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Povezanost polimorfizma gena MBL-2 s parodontitisom kod pacijenata s dijabetesom tipa 2: preliminarno istraživanje

Association of Polymorphism of the MBL2 Gene in Type 2 Diabetic Patients with Periodontitis: A Preliminary Study

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Sažetak

Svrha: Lektin koji veže manozu (MBL) važan je protein urođenog imunološkog sustava. Sposoban je vezati se za šećerne skupine na površini mnogih mikroorganizama i aktivirati komplement u interakciji sa serinskim proteazama. Mutacije ekspresije humanog gena MBL2 povezane su s nekoliko infektivnih bolesti; ovom preliminarnom istraživanju svrha je bila odrediti povezanost polimorfizma na eksonu-1 gena MBL2 i parodontne bolesti kod pacijenata s dijabetesom tipa 2. **Ispitanici i postupci:** Istraživanjem je bilo obuhvaćeno 50 pacijenata s dijabetesom i svi su na početku bili na kliničkom parodontološkom pregledu na kojemu je na šest mjesta na zubu bila određena dubina sondiranja džepova, krvarenje nakon tog postupka, klinička recesija, zubni plak i broj vlastitih zuba kod pojedinog sudionika. Parodontna bolest definira se kao gubitak pričvrstka od pet milimetara ili više na četiri ili više mjesta, a barem na jednomu treba biti izmjeren džep od četiri ili više milimetara (Beckov kriterij). Bilo je provedeno i prikupljanje stanica oralne sluznice brisom, a detekcija polimorfizma obavljena je tehnikom kvantitativnog RT-PCR-a (*real time PCR)* pomoću analize krivulje temperature mekšanja. **Rezultati:** Podaci su pokazali da nema statistički znatnih razlika u frekvenciji genotipova (p=0,564) ili alela (p=0,643) između zdravih ispitanika i onih s parodontitisom. **Zaključak:** U istraživanom uzorku polimorfizam eksona 1 gena MBL2 nije se mogao povezati s parodontnom bolesti.

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Ključne riječi
Lektin, manoza-vezni; polimorfizam, genetički; parodontitis; diabetes mellitus, tip 2

Uvod

Diabetes mellitus metabolički je poremećaj koji karakterizira povišena razina glukoze u krvi zbog neadekvatne sekrecije ili aktivnosti hormona inzu-

Introduction

Diabetes mellitus is a metabolic disorder characterized by increased levels of glucose in the bloodstream because of defective secretion or activity of

lina. Dijabetes tipa 1 nastaje zbog destrukcije beta-stanica gušterače, a uzrok može biti autoimuni ili idiopatski poremećaj. Dijabetes tipa 2, čini se, ne uzrokuje autoimuni poremećaj, jer mikroskopskim pregledom nije pronađena destrukcija stanica gušterače. Dijabetes toga tipa ima jaku genetsku uvjetovanost i povezan je s rezistencijom na inzulin ili defektom u sekreciji hormona (1).

Periferno vaskularno oštećenje kod pacijenata s dijabetesom utječe na proces cijeljenja i uzrokuje fiziološke promjene koje smanjuju kapacitet imunološkog sustava te se tako povećava sklonost infekcijama. Visoka koncentracija glukoze i kalcija u slini povećava količinu kamenca i čimbenika koji iritiraju oralna tkiva, izazivajući pojavu parodontne bolesti, inače najčešće stomatološke manifestacije u usnoj šupljini pacijenata s dijabetesom (2).

Parodontitis je kronična upalna bolest i zahvaća potporna tkiva zuba (3). Zabilježen je kao šesta klasična komplikacija kod pacijenata s dijabetesom (4). Općenito je prihvaćeno mišljenje da su za razvoj pardontitisa potrebne bakterije, ali moraju biti ispunjeni i neki drugi preduvjeti. Sklonost parodontitisu i težinu bolesti određuju imunološki odgovor domaćina, genetski čimbenici i čimbenici u okolišu (5,6). Čini se da je razvoj te bolesti određen odnosom parodontno-patogenog potencijala plaka i imunološkog odgovora domaćina (7).

Urođena imunost odnosi se na otpornost koja postoji već u prvom kontaktu s patogenom. Stečena imunost odnosi se na otpornost koju ne nalazimo kod prve izloženosti patogenu ili je vrlo slaba, a jača zajedno sa simptomima djelovanja istog patogena. Važan dio urođene obrane od infekcije je sustav komplementa koji omogućuje skupina serumskih proteina (8,9). Može se aktivirati na sljedeća tri načina: klasičan oblik aktivacije dio je specifičnog imunološkog odgovora domaćina i ovisi o stvaranju antitijela i kompleksa antigen-antitijelo; alternativni i lektinski put aktivacije dio su urođenog imunološkog odgovora, a njihovo pokretanje ovisi o specifičnim komponentama na površini mikroorganizama (10).

Glavna komponenta lektinske aktivacije jest lektin koji veže manozu (MBL), protein iz obitelji kolektina. Proizvodi ga jetra te cirkulira krvlju, a primarna mu je zadaća prepoznati i vezati se za specifične šećerne skupine na površini mikroorganizama (11). To su: *Neisseria meningitides*, Candida species, *Aspergillus fumigatus*, *Staphylococcus aureus*, beta hemolitički streptokok grupe A i anaerobne bakterije, kao što su *Bifidobacterium bifidum* i *Veillonella dispar*

the hormone insulin. Type 1 diabetes mellitus results from the destruction of pancreatic beta cells and its cause may be idiopathic or due to an autoimmune disorder. Type 2 diabetes mellitus does not seem to have an auto-immune causation, because no destruction of cells of pancreatic islets is seen microscopically. It appears to carry a strong genetic component and to be linked to insulin resistance or a defect in the secretion of the hormone (1).

Peripheral vascular failure in diabetic patients impairs healing and produces physiological changes that decrease the capacity of the immune system, thereby increasing susceptibility to infections. The high concentration of glucose and calcium in saliva increases the amount of calculus and irritating factors for oral tissues, causing the appearance of periodontal disease, which is the most common dental manifestation in the oral cavity of diabetic patients (2).

Periodontitis is a chronic inflammatory disease that affects the supporting tissues of the teeth (3) and has been reported to be the sixth classic complication of the diabetic patient (4). It is generally accepted that the presence of bacteria is a necessary but not sufficient condition for the development of periodontitis. Host immune responses, genetic and environmental factors determine part of the susceptibility and severity of periodontitis (5, 6). The development of periodontal disease seems to be determined by the relationship between the periodontopathogenic potential of the plaque and the immune response of the host (7).

Innate immunity refers to the resistance already present at the time when a pathogen appears for the first time. Acquired immunity refers to the resistance that is absent or weak on initial exposure to the pathogen, but which increases with subsequent manifestations of the same pathogen. An important, specially developed type of antimicrobial innate defense is provided by a group of serum proteins which, in its entirety, is the pathway of complement (8, 9). The complement system may be activated through the following three different pathways. The classic pathway is part of the specific response of the host, and its activation depends on the prior formation of antibodies and the antigen-antibody complex. The alternative pathway and the lectin pathway participate in the innate response, the activation of which depends on specific components present on the surface of microorganisms (10).

The main component of the lectin pathway is the mannose-binding lectin (MBL), a protein be-

(12,13). Zanimljivo je da i parodontni patogeni *Aggregatibacter actinomycetemcomitans* i *Porphyromonas gingivalis* imaju na staničnoj površini polisaharide bogate manoznim ostacima (14, 15). U plazmi je MBL povezan sa serinskim proteazama MASP1, MASP2 i MASP3. Primjerice, kad se MBL veže za manoznu terminalnu skupinu bakterijskih ugljikohidrata, aktiviraju se MASP1 i MASP2 te se u nastavku aktivira klasičan oblik (put) imunološkog odgovora neovisno o antitijelima (11).

MBL je jedna od najsvestranijih komponenti urođenog imunološkog sustava. Zna se kakva mu je zadaća u aktivaciji komplementa (16), u pomaganju opsonofagocitoze neovisne o komplementu (17), podešavanju upalnog odgovora (18), prepoznavanju promijenjenih vlastitih struktura i „čišćenju“ stanica apoptozom (19).

Postoje dva humana MBL gena - MBL-1 (to je pseudogen) i MBL-2 (kodira proteinski proizvod). Funkcijski je MBL-2 lociran na kromosomu 10 (q11,2-q21) i obuhvaća četiri eksona. Ekson 1 kodira signalni peptid, područje bogato cisteinom i dio kolagenog područja s jako mnogo glicina. Ekson 2 kodira ostatak kolagenog područja, a ekson 3 kodira a-helikoidalnu spiralu poznatu kao „vratno“ područje. Ekson 4 kodira CRD, što podrazumijeva globularnu konfiguraciju. Promotor sekvencija gena MBL-a sadržava mnogobrojne regulatorne elemente koji utječu na transkripciju proteina (20-22).

Tri supstitucije od jedne baze smještene na eksonu 1 gena MBL2 na kodonima 52 (Arg3Cys, alel “*D*”), 54 (Gly3Asp, alel “*B*”) i 57 (Gly3Glu, alel “*C*”) rezultiraju prekidom formiranja MBL-peptida u funkcionalne polimere i izrazitim smanjenjem serumske razine funkcionalnog MBL-a (23, 24). Pretpostavlja se da je to povezano s rekurentnim infekcijama (25) i napredovanjem kroničnih virusnih infekcijskih bolesti, kao što je sindrom stečene imunodeficijencije (sida) (26), ateroskleroza (27), cistična fibroza (28), autoimune bolesti kao što su lupus eritematozus (29) i reumatoidni artritis (30) te sa srčane bolesti (20).

Polimorfizmi od jednog nukleotida (SNPs-a) smatraju se moćnim “alatom” za identifikaciju gena uključenih u Mendelovu i poligenske bolesti te bolesti koje određuje više čimbenika. Analizama mutacija toga tipa uspješno se utvrđuju patogeneze mnogih bolesti (31).

U pregledu rizičnih genetskih čimbenika za nastanak parodontitisa, uočeno je da većina istraživanja gena kandidata za razvoj parodontitisa proučava samo jedan polimorfizam (32). Budući da imunolo-

longing to the family of collectins. MBL is primarily produced in the liver and circulates in the blood. It primarily recognizes and binds to specific sugar groups that are displayed on the surface of microorganisms (11). These include *Neisseria meningitides*, Candida species, *Aspergillus fumigatus*, *Staphylococcus aureus*, beta-hemolytis group A streptococci, and anaerobic bacteria such as *Bifidobacterium bifidum* and *Veillonella dispar* (12, 13). Interestingly, the periodontal pathogens *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* also appear to have mannan-rich polysaccharides on the cell surfaces (14, 15). In plasma, the MBL is found associated with the serine proteases MASP1, MASP2 and MASP3. When the MBL is bound to mannose terminal groups in bacterial carbohydrates, for example, MASP1 and MASP2 are activated and continue to activate the classical pathway irrespective of antibodies (11).

The MBL is one of the most versatile components of the innate immune system. It is recognized to have a role in processes as diverse as complement activation (16), promotion of complement-independent opsonophagocytosis (17), modulation of inflammation (18), recognition of altered self-structures and apoptotic cell clearance (19).

There are two human MBL genes, but MBL-1 is a pseudogene and only MBL-2 encodes a protein product. The functional MBL-2 gene is located on chromosome 10 (q11.2-q21) and comprises four exons. Exon 1 encodes the signal peptide, a cysteine-rich region and part of the glycine-rich collagenous region. Exon 2 encodes the remainder of the collagenous region and exon 3 encodes an a-helical coiled-coil structure, which is known as the “neck” region. Exon 4 encodes the CRD, which assumes a globular configuration. The promoter region of the MBL gene contains a number of regulatory elements that affect transcription of the protein (20-22).

Three single-base substitutions positioned in exon 1 of the MBL2 gene at codons 52 (Arg-3Cys, allele “*D*”), 54 (Gly3Asp, allele “*B*”), and 57 (Gly3Glu, allele “*C*”) result in disruption of the formation of MBL peptides in functional polymers and a marked reduction in serum levels of functional MBL (23, 24) and are believed to be associated with recurrent infections (25) and progression of chronic viral diseases, such as acquired immunodeficiency syndrome (26), arteriosclerosis (27), cystic fibrosis (28), autoimmune diseases such as lupus erythematosus (29), rheumatoid arthritis (30) and heart disease (20).

ki sustav ima glavnu ulogu u patogenezi parodontitisa, u našem smo istraživanju određivali povezanost parodontne bolesti s genetskim polimorfizmom eksona-1 (kodoni -52, -54, -57) gena MBL2 tehnikom kvantitativne *real time PCR,* na uzorku brazilskih pacijenata s dijabetesom tipa 2.

Ispitanici i postupci

Istraživanje je bilo obavljeno u Zavodu za endokrinologiju Javne zdravstvene službe u gradu Recifeu u Brazilu. U razdoblju od 1. veljače do 30. svibnja 2008. tamo je pregledano ukupno 50 pacijenata s već postavljenom dijagnozom dijabetesa tipa 2. Uzorak je odabran nasumce u skladu sa zahtjevima klinike. Razgovaralo se sa svim pacijentima koji su u navedenom razdoblju došli na pregled, a za istraživanje su odabrani prema sljedećim kriterijima: nisu smjeli uzimati antibiotike u posljednjih šest mjeseci, morali su imati dijagnosticiran dijabetes tipa 2, nisu smjeli imati većih medicinskih komplikacija (primjerice neku srčanu bolest), morali su imati u usnoj šupljini barem osam vlastitih zuba, nisu smjeli biti parodontološki liječeni u posljednjih šest mjeseci, a žene nisu smjele biti trudne ili dojiti. I svi su se trebali složiti sa sudjelovanjem u istraživanju. Projekt je odobrilo Povjerenstvo za etiku u istraživanjima Federalnog sveučilišta države Pernambuce (broj protokola 285/07).

Nakon što su pacijenti potpisali pristanak, ispunili su upitnik s pitanjima o dobi, spolu, prihodima, stupnju obrazovanja, bračnom statusu, pušenju, uzimaju li inzulin i koliko se dugo liječe od dijabetesa.

Nakon toga su ih na stomatološkom stolcu klinički pregledala dva educirana istraživača, a koristili su se milimetarskom parodontnom sondom North Carolina (Trinity® - São Paulo, SP, Brazil). Mjerenje je bilo obavljeno na šest mjesta (distobukalno, bukalno, meziobukalno, distoralno, oralno i mezioralno) za svaki zub, osim trećih kutnjaka. Bilježi-

The polymorphisms of a single nucleotide (SNPs) are considered to be a powerful tool in the identification of genes involved in Mendelian, polygenic and multifactorial diseases. Analyses of mutations of this type have been used successfully for a better understanding of the pathogenesis of many diseases (31).

In a review of genetic risk factors associated with periodontitis it was observed that the majority of studies concerning candidate genes for periodontitis investigate single polymorphisms (32) and, since the immune system plays a crucial role in the pathogenesis of periodontitis, the present study evaluated the association of periodontal disease with genetic polymorphism of the exon-1 (codons -52, -54, -57) of MBL2 gene using the technique of real-time PCR in a sample of Brazilian patients with type 2 diabetes mellitus.

Material and methods

This study was carried out in the Department of Endocrinology of the public healthcare service in Recife, Brazil. A total of 50 patients with prior diagnosis of type 2 diabetes were evaluated between February 1 and May 30, 2008. The sample was randomly obtained in accordance with the demand at the clinic involved. All patients who presented for consultation during this period were approached and selected using the following inclusion criteria: not using antibiotics for at least 6 months, having type 2 diabetes, not having any major medical complications (e.g. heart disease), having at least eight natural teeth, not having undergone periodontal treatment in the previous 6 months, not being pregnant or breastfeeding and agreeing to participate in the study. The research project received approval from the Ethics in Research Committee of the Federal University of Pernambuco (protocol number 285/07).

After free and informed written consent had been obtained, a questionnaire was completed, on which data on age, gender, income, educational level, marital status, smoking, use of insulin and duration of treatment for diabetes were recorded.

Next, a clinical examination was performed in a dental chair by two previously calibrated researchers using a millimeter North Carolina-type (Trinity® - São Paulo, SP, Brazil) periodontal probe. Six sites per tooth were evaluated (distobuccal, midbuccal, mesiobuccal, distolingual, midlingual and mesiolingual), except for third molars. Probing depth, bleeding on probing, clinical recession, dental plaque and

la se dubina sondiranja, krvarenje nakon sondiranja, klinička recesija, zubni plak i broj zuba.

Parodontna bolest bila je definirana na temelju četiriju ili više mjesta na kojima je nađen gubitak pričvrstka od 5 milimetara ili više, a barem na jednom mjestu dubina džepa trebala je biti četiri milimetra li više (33). Gubitak pričvrstka odgovara udaljenosti najapikalnijeg dijela parodontnog džepa od caklinsko-cementnog spojišta. Sudionici su bili podijeljeni na kontrolnu skupinu (pacijenti dijabetičari bez parodontitisa) i istraživanu skupinu (pacijenti dijabetičari s parodontitisom).

Izolacija DNK

Nakon kliničkog pregleda iz brisa su se prikupile stanice oralne sluznice i to pomoću odgovarajućih četkica (Kolplast® – São Paulo, SP, Brazil) koje su nakon toga pohranjene u jedan mililitar fiziološke otopine 0,9-postotnog NaCl-a (Laboratory Tayuyna Ltda – Nova Odessa, SP, Brazil). Prikupljeni materijal za ekstrakciju DNK čuvao se na temperaturi od 20°C.

Ekstrakcija genetskog materijala bila je obavljena opremom za ekstrakciju i purifikaciju DNK-a Genecleanom® (GENECLEAN® Kit, BIO 101, La Jolla, Ca, USA) prema protokolu koji su predložili Leão i njegovi suradnici (34).

Genotipizacija MBL2

Detekcija polimorfizma gena MBL2 bila je postignuta tehnikom kvantitativnog RT-PCR-a (*real time PCR*) analizom krivulje temperature mekšanja (35).

MBL2 SNP genotipizacija bila je učinjena uporabom sljedećih početnica oblikovanih u programu Primer Express 1,5 software (Applied Biosystems, Foster City, Ca, SAD): *forward* početnica 5'-AGGCATCAACGGCTT CCCA-3' i *reverse* početnica 5'-CAGAA CAGCCCAACACGTACCT-3'. Očekivan je bio produkt umnažanja duljine 90 bp, čija je pretpostavljena temperatura mekšanja 84°C. Amplifikacijska reakcija postignuta je u konačnom volumenu od 25 µL s mješavinom *1X SYBR Green I Amplification Master Mix* (Euroclone, Milano, Italija), 150 pikomola *forward* početnice, 50 pikomola *reverse* početnice i 10 ng genomskog DNK. Uvjeti termocikliranja bili su sljedeći: 95°C - 10 minuta, zatim 95°C - 30 sekundi i 60°C - jednu minutu, sve ponovljeno 40 puta u uređaju Rotor Gene-3000 (Corbett Research Mortlake, Sydney, Australija). Na kraju PCR-postupka disocijacijski je protokol uključivao polagano zagrijavanje od 60°C do 95°C po 0,2°C, s intervalom među zagrijavanjima od 8

number of teeth present were recorded.

Periodontal disease was defined as four or more sites with a loss of attachment of 5 mm or more with one or more of those sites having a pocket of 4 mm or more (33). The loss of attachment corresponds to the distance from the most apical portion of the periodontal pocket to the cemento-enamel junction. Thus the sample was divided into a control group (diabetic patients without periodontitis) and a study group (diabetic patients with periodontitis).

DNA isolation

After clinical examination, the collection of the scaling cells from the oral mucosa was carried out with appropriate cytobrush-type brushes (Kolplast® – São Paulo, SP, Brazil), which were subsequently stored in 1 ml of saline solution of 0.9% chloride sodium (Laboratory Tayuyna Ltda – Nova Odessa, SP, Brazil). The collected material was stored at -20 ° C for extraction of the DNA.

The kit for extraction and purification of DNA, Geneclean®, (GENECLEAN® Kit, BIO 101, La Jolla, CA, USA) was used to extract the genetic material according to the protocol suggested by Leão et al. (34).

MBL2 genotyping

The detection of polymorphism in the MBL2 gene was conducted by the technique of real time PCR (RT-PCR) using the melting temperature curve analysis (35).

MBL2 SNP genotyping was performed using the following primers designed with the Primer Express 1.5 software (Applied Biosystems, Foster City, CA, USA): forward primer 5'-AGGCATCAACGGCTT CCCA-3' and reverse primer 5'-CAGAA CAGCCCAACACGTACCT-3'. The expected amplicon length is 90 bp and its theoretical melting temperature is 84ºC. Amplification reactions were performed in a final volume of 25 µL with 1X SYBR Green I Amplification Master Mix (Euroclone, Milan, Italy), 150 picomoles of the forward primer, 50 picomoles of the reverse primer and 10 ng of genomic DNA. The cycling conditions were as follows: 95ºC for 10 min followed by 95ºC for 30 s and 60ºC for 1 min, repeated 40 times in the Rotor Gene-3000 apparatus (Corbett Research Mortlake, Sydney, Australia). At the end of the PCR, the dissociation protocol included a slow heating from 60º to 95ºC in 0.2ºC steps, with an 8-s interval between steps. Melting curve profiles were obtained

sekundi. Profil krivulje mekšanja dobio se uporabom programa za disocijaciju na uređaju Rotor Gene-3000 (26).

Tri polimorfizma gena MBL2 (na pozicijama 52, 54 i 57 u prvom eksonu) obuhvaćena su u jednu kategoriju (alel 0) zbog njihova velikog učinka na serumski MBL (16); kombinacija triju alela divljeg tipa grupira je kao alel A. Analiza temperature mekšanja omogućila nam je da lako razlikujemo profil mekšanja za tri genotipa MBL2: AA, A0 i 00. Temperature mekšanja za tri genotipa MBL2 bile su sljedeće: A/A (jedan vrh na 83,1 ± 0,1°C), A/0 (dva vrha; na 82,6 ± 0,3 i na 80,7 ± 0,1°C) i 0/0 (jedan vrh na 81,7 ± 0,1°C) (26).

Genotipizacija je bila obavljena preklapanjem krivulja pacijenata s krivuljama triju kontrolnih uzoraka: jednog divljeg, jednog mutanta i jednog od heterozigota. Kad god se krivulja pacijenta preklapala sa standardnom krivuljom, bilo je moguće ustanoviti o kojem je genotipu riječ.

Frekvencije genotipa MBL2 bile su u skladu s Hardy-Weinbergovim modelom ravnoteže. Frekvencije alela računale su se prema direktnom brojenju gena, a razlike su analizirane Fisherovim egzaktnim testom.

Interval pouzdanosti bio je 95 posto, te su prihvaćene p-vrijednosti manje od 0,05. Rezultati koji nisu dosegnuli tu vrijednost nisu se uzimali u obzir. Analiza podataka obavljena je programskim paketom SPSS, verzija 13,0 (SPSS Inc., Chicago, Il, SAD).

Rezultati

U Tablici 1. prikazana je deskriptivna statistika analiziranog uzorka - srednja vrijednost, medijan i standardna devijacija broja zuba, postotka krvarenja nakon sondiranja i postotka zubnog plaka.

Prosječna starost pacijenata kad su se skupljali podaci iznosila je 52,78 godina - minimum 35 i maksimum 74 godine, a sudjelovala su 22 (47,8%) muškarca i 24 (52,2%) žene.

Prevalencija parodontne bolesti u istraživanoj skupini nalazi se u Tablici 2. Šezdeset posto pacijenata imalo je parodontitis, a zdravih je bilo 40 posto.

Prema distribuciji genotipova, u uzorku su bila 24 pacijenta (48%) s genotipom AA, 26 (52%) je imalo genotip A0, a 0,0 posto genotip 00.

Tablica 3. prikazuje povezanost polimorfizma i parodontne bolesti. Učestalost polimorfizma MBL2 pokazuje da je od pacijenata s parodontitisom njih 43,3 posto imalo genotip A, a 56,7 posto genotip

using the dissociation software of the Rotor Gene-3000 apparatus (26).

The three MBL2 polymorphisms (at positions 52, 54, and 57 in the first exon) were grouped together in a single category (allele 0), because they have a substantial effect on serum MBL (16); the combination of three wild type alleles was grouped as allele A. The melting temperature assay allowed us to easily distinguish the melting profiles of the three MBL2 genotypes, which were AA, A0, and 00, respectively. The melting temperatures of the three MBL2 genotypes were as follows: A/A (one peak of 83.1 ± 0.1oC), A/0 (two peaks of 82.6 ± 0.3 and 80.7 ± 0.1oC), and 0/0 (one peak of 81.7 ± 0.1oC) (26).

The genotyping was performed by overlapping the patients' curves with the curves of three control samples: one wild, one mutant and one for a heterozygote. Whenever there was an overlap between the curve of the patient with a standard curve, it was possible to infer the genotype in question.

The MBL2 genotype frequencies were in agreement with the Hardy-Weinberg equilibrium. Allele frequencies were calculated by direct gene counting and the differences analyzed by Fisher's exact test.

The confidence interval considered was 95%, so p-values less than 0.05 were accepted. The results that did not attain this value were considered to be without significance. All data analyses were performed using the statistical package SPSS version 13.0 (SPSS Inc., Chicago, IL, USA).

Results

Table 1 presents the measurements of descriptive analysis that characterize the sample studied. Mean, median and standard deviation of the number of teeth present, percentage of bleeding on probing and percentage of dental plaque are shown.

The mean age of the patients during the data collection was 52.78 years, the minimum was 35 years and the maximum was 74 years, 22/50 (47.8%) being male and 24/50 (52.2%) female.

The prevalence of periodontal disease in the sample studied is presented in Table 2. Sixty per cent of the patients had periodontitis and 40% were healthy individuals.

Regarding the distribution of genotypes, the sample presented 24 subjects (48%) with the AA genotype, 26 (52%) the A0 and 0.0% the 00 genotype.

Table 3 presents the association between polymorphism and periodontal disease. The frequencies of MBL2 polymorphisms reveal that 43.3% of the

Tablica 1. Deskriptivna analiza broja zuba, postotka krvarenja i plaka
Table 1 Descriptive analysis of the number of teeth present, percentage of bleeding and plaque

	Broj zuba • Number of teeth present	Postotak krvarenja nakon sondiranja • Percentage of bleeding on probing	Postotak zubnog plaka • Percentage of dental plaque
Srednja vrijednost • Mean	18,54	32,7880	54,0408
Medijan • Median	19,50	23,1500	47,8500
SD	6,831	24,04883	31,64935

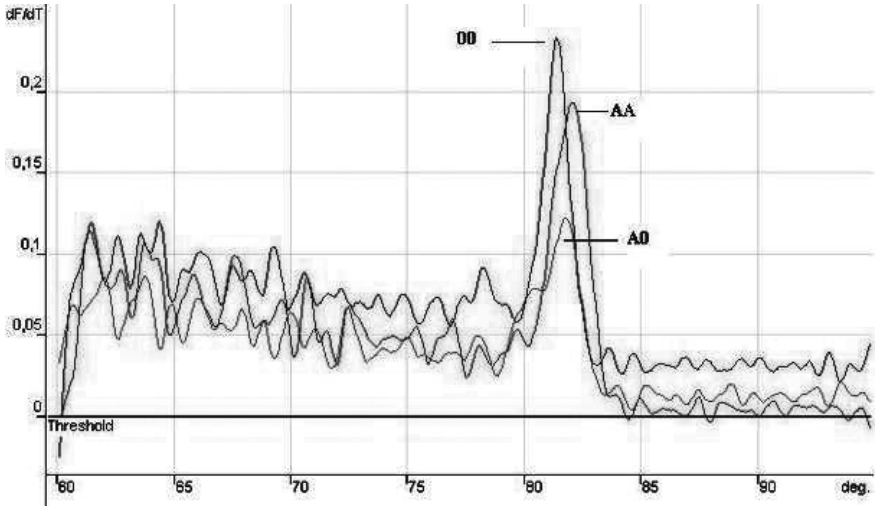
Tablica 2. Učestalost parodontitisa u istraživanoj populaciji dijabetičara tipa 2
Table 2 Frequency of periodontitis in the diabetic type 2 population studied

	Učestalost • Frequency	Postotak • Percentage
Pacijenti s parodontitisom • Periodontitis patients	30	60,0
Zdravi pacijenti • Healthy patients	20	40,0
Ukupno • Total	50	100,0

Tablica 3. Učestalost polimorfizama na eksonu 1 gena MBL2 kod pacijenata s dijabetesom tipa 2 i zdravih kontrolnih ispitanika
Table 3 Polymorphism frequencies in exon-1 of the MBL2 gene in type 2 diabetic patients with periodontitis and healthy controls.

		Periodontitis patients	Healthy Patients	P Value
učestalost genotipova MBL2/ MBL2 genotype frequencies				
A0	N	17	9	
	%	56,7%	45%	$p^{(1)} = 0,564$
AA	N	13	11	
	%	43,3%	55%	
učestalost MBL2 alela/ MBL2 allele frequencies				
A	N	43	31	
	%	71,7%	77,5%	$p^{(1)} = 0,643$
0	N	17	9	
	%	28,3%	22,5%	

(1): Fisherov egzaktni test/ Using Fisher's exact test.



Slika 1. Stapajući profil triju genotipova MBL2. Divlji homozigot (AA), heterozigot (A0) i mutant homozigot (00) dobiveni su uz pomoću programa za disocijacijsku krivulju Rotor Gene-3000.
Figure 1 Melting profile of the three MBL2 genotypes. Wild homozygous (AA), heterozygous (A0), and mutant homozygous (00) obtained using the Rotor Gene-3000 dissociation curve software.

A0, dok je kod zdrave kontrolne skupine čestoća bila 55 posto i 45 posto za iste genotipove. Prema distribuciji genotipova (p=0,564) nije bilo znatne razlike između pacijenata s parodontitisom i onih u kontrolnoj skupini.

Učestalost alela također je u Tablici 3. U skupini s parodontitisom 71,7 posto nosilo je alel A, a 28,3 posto alel 0, a u kontrolnoj skupini opažena čestoća iznosila je 77,5 posto i 22,5 posto za iste alele. Rezultati su pokazali da između zdravih ispitanika i onih s parodontitisom (p=0,643) nema statistički veće razlike u učestalosti alela gena MBL2.

Rasprava

U ovom se istraživanju određivalo koliko je čest polimorfizam na prvom eksonu gena MBL2 na uzorku pacijenata s dijabetesom tipa 2, u gradu Recifeu, u državi Pernambucu u sjeveroistočnom Brazilu.

Godine 1993. Svjetska zdravstvena organizacija (WHO) navela je parodontitis kao šestu klasičnu komplikaciju kod pacijenata s dijabetesom. Diljem svijeta uglavnom je prihvaćeno mišljenje da je zubni biofilm glavni uzročnik parodontne bolesti, no oblikovanje upalnog odgovora potiču lokalni i sistemski čimbenici. Kad je riječ o sistemskim rizičnim čimbenicima, ustanovljeno je da pacijenti s dijabetesom imaju jače izraženu parodontnu bolest nego oni bez dijabetesa (4,36).

Ovo je istraživanje pokazalo da je parodontna bolest vrlo česta u populaciji dijabetičara – imalo ju je čak 60 posto ispitanika. Rezultati se slažu s mnogim istraživanjima o povezanosti dijabetesa tipa 2 i parodontitisa i s uočenom proširenošću parodontne bolesti u toj populaciji (37- 40).

Učestalost strukturnih polimorfizama gena MBL2 varira od naroda do naroda. Mutantni aleli na kodonu 54 češći su u kavkazoidnim populacijama (41), kod Eskima (42) i Kineza (29) s učestalošću gena u rasponu od 0,11 do 0,17. Mutantni alel 57 nađen je samo u populacijama afričkog podrijetla s medijanom čestoće od 0,29 (43). Mutacija na kodonu 52 najrjeđa je strukturna mutacija i javlja se kod bijelaca te u kavkaskim populacijama, s učestalošću gena od 0,05 u objema skupinama. U ovom istraživanju učestalost mutantnog alela u brazilskoj populaciji bila je 0,26.

U novijem preglednom radu o genu MBL2 (44) za dvije kavkaske populacije (dansku i britansku) dane su učestalosti niskoproduktivnog (A0) i deficijentnog genotipa (00) od približno 40 posto. U

periodontitis patients were carrying the AA genotype and 56.7% the A0 genotype, while the healthy controls were carrying 55% and 45%, respectively. There were no significant differences between periodontitis patients and controls in the distribution of the genotypes (p=0.564).

The allele frequency is also shown in Table 3. In the periodontitis group, 71.7% were carrying the A allele and 28.3% the 0 allele versus 77.5% and 22.5%, respectively, of the controls. The results indicated that there were no statistically significant differences in the frequency of the alleles of the MBL2 gene between the healthy and periodontitis individuals (p=0.643).

Discussion

In this study the frequency of polymorphisms in the first exon of MBL-2 gene in a sample of Brazilian type 2 diabetes mellitus patients in, Recife, Pernambuco, northeast Brazil was evaluated.

In 1993, the World Health Organization included periodontal disease as the sixth classic complication in the diabetic patient. Dental biofilm is widely agreed to be the main etiological factor in periodontal disease; however, local and systemic factors have also been implicated in the modulation of the inflammatory response. With regard to these systemic risk factors, it is well established that patients with diabetes exhibit a slight increase in the severity of periodontal disease when compared with non-diabetic ones (4, 36).

The present study showed a high frequency of periodontal disease in the diabetic population, 60% of the sample having periodontal disease. These results are in agreement with many studies that investigated the relationship between type 2 diabetes and periodontitis and observed the widespread existence of periodontal disease in this population (37- 40).

The frequencies of structural polymorphisms in the MBL2 gene vary between different ethnic groups. Mutant alleles at codon 54 are more common in Caucasian populations (41), Eskimos (42) and Chinese (29) with gene frequencies ranging from 0.11 to 0.17. The mutant allele 57 is found only in populations of African origin with a median frequency of 0.29 (43). The mutation in codon 52 is the least common structural mutation and is present in white and Caucasian populations, with a gene frequency of 0.05 in both. This study in the Brazilian population observed a frequency of 0.26 for the mutant allele.

In a recent review of the MBL2 gene (44), frequencies of the low-producing (A0) and deficient

ovom istraživanju provedenom u populaciji dijabetičara tipa 2 u sjeveroistočnom Brazilu, nalazi su bili slični dosad objavljenim frekvencijama - prevalencija genotipa koji kodira nisku razinu MBL-a u plazmi (A0) bila je 52 posto.

Epidemiološka istraživanja upućuju na povezanost MBL-insuficijencije s povećanim rizikom od infekcija, posebice kad je prisutna i imunodeficijencija. Osim toga, varijacije u serumskim razinama MBL-a mogu utjecati na sklonost prema nekoliko vrsta autoimunih bolesti, zatim prema kardiovaskularnim i drugim bolestima te na njihov tijek (45). Važno je istaknuti da zbog genetskog polimorfizma varijacije u razini MBL-a mogu imati ulogu u sklonosti prema parodontitisu. Louropoulou i kolege (46) istraživali su povezanost polimorfizma gena MBL2 i parodontitisa. Uočili su da prevalencija genotipova koji kodiraju nisku i jako nisku razinu MBL-a u plazmi iznosi 44 posto kod pacijenata s parodontitisom, te 35 posto kod zdravih ispitanika u kontrolnoj skupini. U ovom je istraživanju 52 posto pacijenata imalo genotipove koji kodiraju nisku razinu MBL-a u plazmi (A0) - od toga je njih 34 posto imalo parodontnu bolest, a 18 posto je bilo zdravih. Unatoč većoj frekvenciji alela 0 u skupini s parodontnom bolesti, ni naše istraživanje ni ono Louropouloua i suradnika (46) nije moglo ustanoviti statistički znatne razlike u učestalosti genotipova između pacijenata s parodontitisom i zdravih u kontrolnoj skupini. Maffei i suradnici (47) u svojem su istraživanju analizirali razine MBL-a u plazmi u odnosu prema parodontitisu te su dokazali da u parodontitisu razine MBL-a nisu visoke, a MBL-deficijencija nije bila povezana sa sklonošću prema toj bolesti. Ti se rezultati slažu s podacima iz ovog istraživanja i ne daju osnovu za zaključivanje o upletenosti MBL-deficijencije u patogenezu parodontitisa.

Genetičke studije potrebne su kako bi se povezao genetski polimorfizam sa sklonostima prema razvoju različitih bolesti. Ova preliminarna studija bavila se povezanošću polimorfizama na eksonu 1 gena MBL2 i parodontne bolesti u populaciji dijabetičara tipa 2.

Zaključak

Na kraju istaknimo da nema povezanosti između mutiranog alela MBL2 (alel 0) i parodontne bolesti kod pacijenata s dijabetesom tipa 2. Ipak, uočena je pozitivna korelacija, jer su podaci pokazali češći parodontitis kod ispitanika s genotipovima koji

genotypes (00) of around 40% were reported for two Caucasian populations (Danish and British). In this study, in type 2 diabetic population of northeast Brazil, the findings are similar to those of the previously reported frequencies, and the prevalence of genotypes encoding low MBL plasma levels (A0) was 52%.

Epidemiological studies have suggested that MBL insufficiency is associated with increased risk for infections, especially when a coexisting immune deficiency is present. Further, variations in MBL serum levels may influence the susceptibility to and the course of several types of autoimmune, cardiovascular and other diseases (45). Thus, it is important to note that variations in the MBL levels due to genetic polymorphisms may have a role in susceptibility to periodontitis. Louropoulou et al. (46) investigated the correlation of polymorphisms in the MBL2 gene in relation to periodontitis and observed that the prevalence of genotypes encoding for low and very low MBL plasma levels was 44% for periodontitis patients and 35% for healthy controls. In the present study, 52% of the patients displayed genotypes encoding low MBL plasma levels (A0), of whom 34% had periodontal disease and 18% were healthy controls. Nonetheless, despite the greater frequency of the 0 allele in the group with periodontal disease, neither the present study nor that of Louropoulou et al. (46) was able to detect any statistically significant differences in the genotype frequencies between periodontitis patients and healthy controls. In a previous study, Maffei et al. (47) analyzed MBL plasma levels in relation to periodontitis and demonstrated that MBL levels were not high in periodontitis and that MBL deficiency was not related to susceptibility to periodontitis. Those results are in agreement with the data recorded by this study and do not offer a basis for implicating MBL deficiency in the pathogenesis of periodontitis.

Genetic studies are needed to link genetic polymorphism and susceptibility to the development of various diseases. This preliminary study investigated the correlation of polymorphisms in exon-1 of the MBL2 gene with periodontal disease in type 2 diabetic population.

Conclusion

It was concluded that there is no association between the presence of the mutated MBL2 allele (allele 0) and the presence of periodontal disease in type 2 diabetes patients. However, a positive correlation tended to be observed when the data showed a

kodiraju niske razine MBL, te je moguće da je razmjerno mali broj sudionika utjecao na to da ta povezanost nije dosegnula razinu statističke znatnosti. Potrebno je poduzeti daljnja istraživanja, ali na većem uzorku kako bi i zaključci bili relevantniji.

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higher frequency of periodontitis in individuals with genotypes encoding low levels of MBL, and the relatively low number of individuals in this study may have influenced the lack of significance in this association. Thus, further studies with a larger sample of patients will be needed before more substantiated conclusions can be drawn.

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Abstract

Aim: Mannose-binding lectin (MBL) is an important protein of the innate immune system. It is able to bind to sugar groups encountered on the surfaces of a wide range of microorganisms and interacts with serine proteases (MASPs) to effect the activation of complement. Mutations of the expressed human MBL2 gene have been associated with several infections; this preliminary study thus sets out to assess the association between the polymorphism in exon-1 of the MBL2 gene and periodontal disease in type 2 diabetic patients. **Material and Methods:** The sample comprised 50 diabetic patients, who were submitted to a clinical periodontal examination that evaluated in six sites per tooth probing depth, bleeding on probing, clinical recession, dental plaque and the number of teeth present. Periodontal disease was defined as 4+ sites of loss of attachment of 5+ mm, with one or more of those sites having a pocket of 4+ mm (Beck's criteria). The collection of scaling cells from the oral mucosa was carried out and the detection of polymorphism was made by the technique of real time PCR (RT-PCR) using the melting temperature curve analysis. **Results:** The data revealed that no statistically significant differences in the genotype (p=0.564) or the allele (p=0.643) frequencies were observed among the healthy individuals or those with periodontitis. **Conclusions:** The polymorphism in exon-1 of the MBL2 gene was not related to the periodontal disease in the sample evaluated.

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Mannose-Binding Lectin; Polymorphism, Genetic; Periodontitis; Diabetes Mellitus, Type 2

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