Natural Resistance Associated Macrophage Protein 1 Gene Polymorphism is Associated with Chronic Periodontitis Not Peri-Implantitis in an Iranian Population: A Cross Sectional Study

Mahdi Kadkhodazadeh[1], Ahmad Reza Ebadian[1], Reza Amid[2], Parisa Zarnegarnia[3], Fatemeh Mollaverdi[4], and Nika Aghamohammadi[3]

[1] *Dental Research Center, Research Institute of Dental Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

[2] *Department of Periodontics, School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

[3] *School of Dentistry, International Branch, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

[4] *Restorative and Esthetic Dentistry, Dental Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

Received: 08 Oct. 2014; Accepted: 21 Apr. 2015

Abstract- In inflammatory diseases such as peri-implantitis (PI) and chronic periodontitis (CP) both adaptive and innate immunity play a part. Natural resistance associated macrophage protein 1 (*NRAMP1*) has considerable effects on macrophage function (phagocytosis) and host innate immune response against infections. The present study was to investigate the relationship of *NRAMP1* gene polymorphisms with PI and CP in an Iranian population. In this cross sectional study 79 patients with CP, 38 patients with PI and 84 healthy controls presenting to the Periodontology Department of Shahid Beheshti University of Medical Sciences were enrolled. DNA was extracted from fresh blood samples of arm vein of participants and transferred to KBiosience institute (United Kingdom) for genotyping. X2 and Fisher's exact tests were used by SPSS software v.19 for statistical analyzes. Significant differences were detected in the distribution of genotypes between control and CP groups both for rs17235409 and rs2276631 polymorphisms (*P*:0.044 and *P*:0.028 respectively). Distribution of genotypes differed insignificantly in comparison of PI and control groups for rs2276631 (*P*:0.623) and either rs17235409 (*P*:1) polymorphisms. Based on our results, we conclude that presence of G allele in both rs2276631 and rs17235409 location may be a protective factor against CP. More studies with a larger sample size in different populations are required for confirming *NRAMP1* as a genetic determinant in periodontal disorders.

© 2016 Tehran University of Medical Sciences. All rights reserved.

Acta Med Iran, 2016;54(5):323-329.

Keywords: NRAMP1; Periodontitis; Peri-implantitis; Polymorphism; Inflammation; Bone

Introduction

Peri-implantitis (PI) and chronic periodontitis (CP) are multifactorial diseases of dental implant and tooth which are caused by microbial pathogens that result in inflammation of the supporting tissues (1,2). The accumulation of sub-gingival plaque on the implant/tooth surfaces may lead to host inflammatory responses that in turn may progress into peri-implantitis and periodontitis and result in eventual implant/tooth loss (3). Environmental factors and genetic predispositions may be responsible for differences in immune response to periodontal pathogens (4,5).

Peri-implantitis has less prevalent in comparison with chronic periodontitis and the studies have yielded highly variable results for prevalence of peri-implantitis (6-9). Previous studies have shown that patients who have lost one implant are more likely to experience additional failures; this phenomenon may be related to genetic variations (10). Genetic factors contribute to the pathogenesis of periodontitis (11). Many studies have cited the relationship of peri-implantitis and periodontitis with gene polymorphisms of immune response components (12-17).

In inflammatory diseases such as peri-implantitis and periodontitis, both adaptive and innate immunity play a part. Phagocytosis plays a key role in innate immunity to eliminate pathogens through diverse mechanisms

Corresponding Author: A.R. Ebadian
Dental Research Center, Research Institute of Dental Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Tel: +98 915 3156406, Fax: +98 21 22190224, E-mail address: ar_ebadian@yahoo.com

including antimicrobial enzymes, low pH, peptides, and oxidizing reagents (18,19). Natural resistance associated macrophage protein 1(*NRAMP1*) has considerable effects on macrophage function (phagocytosis) and host innate immune response against infections. *NRAMP1* is also a heme binding agent and plays a bacteriostatic role in infections caused by bacteria for whom heme is an essential nutrient such as *Porphyromonas gingivalis* and *Prevotella intermedia* which contribute to peri-implantitis and periodontitis (20).

NRAMP1 is encoded by the solute carrier family 11a member 1 (*SLC11A1*) gene. The location of *SLC11A1* gene is on chromosome 2q35 and has 15 exons spanning about 14 Kb (21,22).

SLC11A1 contains a number of single nucleotide polymorphisms (SNPs), including *rs17235409* (in exon) and *rs2276631* (in intron) that change the protein function (23). There are many studies that have evaluated the association of these SNPs with inflammatory, autoimmune and infectious diseases including visceral leishmaniasis, tuberculosis, inflammatory bowel disease (IBD), multiple sclerosis, leprosy, type 1 diabetes mellitus, Crohn's disease and rheumatoid arthritis (21-24).

The present study was the first in dentistry to investigate the relationship of *NRAMP1* gene polymorphisms with peri-implantitis and chronic periodontitis in an Iranian population.

Materials and Methods

The principles of this cross sectional study were based on STROBE and STREGA statements (25). The study protocol was thoroughly explained to the subjects and written informed consent was obtained from them. The Institutional Clinical Research Ethics Committee of Dental Research Center, Shahid Beheshti University of Medical Sciences approved this study.

We evaluated 2600 individuals who had been treated in our department from 2001 to 2010. A total of 201 people passed our strict criteria and enrolled this study as three groups: Chronic periodontitis (CP), peri-implantitis (PI) and healthy subjects (N).

Previously, we obtained 5 cc blood samples from arm vein of each individual in order to evaluate the effect of OPG gene polymorphism (26). The DNAs was extracted using the DNA extraction kit (CinnaGen Inc., Tehran, Iran), according to the manufacturer's instructions. After genotyping, the remained blood and DNA samples were stored at -4ºC and -70 º C freezers, respectively for further analysis. For genotyping of this study, the DNA samples were re-extracted and were transported to KBiosience Ltd Co (Hoddesdon, UK). Thereby the blood samples from patients and healthy subjects in our previous study (OPG SNPs) (26,27) were used for this study, too.

All individuals were evaluated by clinical and radiographic examinations including probing pocket depth (PPD), plaque index (PI) using standard Williams probes (Hu-Friedy, Chicago, IL, USA), bleeding index (BI) and clinical attachment level (CAL) measurements were carried out at 4 sites around each tooth/implant (mesial, distal, mid-buccal and mid-lingual). Clinical assessments were performed by an expert periodontist with 93% reproducibility based on the intra-class correlation coefficient index (one of the authors, M.K)

Exclusion criteria for all groups were: Oral diseases other than caries and periodontal disease, ongoing orthodontic therapy, smoking, a history of systemic or local disease with influence on the immune system, diabetes mellitus, hepatitis, HIV infection, immunosuppressive chemotherapy, current pregnancy, lactation or orthodontic treatment and non-Iranian population. The criteria for healthy subjects were as follows: Presence of at least 20 teeth in the mouth, no bleeding on probing, plaque index less than 20%, clinical attachment loss less than 1mm on all sites. No periodontal disease, no periodontal treatment except for routine scaling and polishing, no systemic diseases or medication intake, no smoking. If they had implants, they had to have peri-implant probing depth<4mm without radiographic evidence of bone loss, no evidence of peri-implant mucositis or peri-implantitis.

The subjects in chronic periodontitis group had to have at least 5 teeth, except the third molar in each quadrant. The diagnosis of chronic periodontitis was established based on radiographic and clinical parameters, including plaque index (PI>20%), probing pocket depth (PPD), clinical attachment level (CAL) and bleeding on probing (both BOP + or - included). Periodontally diseased individuals (each of generalizing or localize forms) had to have at least three teeth with CAL≥3mm and PPD>3mm in at least two quadrants. Subjects in the peri-implantitis group had to have one or more implants placed and functionally loaded for more than one year. The peri-implant probing depth of at least one site had to be ≥5 mm with or without suppuration / bleeding on probing. Plaque index>20%, radiographically, the crestal bone loss had to be present in at least one site around the implant resulting in exposure of at least two implant threads. Patients who were in class VI, VII and VIII of Implant Success Index

(ISI) were enrolled in this study (Table 1) (28).

The main criteria for disease condition were based on probing pocket depth and radiographic bone loss/clinical attachment loss.

Table 1. Implant Success Index (ISI)

Scores	Soft tissue level (SL)	Hard tissue level (HL)	Clinical Findings
ISI I	SL+, PPD<=4mm, BOP-	HL+	Clinical Healthy
ISI II	SL+, PPD<=4mm , BOP+	HL+	Soft Tissue Inflammation
ISI III	SL+, PPD>4mm , BOP+	HL+	Deep soft tissue pocket
ISI IV	SL+	HL-, RBL<=2mm(<= 20%)	Initiation of Hard Tissue Breakdown
ISI V	SL-	HL-, RBL<=2mm(<= 20%)	Hard Tissue Breakdown Plus Soft Tissue Recession
ISI VI	SL+	HL-, RBL:2-4 mm (<40%)	Notable Hard Tissue Breakdown
ISI VII	SL-	HL-, RBL:2-4 mm (<40%)	Notable Hard Tissue Breakdown Plus Soft Tissue Recession
ISI VIII	--	RBL>+ 40%	Severe Bone Loss
ISI IX	--	Clinical mobility	Clinical failure

SL: Soft Tissue Level, *PPD*: Probing Pocket Depth, *BOP*: Bleeding on Probing, *HL*: Hard Tissue Level, +: tissue level located at or coronal to the reference line, - : level apical to the reference line, *RBL*: Radiographic Bone Loss detected via long cone Parallel peri-apical technique.
Note: If the peri-apical of implants have a bone loss/radiolucent view (retrograde peri-implantitis) it will be identified by placing the letter R (e.g. ISI IR, ISI IIR, ISI IIIR, etc.).

DNA extraction and genotyping

DNA extraction was performed using Bioneer DNA purification kit (Bioneer, Daejeon, Korea). Briefly, 100 µl of blood sample was suspended in 400 µl of lysis buffer and after vortex at maximum speed for 20 seconds, 300 µl precipitation solutions was added to the lysate. After vortexing and centrifugation, the resulting solution was transferred to a spin column and centrifuged for 1 min at 12000 rpm at room temperature. The column was washed with washing buffers I and II respectively and then 30 µl preheated elution buffer (65 ºC) was added to elute the DNA. The concentration of DNAs was assessed by a spectrophotometer as 75nanogram. Then we transferred our DNA samples to the company of KBioscience for SNP studies. In this study, the rs2276631and rs17235409 SNPs of Nramp1 genes were genotyped by KASP method. The details of this genotyping are available at URL: www.kbioscience.co.uk/reagents/KASP.html

The NCBI sequence used for evaluation of rs2276631 polymorphism was

GGGTCCAGGAAAGCAATGCTCATGAG[A/G]AAGCCAGGCCCCGTGAAGGCCCATA

The NCBI sequence used for evaluation of rs17235409 polymorphism was

ACTTCCTGTATGGGCTCCTTGAAGAG[A/G]ACCAGAAAGGGGAGACCTCTGGCTA

Statistical analysis was performed by SPSS version 19.5 software using *Chi*-square and Fisher's exact tests. $P<0.05$ was considered statistically significant.

Results

From people attended to the Periodontology Department of Shahid Beheshti University of Medical Sciences, 463 individuals selected for the study. Nonetheless, according to our strict criteria only 201participants including 79 patients with chronic periodontitis, 38 patients with peri-implantitis and 84 healthy controls were enrolled.

Clinical data and demographic characteristics of patients and controls are presented in Table 2.

Table 2. Demographic and clinical characteristics of subjects

Objectives / Groups	Age	Sex		Probing pocket depth (Mean ± SD)	Attachment bone loss
		Female	Male		
Chronic periodontitis (n:79)	Mean: 48.3 (26 to 60)	N: 36	N: 43	5.95±0.75	5.34±0.85
Healthy periodontium (n:84)	Mean: 38.4 (31 to 55)	N: 41	N: 43	1.83±0.68	0.17±0.11
Peri-implantitis (n: 38)	Mean: 50.2 (29 to 60)	N: 20	N: 18	6.81±0.52	4.44±1.89

Some of our samples were missed during the genotyping process as reported in Table 3 and 4.

In evaluating rs17235409 (Table 3), the results showed that significant differences were detected within the distribution of genotypes between healthy controls and CP group (*P*=0.044). While the Fisher' exact test revealed no significant differences between control and PI groups in the distribution of genotypes (*P*=1). All PI patients (n=38) had GG genotype and the frequency of AA and GA were 0. There were insignificant differences between genotype distributions of CP and PI (*P*=0.298).

Chi-square test showed significant differences between genotypes regarding rs2276631 polymorphism in control and CP groups (Table 3, *P*-value=0.028). The Distribution of genotypes differed insignificantly in comparison of PI and control groups (*P*=0.623). The genotype comparison of CP and PI revealed no significant differences (*P*=0.065). The frequencies of AA, GA and GG genotypes in PI group were 0(0.0%), 10(27%) and 28(73%), respectively.

Table 3. Genotype and allele frequencies of the NRAMP1 rs17235409 polymorphism

rs17235409 genotypes	Chronic periodontitis n (%)	Controls n (%)	*P*.value
A:A	0 (0.0)	0 (0.0)	Fisher's exact test: *P* value equals 0.0447
G:A	4 (5.0)	0 (0.0)	
G:G	69 (87.5)	84 (100)	
Missed	6(7.5)	0(0.0)	
Total	79 (100)	84 (100)	

Table 4. Genotype and allele frequencies of the NRAMP1 rs2276631 polymorphism

Genotypes rs2276631	Chronic periodontitis n (%)	Controls n (%)	*P*.value
A:A	1 (1.3)	0 (0)	Chi squared equals 4.789 *P*.value=0.0286
G:A	26 (34.7)	15 (17)	
G:G	48 (64)	64 (76)	
Missed	0(0.0)	6 (7)	
Total	79 (100)	84 (100)	

Discussion

Chronic periodontitis and peri-implantitis are characterized by the presence of bacteria, inflammation, and bleeding of gingiva, bone loss and deep periodontal/peri-implant pockets with or without pus. *NRAMP1* plays a significant role in innate immunity (especially in phagocytosis of macrophages). Also, *NRAMP1* is a bacteriostatic agent. Its mechanism of action is through consuming the heme, an essential nutrient for two common periodontal pathogens (P. gingivalis and P. intermedia). Evidence has shown that some gene polymorphisms of *NRAMP1* could alter its function. This study was the first to evaluate the correlation of two functional SNPs of *NRAMP1* with CP and PI. Our results indicated that rs17235409 and rs2276631 (A to G allele substitution in *NRAMP1* gene) are associated with chronic periodontitis in an Iranian population. The more prevalence of G allele could be detected in more healthy samples.

Statistically, significant differences were observed in genotypes of rs17235409 between controls and patients with chronic periodontitis. This finding should be interpreted with caution because in our study group we did not detect any AA genotype (in controls and patients). The differences in the frequency of genotypes of rs2276631 among the study groups were also statistically significant between CP and control groups.

To the best of our knowledge, no study has evaluated the *NRAMP1* polymorphism in dentistry; thus, comparison of results was not possible. However, SNPs in other components of immune response have been reported in different populations with controversial results. Lachmann and colleagues found no correlation between IL1composite genotype and peri-implantitis (29). Bormann *et al*., in their review concluded that IL1

polymorphism alone is not a risk factor for peri-implantitis while in combination with smoking can significantly enhance peri-implantitis (30). Menezes and colleagues failed to find an association between CP and tumor necrosis factor alpha (*TNFa*) polymorphism (31). Campos *et al*., studied the SNPs in *IL2, IL6*, transforming growth factor beta (*TGFb*) and matrix metalloproteinase (*MMP*) genes in early failed implants and detected no relationship (32,33). On the contrary, many studies reported significant associations between relevant diseases and SNPs. For instance, Santos and colleagues cited that there may be an association between early implant loss and *MMP1* SNP in Brazilians (34). Costa-Junior *et al*., reported that promoter polymorphism of *MMP8* has a significant relationship with the failure of osseointegration (35). Dereka and colleagues in their review showed that SNP of *IL1* receptor antagonist *(IL1RN*), *IL1*a and *IL1b* were correlated with increased peri-implant tissue infection and destruction (36). Baradaran *et al*., found that aggressive periodontitis is associated with SNP of *IL-1RN* (37). Martelli and colleagues reported that *VDR* polymorphism is associated with chronic periodontitis (38). Andia *et al*., concluded that SNP of *IL8* may be a genetic determinant for periodontitis. The SNP of *NRAMP1* has not yet been evaluated in dentistry (39). However, there are published studies regarding its association with other diseases with similar pathogenesis (infectious, inflammatory or autoimmune diseases). Stages and colleagues reported the significant association of SNP in *NRAMP1* gene with pulmonary tuberculosis in a Greek population (40). This finding is supported by the results of a review article conducted by Ates *et al*., (41). Rheumatoid arthritis was also reported to be correlated with SNP of *NRAMP1* (rs3731865) (41). Another study by Ates and colleagues showed that Behçet's syndrome is associated with polymorphism of *NRAMP1* in a Turkish population (42). Conversely, there are some studies reporting negative association of *NRAMP1* SNPs with pulmonary tuberculosis, (43) multiple sclerosis, (44) leprosy, (45) and etc.

The present study had some limitations regarding peri-implantitis (PI) that necessitate more relevant research in this respect. These limitations are as follows: Low prevalence of genotypes that may be due to the small sample size resulted from our strict criteria, our control group comprised both healthy teeth and healthy implants, lack of sufficient evidence for the etiology of PI which results in unsuccessful preventive efforts, lack of an accredited and unique definition and classification for PI in order to achieve a correct clinical judgment, the multifactorial nature of the PI and CP diseases necessitates further research on other factors such as the impact of genetic predispositions of the immune components upon tissue presentation of them, in addition, there are other contributing factors that affect the diagnosis, treatment planning and treatment of patients including the time of implant loading, expertise of the clinician, patient's oral health status and local habits, the time of visits, the expertise of the observers (inter-examiner error) and etc.

Based on our results, we conclude that presence of G allele in both rs2276631 and rs17235409 location may be a protective factor against chronic periodontitis. More studies with a larger sample size in population of different origins are required for confirming *NRAMP1* as a genetic determinant in dental disorders.

Acknowledgment

This study was supported by Dental Research Center, Shahid Beheshti University of Medical Sciences.

References

1. Socransky SS, Haffajee AD. The bacterial etiology of destructive periodontal disease: current concepts. J Periodontol 1992;63(4 Suppl):322-31.
2. Leonhardt A, Berglundh T, Ericsson I, et al. Putative periodontal pathogens on titanium implants and teeth in experimental gingivitis and periodontitis in beagle dogs. Clin Oral Implants Res 1992;3(3):112-9.
3. Laine ML, Leonhardt A, Roos- Jansa ker AM, et al. IL-1RN gene polymorphism is associated with peri-implantitis. Clin Oral Implant Res 2006;17(4):380-385.
4. Offenbacher S. Periodontal disease: pathogenesis. Ann Periodontal 1996;1(1):821-78.
5. Albandar JM. Global risk factors and risk indicators for periodontal disease. Periodontol 2000. 2002;29:177-206.
6. Ebadian AR, Kadkhodazadeh M, Zarnegarnia P, et al. Bacterial analysis of peri-implantitis and chronic periodontitis in Iranian subjects. Acta Med Iran 2012;50(7):486-92.
7. Mombelli A, Lang NP. The diagnosis and treatment of peri-implantitis. Periodontol 1998;17(1):63-76.
8. Bregger U, Aeschlimann S, Burgin W, et al. Biological and technical complications and failures with fixed partial dentures on implants and teeth after four to five years of function. Clin Oral Implants Res 2001;12(1):26-34.
9. Lindhe J, Meyle J. Peri-implant diseases: Consensus report of the Sixth European Workshop on Periodontology. J Clin Periodontol 2008;35(Suppl 8):282-5.

10. Weyant RJ, Burt BA. An assessment of survival rates and within-patient clustering of failures for endosseous oral implants. J Dent Res 1993;72(1):2-8.
11. Michalowicz BS, Diehl SR, Gunsolley JC. Evidence of a substantial genetic basis for risk of adult periodontitis. J Periodontol 2000;71(11):1699-707.
12. Santos MC, Campos MI, Souza AP, et al. Analysis of MMP-1 and MMP-9 promoter polymorphisms in early osseointegrated implant failure. Int J Oral Maxillofac Implants 2004;19(1);38-43.
13. Shimpuku H, Nosaka Y, Kawamura T, et al. Genetic polymorphisms of the IL-1 gene and early marginal bone loss around endosseous dental implants. Clin Oral Implants Res 2003;14(4):423-9.
14. Hall J, Britse AO, Jemt T, Friberg B. A controlled clinical exploratory study on genetic markers for peri-implantitis. Eur J Oral Implantol. 2011;4(4):371-82.
15. Kadkhodazadeh M, Amid R, Ebadian AR, et al. TRAF family member-associated NF-KB activator (TANK) gene polymorphism in chronic periodontitis and peri-implantitis patients. J Long Term Eff Med Implants 2012;22(2):127-36.
16. Kdkhodazadeh M, Hajilooi M, Houshmand B, et al. Evaluation of PECAM-1 gene polymorphism in patients with periodontal disease and healthy individuals. ISRN Dent 2012;2012:751920.
17. Kinane DF, Shiba H, Hart TC. The genetic basis of periodontitis. Periodontol 2000;39:91-117.
18. Djaldetti M, Salman H, Bergman M, et al. Phagocytosis the mighty weapon of the silent warriors. Microsc Res Tech 2002;57(6):421-31.
19. Champion OL, Karlyshev A, Cooper IA, et al. Yersinia pseudotuberculosis mntH functions inintracellular manganese accumulation, which is essential for virulence and survival in cells expressing functional Nramp1. Microbiology 2011;157(Pt 4):1115-22.
20. McDermid JM, Prentice AM. Iron and infection: effects of host iron status and the iron-regulatory genes haptoglobin and NRAMP1 (SLC11A1) on host-pathogen interactions in tuberculosis and HIV. Clin Sci (Lond) 2006;110(5):503-24.
21. Canonne-Hergaux F, Gruenheid S, Govoni G, e al. The Nramp1 protein and its role in resistance to infection and macrophage function. Proc Assoc Am Physicians 1999;111(4):283-9.
22. Li XW, Yang Y, Zhou F, et al. SLC11A1 (NRAMP1) polymorphisms and tuberculosis susceptibility: updated systematic review and meta-analysis. PLoS One 2011;6(1):e15831.
23. Gazouli M, Atsaves V, Mantzaris G, et al. Role of functional polymorphisms of NRAMP1 gene for the development of Crohn's disease. Inflamm Bowel Dis 2008;14(10):1323-30.
24. Donninger H, Cashmore TJ, Scriba T, et al. Functional analysis of novel SLC11A1 (NRAMP1) promoter variants in susceptibility to HIV-1. J Med Genet 2004;41(4):e49.
25. Little J, Higgins JPT, Ioannidis JPA, et al. Strengthening the reporting of genetic association studies (STREGA) – an extension of the STROBE statement. Eur J Epidemiol 2009;24(1):37-55.
26. Kadkhodazadeh M, Alizadeh Tabari Z, Talebi Ardakani MR, et al. Analysis of osteoprotegerin (OPG) gene polymorphism in Iranian patients with chronic periodontitis and periimplantitis. Eur J Oral Implantol 2012;5(4):381-8.
27. Kadkhodazadeh M, Ebadian AR, Gholami GA, et al. Analysis of RANKL gene polymorphism (rs9533156 and rs2277438) in Iranian patients with chronic periodontitis and periimplantitis. Arch Oral Biol 2013;58(5):530-6.
28. Kadkhodazadeh M, Amid R. Evaluation of peri-implant tissue health using a scoring system. J Implant Adv Clin Dent 2012;4(1):51-7.
29. Lachmann S, Kimmerle-Müller E, Axmann D, et al. Associations between peri-implant crevicular fluid volume, concentrations of crevicular inflammatory mediators, and composite IL-1A -889 and IL-1B +3954 genotype. A cross-sectional study on implant recall patients with and without clinical signs of peri-implantitis. Clin Oral Implants Res 2007;18(2):212-23.
30. Bormann KH, Stühmer C, Z'Graggen M, et al. IL-1 polymorphism and periimplantitis. A literature review. Schweiz Monatsschr Zahnmed 2010;120(6):510-20.
31. Menezes NG, Colombo AP. Lack of association between the TNF-alpha -308 (G/A) genetic polymorphism and periodontal disease in Brazilians. Braz Oral Res 2008;22(4):322-7.
32. Campos MI, Godoy dos Santos MC, Trevilatto PC, et al. Interleukin-2 and interleukin-6 gene promoter polymorphisms, and early failure of dental implants. Implant Dent 2005;14(4):391-6.
33. Dos Santos MC, Campos MI, Souza AP, Scarel- Caminaga RM, et al. Ansalysis of the transforming growth factor-beta 1 gene promoter polymorphisms in early osseointegrated implant failure. Implant Dent 2004;13(3):262-9.
34. Santos MC, Campos MI, Souza AP, et al. Analysis of MMP-1 and MMP-9 promoter polymorphisms in early osseointegrated implant failure. Int J Oral Maxillofac Implants 2004;19(1):38-43.
35. Costa-Junior FR, Alvim-Pereira CC, Alvim-Pereira F, et al. Influence of MMP-8 promoter polymorphism in early osseointegrated implant failure. Clin Oral Investig 2013;17(1):311-6.

36. Dereka X, Mardas N, Chin S, et al. A systematic review on the association between genetic predisposition and dental implant biological complications. Clin Oral Implants Res 2012;23(7):775-88.
37. Baradaran-Rahimi H, Radvar M, Arab HR, Tavakol-Afshari J, et al. Association of interleukin-1 receptor antagonist gene polymorphisms with generalized aggressive periodontitis in an Iranian population. J Periodontol 2010;81(9):1343-7.
38. Martelli FS, Mengoni A, Martelli M, et al. VDR TaqI polymorphism is associated with chronic periodontitis in Italian population. Arch Oral Biol 2011;56(12):1494-8.
39. Andia DC, de Oliveira NF, Letra AM, et al. Interleukin-8 gene promoter polymorphism (rs4073) may contribute to chronic periodontitis. J Periodontol 2011;82(6):893-9.
40. Stagas MK, Papaetis GS, Orphanidou D, et al. Polymorphisms of the NRAMP1 gene: distribution and susceptibility to the development of pulmonary tuberculosis in the Greek population. Med Sci Monit 2011;17(1):PH1-6.
41. Ates O, Dalyan L, Müsellim B, et al. NRAMP1 (SLC11A1) gene polymorphisms that correlate with autoimmune versus infectious disease susceptibility in tuberculosis and rheumatoid arthritis. Int J Immunogenet 2009;36(1):15-9.
42. Ateş O, Dalyan L, Hatemi G, et al. Genetic susceptibility to Behçet's syndrome is associated with NRAMP1 (SLC11A1) polymorphism in Turkish patients. Rheumatol Int 2009;29(7):787-91.
43. Niño-Moreno P, Portales-Pérez D, Hernández-Castro B, et al. P2X7 and NRAMP1/SLC11 A1 gene polymorphisms in Mexican mestizo patients with pulmonary tuberculosis. Clin Exp Immunol 2007;148(3):469-77.
44. Ates O, Kurt S, Bozkurt N, Karaer H. NRAMP1 (SLC11A1) variants: genetic susceptibility to multiple Sclerosis. J Clin Immunol 2010;30(4):583-6.
45. Velayati AA, Farnia P, Khalizadeh S, et al. Interferon-gamma receptor-1 gene promoter polymorphisms and susceptibility to leprosy in children of a single family. Am J Trop Med Hyg 2011;84(4):627-9.