ORIGINAL ARTICLE

Periodontics

Haptoglobin gene polymorphisms in peri-implantitis and chronic periodontitis

Ahmad R. Ebadian[1], Mahdi Kadkhodazadeh[2], Seyed Hamid Hosseini Naghavi[3], Maryam Torshabi[4] & Mahmood Tamizi[5]

1 Iranian General Dentists Association, Tehran, Iran
2 Department of Periodontics, Dental School, Shahid Beheshti University of Medical Sciences, Tehran, Iran
3 Dental School, Shahid Beheshti University of Medical Sciences, Tehran, Iran
4 Department of Dental Biomaterial, Dental School, Shahid Beheshti University of Medical Sciences, Tehran, Iran
5 Iranian Academy of Periodontology, Tehran, Iran, the Research Deputy of this university

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Correspondence
M. Kadkhodazadeh, Department of Periodontics, Dental School, Shahid Beheshti University of Medical Sciences, Evin, Tehran, Iran.
Tel: +989127608346
Fax: +982122173754
Email: Kadkhodazadehmahdi@yahoo.com

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Abstract

Aim: The haptoglobin–hemoglobin (Hp–Hb) complex plays a significant role in regulating immune responses and acts as a bacteriostatic agent. Haptoglobin polymorphisms result in different Hb binding affinities. This study sought to assess whether Hp 2-2 could be a genetic determinant for increasing the risk of peri-implantitis and chronic periodontitis.

Methods: Of the Iranian subjects referred to the Department of Periodontics, Shahid Beheshti University, Tehran, 203 were entered into the study, including 117 patients and 86 periodontally healthy individuals. Polymerase chain reaction (PCR) was performed for genotyping. Data were analyzed by Kruskal–Wallis test using the SPSS statistics software package.

Results: The prevalence of Hp 2-2, 2-1, and 1-1 did not differ significantly between patients and healthy subjects ($P > 0.05$). The highest frequencies of Hp 1-1, 2-1, and 2-2 genotypes were seen in the control (7%), peri-implantitis (51%) and periodontitis (64%) groups, respectively.

Conclusions: Haptoglobin polymorphisms may not play a role in development of peri-implantitis or chronic periodontitis among Iranians but we strongly suggest researchers to evaluate this polymorphism in further studies on larger sample sizes, different populations, and other types of periodontitis.

Introduction

Haptoglobin (Hp) is a positive acute-phase glycoprotein which is produced mainly by liver adipocytes, binds to hemoglobin (Hb) and is known as a "heme-scavenger."[1,2] Haptoglobin synthesis is increased by cytokines, such as interleukin-6 (IL6), IL1, and tumor necrosis factor (TNF).[3] The Hp–Hb complex influences the balance between T-helper lymphocyte 1 (Th1) and Th2 cytokines, and thereby plays a significant role in immune response and inflammation.[4–6] Haptoglobin also inhibits biosynthesis of prostaglandins via down-regulation of cyclooxygenase and lipoxygenase and is also an angiogenic and antioxidant factor.[7–9] In other words, our defense system contains two components: a specific immune system and a non-specific Hp defense system.[9]

Periodontitis and peri-implantitis are multifactorial diseases of the tooth and implant supporting tissues. They are characterized by bacterial accumulation on the adjacent tooth or implant, innate immune response to this invasion, inflammation phenomenon, destruction of the alveolar bone and periodontal ligament, apical migration of the epithelial attachment resulting in the formation of periodontal pockets, and ultimately loosening and exfoliation of the tooth or implant.[10–12] Factors responsible for the development of these inflammatory diseases are not

easily determined. While epidemiological evidence clearly points to an environmental influence, not all individuals in these environments develop the same disease. Susceptibility to periodontitis and peri-implantitis may have a genetic component in the innate immune system, inflammation, or bone resorption.[13–15] Nonetheless, neither the environment nor genetics are the only determining factor.

There are three possible Hp genotypes: 1.1, 1.2, or 2.2.[16] In fact, Hp consists of two protein chains of α and β, and the polymorphisms arise from different amino acids in the α chain on chromosome 16q22 as follows: α1S (slow), α1F (fast), and α2.[17] According to Figure 1: Hp 1.1 is a small protein (89 kDa) made of α1-β; Hp 2.1 (86–300 KDa) is made of α1-β + α2-β; and Hp 2.2 (170–900 kDa) is made of α 2-β.[18] These genotypes have different Hb binding affinities. People carrying the 1.1 genotype have the highest binding affinity while some inflammatory diseases (e.g. atherosclerosis, inflammatory bowel disease, diabetes, celiac disease, Parkinson's disease, recurrent seizures, etc.) are more prevalent in patients carrying the 2.2 genotype.[18–23] Moreover, Heme is an essential nutrient for some bacteria involved in pathogenesis of periodontitis and peri-implantitis like *Porphyromonas gingivalis* and *Prevotella intermedia*.[24] In other words "heme-scavenger" Hp, seems to play a bacteriostatic role in relevant diseases.[25]

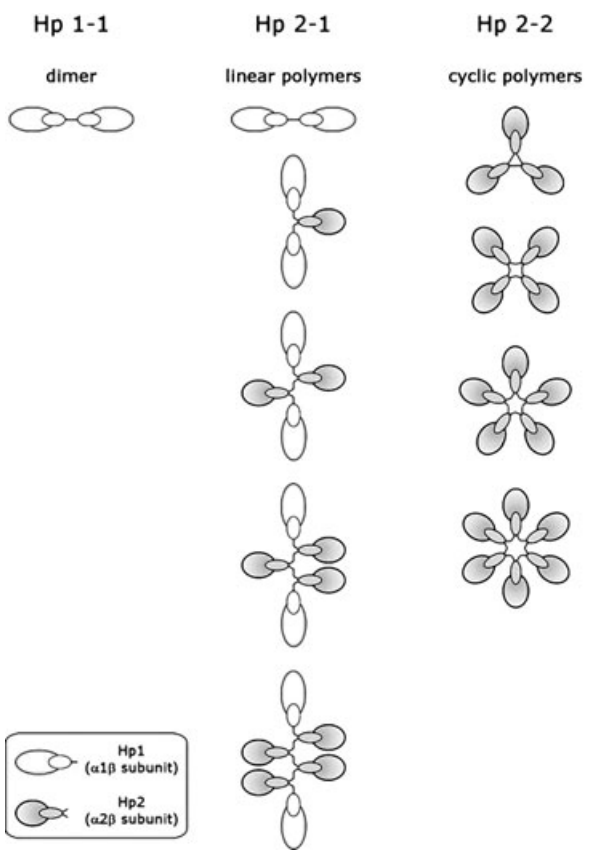

Figure 1. Structural differences and subunit arrangement of Hp phenotypes. The Hp 1 monomer (α1-β) is monovalent and thus can associate only with one other Hp monomer, whereas the Hp 2 monomer (α2-β) is bivalent and associates with two different Hp monomers. Consequently, the three Hp genotypes express entirely different molecular structures at the protein level.

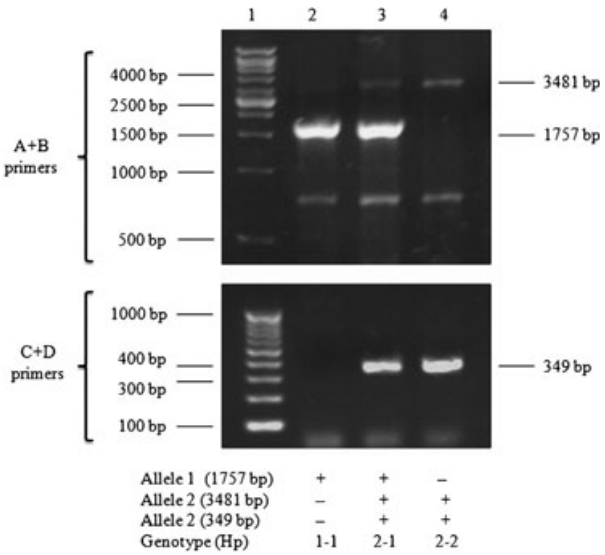

Figure 2. Haptoglobin genotyping with primers A + B and C + D. In a PCR reaction with primers A and B, alleles Hp 1 and Hp 2 were characterized by amplification bands of 1757 and 3481 bp, respectively. The Hp-2-specific amplification product was characterized by the 349 bp band. Lane 1, DNA size marker; lane 2, genotype Hp 1-1; lane 3, genotype Hp 2-1; lane 4, genotype Hp 2-2.

Accordingly, evaluation of the role of Hp in dental conditions is of benefit due to its capability in eliminating two factors involved in periodontitis and peri-implantitis, that is, inflammation and bacterial invasion. This study was the first to assess the role of Hp genotypes in peri-implantitis and chronic periodontitis. We hypothesized that people carrying Hp 2 may be more susceptible to periodontitis and peri-implantitis.

Methods

This cross-sectional study evaluated the role of Hp genotypes in peri-implantitis and periodontitis, from July 2009 to Feb. 2012 at Shahid Beheshti Dental School, Tehran, Iran according to the STREGA statement.[26]

From individuals referred to our Periodontology Department, a total of 463 were selected for the study after clinical and radiographic observations of four points around each tooth/implant. Use of strict inclusion and exclusion criteria resulted in enrollment of 203 participants, including 117 patients (43 peri-implantitis, 74

chronic periodontitis) and 86 periodontally healthy subjects. The final examination was done by an expert periodontist with reproducibility of 93% (based on the intraclass correlation coefficient). All individuals provided written informed consent and filled out a questionnaire containing demographic characteristics, and medical and dental history. The Ethics Committee of Shahid Beheshti University of Medical Sciences approved this study.

The exclusion criteria were as follows: oral diseases other than caries and periodontal disease, ongoing orthodontic therapy, smoking, history of systemic or local disease impacting on the immune system, diabetes mellitus, hepatitis, HIV infection, immunosuppressive chemotherapy, current pregnancy or nursing, and non-Iranian population.

The inclusion criteria were as follows: participants in the chronic periodontitis group had to have at least five teeth, except for the third molar in each quadrant. The diagnosis of chronic periodontitis was established based on radiographic and clinical parameters, including plaque index (PI), probing pocket depth (PPD), clinical attachment level (CAL), and bleeding index (BI). Periodontally diseased individuals had to have at least three teeth with CAL $\geq$ 3 mm and PPD > 3 mm, in at least two quadrants.[27,28] The subjects in the peri-implantitis group had to have no history of periodontitis and have one or more implants with more than 12 months of loading. A clinical diagnosis of peri-implantitis was made when the peri-implant probing depth of at least one measurement site was equal to or more than 5 mm, with bleeding on probing and/or suppuration. There had to be radiographic evidence of crestal bone loss in at least one site around the implant. Crestal bone loss had to expose at least two threads of the implants. Implant Success Index (ISI) ratings of VI, VI,I and VIII were considered for this study.[29]

The control group comprised subjects with at least 20 teeth in their mouth with a minimum of five teeth in each quadrant, no symptom and history of periodontitis, no evidence of bone loss and PD < 3 mm. They did not have oral diseases other than caries, ongoing orthodontic therapy, history of smoking, systemic or local disease impacting on the immune system, diabetes mellitus, hepatitis, HIV infection, immunosuppressive chemotherapy, current pregnancy or nursing and were Iranians akin to the other two groups. If these subjects had implants, they had to have peri-implant probing depth <4 mm, and no radiographic evidence of bone loss.

For genotyping to occur, we obtained a 5 cc blood sample from an arm vein of each individual at the Periodontology Department. DNA extraction was performed using a Bioneer DNA purification kit (Bioneer, Daejeon, Korea) at the Cellular and Molecular Biology Department of Shahid Beheshti Dental School. The remaining blood samples were stored at −4°C and DNA samples were stored at −70°C for further analysis. Primer sequences and PCR condition were in accordance with the Kotch *et al.*[30] description (Table 1).

DNA extraction and PCR reactions were performed by one of the authors as follows: genomic DNA was extracted from whole blood using a SinaPure DNA kit (SinaClon, Tehran, Iran). Briefly, 100 μL of blood sample was suspended with 400 μL of lysis buffer and after vortex at maximum speed for 20 sec, 300 μL precipitation solution was added to the lysate. After vortexing and centrifugation, the resulting solution was transferred to a spin column and centrifuged for 1 min at 11.269 *g* at room temperature. The column was washed with washing buffers I and II and then 30 μL of preheated elution buffer (65°C) was added to elute the DNA. Two sets of oligonucleotide primers were synthesized by Sinaclone (Table 1).[31] Primers A and B were used for amplification of a 1757-bp Hp allele-one specific sequence (Hp 1) and a 3481-bp Hp allele- two specific sequence (Hp 2). Primers C and D (confirmation primers) were used to amplify a 348-bp Hp2 allele-specific sequence. The 20-μL reactions contained 10 μL PCR master mix (SinaClone, Tehran, Iran), 1 μL DNA, 1 μL (0.5 μM) of each primer and 7 μL nuclease-free water (5 prime, Hamburg, Germany). The thermocycler used was PeQlab (Wilmington, DE, USA) and the PCR was performed using the following program: 95°C for 2 min, 35 cycles of denaturation at 95°C for 30 sec, annealing at 63°C (A + B primers) and 61°C (C + D primers) for 30 sec, extension at 72°C for 1 min (C + D primers) and 2 min (A + B primers), and a final extension at 72°C for 7 min. For genotype assignments (Figure 2), the PCR products were analyzed on 1% agarose gels (KBC, Tehran, Iran) and UV illumination (Vilber Lourmat, Marne La Vallée, France). Genotype determinations were undertaken without knowledge of the phenotyping results and more than 50% of samples were reanalyzed and reconfirmed randomly.

Table 1. Primer pairs for amplification of haptoglobin (Hp 1 and 2)

Gene	Primer sequence (5′→3′)		Annealing temperature (°C)	Product size (bp)
HP-1	F = A	GAGGGGAGCTTGCCTTTCCATTG	63	1757
HP-2	R = B	GAGATTTTTGAGCCCTGGCTGGT	63	3481
HP-2	F = C	CCTGCCTCGTATTAACTGCACCAT	61	349
	R = D	ACATGGCTATGTGGAGCACTCGG		

Statistical analysis

Data were analyzed to find significant differences (P-value $\leq$ 0 .05), if any, in genotype and allele distribution between three groups by the Kruskal–Wallis test using SPSS Version 19 statistical software package (IBM, Armonk, NY, USA).

Results

Of 463 subjects selected for this study, 203 met our criteria and volunteered for blood sampling. There were three groups: periodontally healthy (n = 86), chronic periodontitis (n = 74) and peri-implantitis (n = 43). From individuals in the control group, 42 were females and 41 were males, with a mean age of 44 years (range 30–55 years). There were 34 females and 40 males with a mean age of 47 years (range 30–60 years) in the chronic periodontitis group. In the peri-implantitis group, there were 22 females and 21 males, with a mean age of 40 years (range 31–57 years). Ordinal logistic regression analysis showed no significant differences in age and sex distribution (P-values > 0.05).

We assessed the frequencies of Hp genotypes in healthy individuals, chronic periodontitis, and periimplantitis patients after confirming the Hardy–Weinberg disequilibrium. Statistical analyses results are summarized in Tables 2 and 3.

The highest frequency of Hp 1-1 genotype was seen in the control group, the uppermost frequency of Hp 2-1 genotype was observed in peri-implantitis group, and the topmost frequency of Hp 2-2 genotype was detected in the chronic periodontitis group. Nonetheless, these frequencies did not differ significantly (P-value > 0.05) when patients were compared to controls. However, a significant difference was found when Hp 1-1 was compared to Hp 2-1 and Hp 2-2 (P-value < 0.05). We also detected a comparable tendency for frequency of Hp alleles in the three groups (Table 3).

Table 2. Genotype frequencies in the study groups

Group	Hp 1-1 genotype *n* (%)	Hp 2-1 genotype*n* (%)	Hp 2-2 genotype *n* (%)	Total *n* (%)
Control	6 (7)	34 (40)	46 (54)	86 (100)
Peri-implantitis	1 (2)	22 (51)	20 (47)	43 (100)
Chronic periodontitis	1 (1)	28 (38)	45 (61)	74 (100)
P–value	0.286			

Table 3. Allele frequencies among the study groups

Group	Hp 1 allele *n* (%)	Hp 2 allele *n* (%)	Total *n* (%)
Control	46 (26.7)	126 (73.3)	172 (100)
Peri-implantitis	24 (27.9)	62 (72.1)	86 (100)
Chronic periodontitis	30 (20.3)	118 (79.7)	148 (100)
P-value	0.375		

Discussion

Considering the anti-inflammatory and antibacterial properties of the Hp–Hb complex and based on the different Hb binding affinities of Hp genotypes and their subsequent altered function among individuals, we decided to find out whether people carrying low affinity Hp 2 are more susceptible to peri-implantitis or chronic periodontitis compared to those carrying Hp 1. Our study results failed to find any significant correlation in this respect among the Iranian population.

This study is the first to assess the correlation of Hp polymorphisms with "chronic periodontitis" and the less-prevalent but important disease "peri-implantitis." Eradication of such diseases is especially important in improving the quality of life. Achieving this goal necessitates proper strategies for diagnosis and prevention of these conditions. Although there is no controversy regarding the unique criteria for periodontitis, there are different criteria used in the literature for peri-implantitis, resulting in conspicuous variability in reported prevalence from 8.6% to 56%.[31–33] We used a new classification namely "ISI" for peri-implantitis.[29] For detecting the Hp polymorphisms we performed PCR to evaluate a probable risk determinant. Our data indicate an increased tendency towards Hp 2 among patients. Hp 2-2 and Hp 2-1 had the highest frequencies in chronic periodontitis (61%) and peri-implantitis (51%). The highest frequency of Hp 1-1 was observed among healthy individuals (7%). However, no significant difference was detected in this regard among the three groups (P > 0.05).

To our knowledge, Hp genotypes have not been assessed in peri-implantitis and chronic periodontitis patients; thus, we could not compare our results with those of other relevant studies. But there are several studies that have evaluated the role of Hp polymorphisms in other diseases. In our populace, Hp polymorphisms did not play an important role in periodontitis and peri-implantitis. McDermid and Prentice evaluated HIV and tuberculosis in UK and found no relationship with Hp polymorphisms.[34] Wobeto and colleagues cited that cardiovascular diseases do not increase by Hp polymorphisms in Brazilians suffering from diabetes mellitus.[35] Mavondo *et al.*[36] declared that Hp polymorphisms in blacks of Botswana are not associated with prostate cancer. On the contrary, in inflammatory diseases (similar to the nature of peri-implantitis and peri-

odontitis) Hp polymorphisms have been reported as a positive genetic determinant. Papp and colleagues found that Hp 2-1 was associated with a significant risk ($P = 0.0006$) of celiac disease in Hungarians.[18] Delanghe *et al.*[37] cited that vitamin C deficiency and scurvy are not only dietary diseases but are codetermined by Hp polymorphisms. They declared that Hp 2 (the most common Hp in Asians) leads to instability, low concentration, and higher susceptibility to oxidation of vitamin C. Costa-Mallen and colleagues concluded that Hp polymorphisms are associated with idiopathic Parkinson's disease in American women.[22] Sadrzadeh *et al.*[23] determined that Hp 2-2 significantly ($P < 0.001$) increases the risk of recurrent seizures in epilepsy.

Genetic alterations in populations of different origin, small prevalence of Hp 1 in Asians, and the low sample size of our peri-implantitis group may justify the irrelevance of Hp 2 and peri-implantitis or chronic periodontitis.[17] Our small sample size was due to the particularly low prevalence of peri-implantitis, our strict inclusion and exclusion criteria, and our time limitation for sampling.

In conclusion, although this pilot study was unsuccessful in proving the role of Hp polymorphisms in Iranians suffering from peri-implantitis and chronic periodontitis, we strongly suggest further similar research on a larger sample size, in other populations, on aggressive periodontitis patients, and as a multicenter research.

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Competing interests

The authors declare that they have no competing interests.

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