was obtained from all the patients who participated in the study.

Sample collection

A volume of 2 ml of venous blood was collected from antecubital fossa and dispersed into a sterile tube containing a pinch of Ethylene Diamine Tetra Acetic Acid (EDTA). It was mixed thoroughly to avoid clot formation. DNA isolation was performed according to the modified in 1998 protocol [**40**].

Polymerase chain reaction and restriction endonuclease digestion

IL33 receptor gene polymorphism (rs1929992) was assessed by ARMS PCR amplification and digestion. The following primers,forwardprimerF1:5'GGCCAACCTAGGATGTTGTTA -3',F2:5'- GGCCAACCTAGGATGTTGTTG-3' and reverse primer: 5'-GATCTGCCCGCCTCAGC-3' were used for amplification of DNA polymorphic site, of the BRAF receptor gene.

The amplification of DNA was performed in 20 µl volumes using 10 ng of genomic DNA, 5 pmol/µl each of forward and reverse primers along with PCR master mix (Takara, Japan). The cycling conditions were as follows: initial denaturation at 94℃for 5 min, denaturation at 94℃ for 35 sec, annealing at 60℃for 35 sec, extension at 72℃for 35 sec, and a final extension at 72℃ for 5 min. 5 µl of PCR product was checked on a 2%agarose gel[Figure 1].

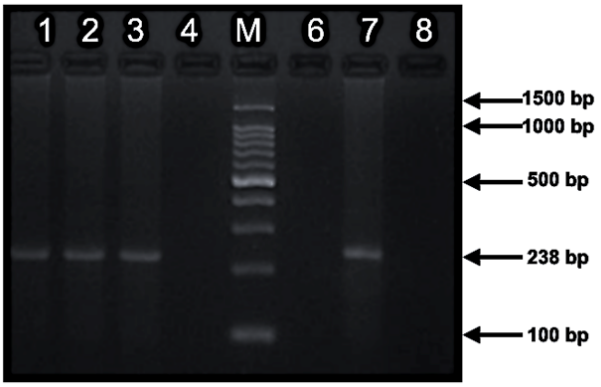

Figure 1: *C/T polymorphism (rs10487888) of BRAF: Allele specific PCR amplification (238 bp) demonstrating the genotypes [Lane 4: M = 100 bp DNA marker] Lane 1 and 2: Sample amplified with both sets of primers, hence CT heterozygous. Lane 3 and 4: Same sample amplified by both the sets of primers, amplification seen only with C allele specific primer (Lane 3), hence CC homozygous; Lane 6 and 7: Same sample amplified by both the sets of primers, amplification seen only with T allele specific primer, hence TT homozygous (Lane 7), Lane 8: Negative control.*

Statistical analysis

All statistical analysis was performed using the Statistical Package for the Social Sciences Version 23.0 for Windows (SPSS Inc., Chicago, IL). The distribution of genotypes and allele frequencies in the chronic periodontitis and control groups were compared using the chi-square test. The risk associated with individual alleles or genotypes was calculated as the Odds Ratio (OR) with 95% confidence intervals. Statistical significance in all tests was determined at $P<0.05$.

Results

The clinical characteristics of the subjects in CP and control groups. The genotype and allele frequencies of the group. The genotype frequency and distributions of BRAF receptor did not differ significantly at 2df (P =.794). Our study results showed that homozygous and heterozygous mutant genotypes had no significant difference (CC *vs* CT+TT) between the CP and healthy control with a P-value of 0.5487. The detected frequency of CT (36% *vs* 34%) and TT (18% *vs* 14%) genotype showed no significant difference between healthy control and CP group. There was no significant difference in C allele (64% *vs* 69%) and T allele (36% *vs* 31%) between the CP and healthy control group.

Discussion

The genetic polymorphism influences susceptibility of periodontitis and there are various gene polymorphisms which are shown to play a role in periodontitis. Experimental evidence gathered from various studies have identified genes encoding immune-regulatory and immune-modulatory molecules such as chemokines (CXCR2), cytokines(interleukin), surface receptors (vitamin D receptors), antigen recognition proteins (FC gamma) etc [41].

BRAF is a member of the Raf kinase family of serine-threonine kinases. BRAF activates the Mitogen Activated Protein Kinase (MAPK) pathway involved in cell division, inflammation, and heat-shock response. Antibodies to BRAF have been described in conditions such as melanoma, in which BRAF is often mutated [42,43]. Indeed, BRAF mutations activate Raf/MEK signaling. Study done on rheumatoid arthritis patients has shown they have p.Val600Ala mutation in BRAF gene. This mutation activates the kinase activity of BRAF. The p.Val600Ala mutation could activate the MAPK pathway, leading to activation of T lymphocytes [44]. Previous studies have shown that BRAF played an important role in the inflammatory process. Studies correlating polymorphism of BRAF gene and papillary thyroid carcinoma have showed that polymorphism of rs3748093*A was significantly correlated with an increased risk of papillary thyroid carcinoma in a chinese population [45] Quaye et al found that three SNPs of BRAF (rs17695623, rs1267622 and rs10487888) were associated with the risk of mucinous ovarian cancer [46]. BRAF mutations are found in 4% of non-small cell lung cancers, and half of these mutations are non V600E [47]. Several other reports documented the identification of BRAF in both small-cell and non-small-cell lung cancer pathogenesis. BRAF mutation is also suggested to be poor prognostic factors in CRC patients with synchronous liver metastasis [48,49] Similarly, it was reported that the melanoma was also correlated with variations of BRAF [50].

Tumour Necrosis Factor (TNF)-alpha has an important role in the pathogenesis of periodontitis. The p38 MAPK pathway is distinct from the MAPK pathway involving BRAF51. In contrast to the MAPK pathway in which TNF alpha induces expression of p38, BRAF induces expression of nuclear factor kappa B, a pro-inflammatory cytokine that can induce inflammation and stimulate production of TNF alpha [52]. Thus TNF alpha is the result of MAPK activation in the BRAF pathway than the stimulus for MAPK activation as in the p38 MAPK pathway. Thus it's relevant to probe the association of BRAF polymorphism in chronic periodontitis patients.

Our study results showed that homozygous and heterozygous mutant genotypes had no significant difference (CC vs CT +TT) between the CP and healthy control with a P-value of 0.5487. The detected frequency of CT (36% vs 34%) and TT (18% vs 14%) genotype showed no significant difference between healthy control and CP group. There was no significant difference in C allele (64% vs 69%) and T allele (36% *vs* 31%) between the CP and healthy control group.

Present study results were in accordance with the study done by Kadkhodazadeh et al. where they found that BRAF rs10487888 polymorphism didn't have association with chronic periodontitis patients and peri implantitis among Iranian Population [53]. There is minimal literature evidence on the association of BRAF gene polymorphism in chronic periodontitis patients.

Hence, studies in a large population size including various ethnic groups at multicenter are required to arrive at a statistically significant observation. BRAF polymorphism has been important in understanding its role in periodontitis, how BRAF and other members of RAS/RAF cascade play in disease and detailed mechanistic study can help to approach effective BRAF inhibitors for periodontal disease. Future scope will be targeting these genes through gene therapy.

The limitation of the present study the sample included in the present study were serum it could be one of the reasons for negative association between chronic periodontitis and BRAF receptor gene polymorphism, collection of tissue samples would have better results in determining genetic polymorphism between BRAF receptor and chronic periodontitis patients. The present study was limited to genetic polymorphism in chronic periodontitis patients; future studies need to be done in aggressive periodontitis patients using the tissue samples as a multicentered study, different ethnic group in a larger study sample.

Conclusion

The present study denotes that BRAF gene polymorphism is not associated with CP in the study group analyzed. Further studies are required to explore the interaction of BRAF receptor genes with microbial and environmental factors in the etiopathogenesis of Periodontitis and link between BRAF receptor genes in chronic periodontitis patients with systemic diseases.

Acknowledgment

The authors are thankful to the management of Saveetha Institute of Medical and Technical Science (SIMATS), Chennai, India and department of BRULAC for the facilities, encouragement and constant support to carry out this work.

Consent for Publication

The patient has given valid and informed consent for publication of this case and related images.

Declaration of Patient Consent

The authors certify that we have obtained all appropriate patient consent forms. In the form the patient has given his consent for his image and other clinical information to be reported in the journal. The patient understands the name and initials will not be published and due efforts will be made to conceal the identity, but anonymity cannot be guaranteed.

Conflict of Interest

No Conflict of interest.

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