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Interleukin-6 and Interleukin-10 Gene Polymorphisms in Patients with Chronic Periodontitis and Response to Treatment after 3 Years

Polimorfizam gena za interleukin-6 i interleukin-10 kod pacijenata s kroničnim parodontitisom te odgovor na liječenje nakon tri godine

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Abstract

Objective: The aim of this study was to investigate whether genetic susceptibility to chronic periodontitis, conferred by the presence of the IL-6 -572GG genotype or the IL-10 -592A allele, influences the outcomes following a non-surgical periodontal therapy (NSPT)over a long period of time. **Material and methods:** Thirty-seven chronic periodontitis patients were divided into two groups according to genotype as susceptible (SCP) and non-susceptible (NSCP). All subjects were clinically evaluated at baseline and 3 years following NSPT. Blood samples were collected at baseline from the individuals who fulfilled the inclusion criteria. All participants received NSPT from a single periodontist who was blind to the genotype status of each patient. A statistical analysis was performed by comparing the variables between groups using the Mann-Whitney U test and between baseline and 3 years for each group using the Wilcoxon test. **Results:** The mean age of the population was estimated to be 47.68±8.64 years and it included 51.4% females, 48.6% smokers, and 45.9% alcohol consumers. Following a genetic analysis, 70.3% of patients were homozygous carriers of the IL-6 -572G (IL-6 SCP), and 46.0% of them were carriers of the IL-10 -592A allele (IL-10 SCP). NSPT reduced all studied parameters (probing depth, attachment loss, bleeding on probing, percentage of sites with 4-6mm and ≥7mm pocket depth and attachment loss) to all participants, but the treatment outcome was not associated with the genotype. The SCP and NSCP individuals showed similar clinical parameters at baseline and at 3 years. **Conclusions:** Within the limitations of this 3-year prospective cohort study in Caucasians diagnosed with chronic periodontitis, individuals susceptible to periodontal disease as determined by the presence of the IL-6 -572GG genotype or the IL-10 -592A allele showed similar treatment outcome following NSPT.

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Introduction

Periodontitis is a non-communicable multifactorial inflammatory disease associated with dysbiotic plaque biofilms resulting in progressive destruction of the tooth-supporting tissues and eventually tooth loss (1). Periodontal disease onset and progression are a result of the interaction between the dysbiosis of the commensal oral microbiota and the host response (2). In the 2017 World Workshop on the Classification of Periodontal and Peri-implant Diseases and Conditions it was recognized that population subgroups may exhib-

Uvod

Parodontitis je nezarazna multifaktorijalna upalna bolest povezana s biofilmom s disbiotičkim plakom, što rezultira progresivnom uništavanjem tkiva koje podupire zub i na kraju uzrokuje njegov gubitak (1). Pojava i progresija parodontne bolesti rezultat su interakcije između disbioze komenzalne oralne mikrobiote i odgovora domaćina (2). Na Svjetskoj radionici o klasifikaciji parodontontalnih i periimplantantnih bolesti i uvjeta, održanoj 2017. godine, istaknuto je da populacijske podskupine mogu pokazati različi-

it distinct disease conditions due to differences with respect to disease susceptibility and exposure (1).

Susceptibility to periodontal disease and response to treatment vary among individuals and depend on different factors. Disease susceptibility depends on environmental and host risk factors, either modifiable such as smoking, diabetes mellitus, obesity and oral hygiene or unmodifiable including genetic predisposition (3). In a study based on 110 pairs of adult twins, 38-82% of the population variance for periodontal clinical parameters may be attributed to heredity of genetic factors highlighting the role of host genetic influences (4). In recent decades, multiple studies have examined the association between gene polymorphisms and the risk of periodontitis development (5).

Polymorphisms in the promoter region of the Interleukin (IL)-6 gene affect the transcription and expression of IL-6 in individuals leading to the up-regulation of IL-6 levels in serum (6) and gingival tissues (7). Similarly, polymorphisms in the promoter region of the IL-10 gene may change the expression of IL-10 in response to inflammatory disease, thus influencing the disease outcome (8,9). A meta-analyses aimed to determine whether IL-6 and IL-10 polymorphisms confer susceptibility to periodontitis have indicated a significant association between the IL-6 -572 G allele and IL-10 -592 A allele and AA genotype with chronic periodontitis (10-13).

The treatment of periodontitis aims to arrest disease progression by reducing the bacterial load, in order to reduce the risk of tooth loss and to prevent the disease recurrence (14). A reasonable endpoint of non-surgical periodontal treatment should include the absence of clinical signs of gingival inflammation, shallow pocket depth and low levels of plaque accumulation. Studies have demonstrated that periodontal treatment can lead to positive clinical outcomes and a supportive periodontal therapy is effective in maintaining periodontal health and preventing a long-term tooth loss (15-17).

Although periodontal therapy is highly predictable, a patient- and tooth-related factors have been associated with tooth loss and further disease progression during supportive periodontal therapy including age, smoking, systemic conditions and genetics (18,19). Rapid progression from gingivitis to periodontitis has been detected in 10-15% of the population and 8-12% of patients within a population exhibit a number of sites that do not respond to a routine periodontal treatment (20,21). Identifying host-related factors such as gene polymorphisms associated with the development and progression of periodontal diseases may lead to early recognition of patients unresponsive to periodontal treatment (22). Although environmental factors have been widely investigated as possible predictors of disease progression in individuals who received periodontal therapy, information concerning genetic factors is scarce.

A meta-analysis that examined the effects of several susceptible genotypes to chronic periodontitis with the periodontal treatment outcome and tooth loss concluded that pocket depth reduction in the first three and six months after a non-surgical periodontal therapy was associated with susceptible genotypes (23). However, the number of available studies was insufficient to draw clear conclusions and further studies are

ta stanja iste bolesti zbog razlika uvjetovanih osjetljivošću i izloženosti bolesti (1).

Osjetljivost na parodontnu bolest i odgovor na liječenje razlikuju se od pojedinaca do pojedinca i ovise o različitim čimbenicima. Osjetljivost ovisi o čimbenicima rizika kad je riječ o okolišu i domaćinu, bilo da je promjenjiva poput pušenja, dijabetesa melitusa, pretilosti i oralne higijene, ili nepromjenljiva, uključujući i genetsku predispoziciju (3). U istraživanju na temelju 110 parova odraslih blizanaca, od 38 do 82 % opaženih varijanca populacije za parodontne kliničke parametre može se pripisati nasljeđivanju čimbenika koji ističu ulogu genetskih utjecaja domaćina (4). Tijekom posljednjih desetljeća u više su istraživanja autori proučavali povezanost između polimorfizama gena i rizika od razvoja parodontitisa (5).

Polimorfizmi u promotorskoj regiji gena *interleukina* (*IL*) utječu na transkripciju i ekspresiju *IL*-6 kod pojedinaca, što potiče porast regulacije razine *IL-6* u serumu (6) i gingivnom tkivu (7). Slično tomu, polimorfizmi u promotorskoj regiji gena *IL-10* mogu promijeniti njegovu ekspresiju kao odgovor na upalnu bolest i tako utjecati na konačni rezultat (8, 9). U metaanalizama u kojima se željelo ustanoviti omogućuju li polimorfizmi *IL-6* i *IL-10* osjetljivost na parodontitis, pokazala se značajna povezanost alela *IL-6-572 G* i genotipa i *IL-10-592 A AA* s kroničnim parodontitisom (10 – 13).

Svrha liječenja parodontitisa jest zaustaviti progresiju bolesti smanjenjem bakterijskog opterećenja kako bi se smanjio rizik od gubitka zuba i spriječio ponovni nastanak bolesti (14). Razumna krajnja točka nekirurškoga parodontalnog liječenja trebala bi uključivati odsutnost kliničkih znakova upale gingive, plitke džepove i nisku razinu nakupljanja plaka. Istraživanja su pokazala da se parodontološkim liječenjem mogu postići pozitivni klinički ishodi, a potporna terapija učinkovita je u održavanju zdravlja parodonta i sprječavanju dugoročnog gubitka zuba (15 – 17).

Iako je parodontološka terapija vrlo predvidiva, čimbenici koji su povezani s pacijentima i zubom statistički su značajno povezani s gubitkom zuba i daljnjim napredovanjem bolesti tijekom liječenja, uključujući dob, pušenje, sistemska stanja i genetiku (18, 19). Brza progresija gingivitisa u parodontitis otkrivena je kod 10 do 15 % populacije, a od 8 do 12 % pacijenata u sklopu populacije ima brojna mjesta koja ne reagiraju na rutinsko parodontološko liječenje (20, 21). Utvrđivanje čimbenika povezanih s domaćinom, poput genskih polimorfizama povezanih s pojavom i progresijom parodontnih bolesti, može pomoći da se rano uoče pacijenti koji ne reagiraju na liječenje (22). Iako su okolišni čimbenici široko istraženi kao mogući prediktori progresije bolesti kod pojedinaca koji su primali parodontološku terapiju, informacija o genetskim čimbenicima je malo.

U metaanalizi, u kojoj su se ispitivali učinci nekoliko osjetljivih genotipova na kronični parodontitis s ishodom parodontološkog liječenja i gubitkom zuba, zaključeno je da je smanjenje dubine džepa u prva tri mjeseca i šest mjeseci poslije nekirurške parodontološke terapije povezano s osjetljivim genotipovima (23). No broj dostupnih istraživanja nije bio dovoljan za nedvojbene zaključke pa su potrebna daljnja istraživanja (21). U prospektivnoj studiji s kratkim raz-

needed (21). In a prospective study with a short follow-up period (45 days following non-surgical periodontal therapy), IL-6 -572 G/C and IL-10 -592 C/A polymorphisms did not influence the treatment outcome of chronic periodontitis (24). Additional methodologically sound studies are needed to contribute to the prediction of periodontal treatment response.

There is still lack of long-term data regarding the treatment outcome and disease susceptibility in patients with chronic periodontitis. To the best of our knowledge, there are currently no published clinical studies that evaluate the effect of gene polymorphisms on the outcome of non-surgical periodontal therapy over a long period of time. Hence, the aim of this study was to investigate whether genetic susceptibility to chronic periodontitis, conferred by the presence of the IL-6 -572GG genotype or the IL-10 -592A allele, influences the clinical outcomes of non-surgical long-term periodontal therapy.

Material and methods

This prospective cohort study included consecutive chronic periodontitis patients from a private practice limited to Periodontics and Implant Dentistry in Thessaloniki, Greece between September 2014 and June 2015. All eligible patients consented to the study protocols. Out of 68 treated subjects, 67 were re-evaluated 45 days following the treatment and the results of the analysis have been published separately (24). The subjects were encouraged to attend supportive periodontal therapy on a 3- or 4- month recall protocol based on their individual needs. A total of 37 out of 67 re-evaluated at 45 days patients presented at their 3-year follow-up appointment. The remaining 30 subjects moved away from the area, did not attend a number or all of the maintenance appointments, or had a surgical periodontal treatment as part of their treatment plan.

Patients were included in the study on the basis of the following inclusion criteria:

a. Diagnosis of chronic periodontitis based on the 1999 classification system for periodontal disease (25). Two or more non-adjacent sites with interproximal clinical attachment loss (CAL) ≥ 3 mm, probing pocket depth (PPD) ≥ 5 mm and bleeding on probing (BOP).
b. Attendance of supportive periodontal therapy on a 3-4 month recall protocol.
c. Completion of periodontal re-evaluation 3 years following the active periodontal treatment.
d. Systemic health.
e. Age of 30-70 years.
f. Presence of ≥ 16 teeth at initial appointment.

The exclusion criteria consisted of:

a. Any periodontal treatment within the last 12 months from the study initiation.
b. Poor attendance of follow-up/recall appointments (failure to attend the recommended supportive periodontal therapy 3-4 month recall protocol).
c. Lack of data at the follow-up examination 3 years after active periodontal treatment.
d. Need for periodontal surgery (resective or regenerative) following the non-surgical periodontal therapy.

dobljem praćenja (45 dana poslije nekirurške parodontološke terapije), polimorfizmi *IL-6 -572 G/C i IL-10 -592 C/A* nisu utjecali na ishod liječenja kroničnog parodontitisa (24). Potrebne su dodatne metodološki ispravne studije da bi se pridonijelo predviđanju reakcije na parodontološko liječenje.

Još uvijek nedostaju dugoročni podatci o ishodu liječenja i osjetljivosti na bolest kod pacijenata s kroničnim parodontitisom. Koliko nam je poznato, trenutačno nema objavljenih kliničkih istraživanja u kojima autori procjenjuju utjecaj genetskih polimorfizama na ishod nekirurške parodontološke terapije tijekom duljeg razdoblja. Dakle, cilj ovog istraživanja bio je istražiti utječe li genetska osjetljivost na kronični parodontitis, dodijeljena prisutnošću genotipa *IL-6 -572GG* ili alela *IL-10 -592A*, na dugoročne kliničke rezultate nekirurške parodontološke terapije.

Materijali i metode

U ovo prospektivno kohortno istraživanje bili su između rujna 2014. i lipnja 2015. uključeni pacijenti s kroničnim parodontitisom iz privatne prakse ograničene na parodontološku terapiju i stomatologiju implantata u Solunu u Grčkoj. Svi odgovarajući pacijenti pristali su na protokol istraživanja. Od 68 liječenih ispitanika, 67 je ponovno procijenjeno 45 dana poslije tretmana, a rezultati analize objavljeni su odvojeno (24). Ispitanici su poticani da sudjeluju u podupirućoj parodontološkoj terapiji i na 3-mjesečni do 4-mjesečni protokol ponovnog pozivanja na temelju njihovih individualnih potreba. Ukupno 37, od 67 osoba procijenjenih 45 dana poslije terapije, praćeno je i procijenjeno tijekom njihovih trogodišnjih kontrola. Preostalih 30 napustilo je područje i/ili nije došlo na određeni broj termina ili uopće nisu dolazili, ili su isključeni zato što su bili podvrgnuti kirurškom parodontološkom liječenju u sklopu njihova plana liječenja.

Pacijenti su bili uključeni u studiju na temelju sljedećih kriterija:

a. dijagnoze kroničnog parodontitisa postavljene na temelju klasifikacijskog sustava za parodontnu bolest iz 1999. godine (25); dva ili više susjednih mjesta s interproksimalnim gubitkom kliničkoga epitelnog pripoja (CAL) ≥ 3 mm, dubinom sondiranja džepova (PPD) ≥ 5 mm i krvarenjem poslije sondiranja (BOP)
b. sudjelovanja u potpornoj parodontološkoj terapiji na temelju 3-mjesečnog i 4-mjesečnog protokola kontrolnih pregleda
c. završetka ponovne procjene parodonta tri godine nakon aktivnoga parodontološkog liječenja
d. zadovoljavajućeg općeg zdravlja
e. dobi između 30 i 70 godina
f. prisutnosti ≥ 16 zuba u prvom terminu.

Kriteriji za isključenje bili su:

a. svako parodontološko liječenje u posljednjih 12 mjeseci od početka ispitivanja
b. rijetko dolaženje ili nedolaženje na kontrole (neuspjeh u preporučenoj potpornoj parodontološkoj terapiji 3-mjesečne i 4-mjesečne kontrole)

e. Presence of systemic diseases or severe medical conditions.
f. Need for antibiotic prophylaxis.
g. Systemic antimicrobial therapy within the past 3 months from the study participation.
h. Pregnant or lactating females.

Clinical examination and periodontal therapy

Details of the clinical examination and treatment sequence were described previously (24). In brief, the periodontal status was assessed including PPD, CAL and BOP by an independent, blinded to the genetic analysis and calibrated (intra-examiner agreement=0.88) periodontist (E.D). The clinical examination was performed using a manual periodontal probe (15 UNC probe, Hu-Friedy, Chicago, IL, USA) at six sites per tooth apart from third molars. All patients enrolled in the study were treated according to a comprehensive periodontal treatment plan including case presentation, oral hygiene instructions and non-surgical periodontal therapy under local anesthesia. Hand instruments (Hu-Friedy, Chicago, USA) and ultrasonic scalers (KaVo SONOsoft LUX, Kavo, Germany) were utilized.

The treated patients were re-assessed at 6 weeks following the non-surgical periodontal therapy and clinical examination was completed by the same clinical examiner as carried out at the baseline examination. Supportive periodontal therapy was scheduled on a 3-4 month basis including assessment of PPD, CAL and BOP, re-instruction and re-motivation for effective plaque control, professional tooth cleaning with hand instruments and ultrasonic scalers, and subgingival instrumentation in areas with PPD ≥ 5 mm. The last clinical examination was performed at 3 years following the active periodontal treatment.

Study groups

The recruited individuals were grouped based on the IL-6 -572 G/C and IL-10 -592 C/A polymorphisms: IL-6 SCP: Susceptible to chronic periodontitis conferred by the presence of the IL-6 -572GG genotype; IL-10 SCP: Susceptible to chronic periodontitis conferred by the presence of the IL-10 -592A allele; IL-6 NSCP: Non-susceptible to chronic periodontitis carrying the non-susceptible genotype IL-6 -572C allele; IL-10 NSCP: Non-susceptible to chronic periodontitis carrying the non-susceptible genotype IL-10 -592CC genotype.

Blood samples and genotyping

Blood drops collected from each patient were used to extract genomic DNA using a commercially available genomic DNA isolation kit following the manufacturer's instructions (QIAamp, DNA mini blood kit, QIAGEN, Germany). Genotyping of IL-6 -572 G/C (rs1800796) and IL-10 -592 C/A (rs1800872) polymorphisms were determined by polymerase chain reaction and restriction fragment length polymorphism techniques in a final volume of 25 ul using a stan-

c. nedostatak podataka tijekom naknadnog pregleda tri godine poslije aktivnoga parodontološkog liječenja
d. potreba za parodontnom operacijom (resektivnom ili regenerativnom) nakon nekirurške parodontološke terapije
e. sistemske bolesti ili teško zdravstveno stanje
f. potreba za antibiotskom profilaksom
g. sustavna antimikrobiološka terapija u posljednja tri mjeseca nakon sudjelovanja u istraživanju
h. trudnice ili dojilje.

Klinički pregled i parodontološka terapija

Pojedinosti o kliničkom pregledu i redoslijedu liječenja već su opisane (24). Ukratko, parodontni status procijenjen je uključujući PPD, CAL i BOP, a obavio ga je neovisan, *slijep* za genetsku analizu i kalibriran specijalist parodontologije E. D. (podudarnost unutar ispitivača = 0,88) Klinički pregled obavljen je ručnom parodontnom sondom (15 UNC sonda, Hu-Friedy, Chicago, IL, SAD) na šest mjesta po zubu, osim trećih kutnjaka. Svi pacijenti uključeni u istraživanje liječeni su prema sveobuhvatnom parodontološkom planu liječenja, uključujući prikaz slučaja, upute za oralnu higijenu i nekiruršku terapiju pod lokalnom anestezijom. Korišteni su ručni instrumenti (Hu-Friedy, Chicago, SAD) i ultrazvučni strugači (KaVo SONOsoft LUX, Kavo, Njemačka).

Liječeni pacijenti ponovno su procijenjeni šest tjedana nakon nekirurške parodontološke terapije, a klinički pregled obavio je isti klinički ispitivač kao i tijekom inicijalnog pregleda. Određena je potporna parodontološka terapija u intervalima od 3 do 4 mjeseca, uključujući procjenu PPD-a, CAL-a i BOP-a, ponovno upućivanje i ponovna motivacija za učinkovitu kontrolu plaka, profesionalno čišćenje zuba ručnim instrumentima i ultrazvučnim strugačima te subgingivno instrumentiranje u područjima s PPD-om ≥ 5 mm. Završni klinički pregled obavljen je tri godine poslije aktivnoga parodontološkog liječenja.

Skupine ispitanika

Odabrani pojedinci grupirani su na osnovi polimorfizama *IL-6 -572 G/C i IL-10 -592 C/A:* skupna *IL-6 SCP*: sudionicima podložnima na kronični parodontitis dodijeljen je status na temelju prisutnosti genotipa *IL-6 -572GG;* skupina *IL-10 SCP*: sudionicima podložnima na kronični parodontitis koji je potvrđen i prisutnošću alela *IL-10 -592A*; podskupina IL-6 NSCP: sudionicima otpornima na kronični parodontitis dodijeljen je status jer su nositelji neosjetljivog genotipa IL-6 -572C; podskupina s alelom IL-10 NSCP: sudionici neosjetljivi na kronični parodontitis su i nositelji neosjetljivog genotipa *IL-10 -592CC.*

Uzorci krvi i genotipizacija

Prikupljene su kapi krvi od svakog pacijenta i korištene za vađenje genomskoga DNK s pomoću komercijalno dostupnog kompleta za gensku izolaciju u skladu s uputama proizvođača (QIAamp, DNA miniset za krv, QIAGEN, Njemačka). Genotipizacija polimorfizama IL-6-572 G/C (rs1800796) i IL-10-592 C/A (rs1800872) određena je tehnikom lančane reakcije polimeraze i polimorfizmom duljine ograničenja, u konačnom volumenu od 25 ul, koristeći se standardnim već

dard protocol which was described previously (24). The IL-6 and IL-10 polymorphisms were determined by using primers to generate a PCR product which was then digested and separated on polyacrylamide gels stained with silver nitrate (24).

Statistical analysis

The aim of this study was to assess the treatment outcome following non-surgical periodontal therapy between susceptible and non-susceptible individuals to periodontitis. PPD was considered the primary outcome and the sample size calculation was performed on the basis of a minimum difference of 1 mm in the mean full-mouth PPD values of each patient and a standard deviation of 0.5 mm. Aiming to achieve a 95% power of the study, it was determined that 6 individuals per group would be essential. The distribution of the clinical parameters was tested for normality using the Shapiro-Wilk test. The chi-square test was utilized to determine whether the SCP and NSCP groups were composed of similar proportions in regards to gender, smoking status and alcohol consumption while the Mann-Whitney U test was used for age and the number of teeth. To determine the differences between the timepoints (baseline and 3 years) for each group, the Wilcoxon test was used. The Mann-Whitney test was utilized to compare SCP and NSCP groups at baseline and 3 years. A multiple logistic regression analysis was used to identify possible associations between the genotypes, clinical and demographic parameters. To avoid biased results from multiple comparisons, the results were adjusted according to the Bonferroni correction for the 8 variables analyzed. P-value <0.05 was considered statistically significant. The statistical analysis was completed using SPSS v.24.0, IBM, Armonk, NY, USA.

Results

The population investigated in this study comprised a total of 37 Caucasians with a mean age of 47.68± 8.64 and a diagnosis of chronic periodontitis. It was divided into groups of similar age according to their genotype. The demographic characteristics of the study population are presented in Table 1. The IL-6 SCP and NSCP groups demonstrated similar smoking (p = 0.64) and alcohol consumption (p = 0.50) habits with no significant differences in regards to the mean number of teeth present (p = 0.11). Smoking habits remained unchanged throughout the study duration. In the examined population, more male participants were susceptible to periodontal disease than females (p = 0.02). No differences were found between the IL-10 groups with respect to age (p=0.30), gender (p=0.86), alcohol consumption (p=0.90) and number of teeth (p=0.96), whereas non-smokers were more likely to be susceptible to periodontal disease (p=0.03).

Clinical outcomes of periodontal treatment and IL-6 genotype

The clinical parameters of the IL-6 SCP and NSCP patients at baseline and 3 years following periodontal therapy are shown in Table 2. All clinical variables of the SCP and NSCP groups were similar at baseline irrespective of the genotype (p>0.05) and all decreased significantly three years following a non-surgical periodontal treatment in both groups

opisanim protokolom (24). Polimorfizmi *IL-6* i *IL-10* određeni su korištenjem prajmera za generiranje PCR produkta koji se zatim digestirao i odvojio elektroforezom na poliakrilamidne gelove obojene srebrnim nitratom (24).

Statistička analiza

Svrha ovog istraživanja bila je procijeniti ishod liječenja nakon nekirurške parodontološke terapije između osjetljivih osoba i osoba otpornih na parodontitis. PPD je smatran primarnim ishodom, a izračunavanje veličine uzorka provedeno je na temelju minimalne razlike od 1 mm u srednjim vrijednostima PPD-a za cijela usta svakog pacijenta i standardnog odstupanja od 0,5 mm. Kako bi se postiglo 95 % snage testa, utvrđeno je da u svakoj skupni mora biti šest pojedinaca. Raspodjela kliničkih parametara testirana je na normalnost korištenjem Shapiro-Wilkova testa. Hi-kvadrat test upotrijebljen je da se utvrdi jesu li SCP i NSCP skupine bile slične u omjeru kad je riječ o spolu, pušačkom statusu i konzumaciji alkohola, a Mann-Whitneyjevim U-testom određivali su se dob i broj zuba. Da bi se ustanovile razlike između vremenskih točaka (ishodišna vrijednost i poslije tri godine), za svaku skupinu korišten je Wilcoxonov test. Mann-Whitneyjev test promijenjen je za usporedbu SCP i NSCP skupina na početnoj točki i nakon tri godine. Za analizu mogućih povezanosti genotipova te kliničkih i demografskih parametara korištena je višestruka logistička regresijska analiza. Da bi se izbjegli pristrani rezultati iz više usporedba, za osam analiziranih varijabli rezultati su prilagođeni prema Bonferronijevoj korekciji. P-vrijednost < 0,05 smatrala se statistički značajnom. Statistička analiza obavljena je u sustavu SPSS v.24.0, IBM, Armonk, NY, SAD.

Rezultati

Populacija uključena u ovo istraživanje sastojala se od ukupno 37 bijelaca prosječne dobi od 47,68 ± 8,64 godina i s dijagnozom kroničnoga parodontitisa. Podijeljeni su u skupine slične prema dobi i ustanovljenom genotipu. Demografska obilježja ispitivane populacije prikazana su u tablici 1. Za skupine *IL-6* SCP i NSCP zabilježene su slične navike kad je riječ o pušenju (p = 0,64) i konzumaciji alkohola (p = 0,50), bez značajnih razlika s obzirom na srednji broj prisutnih zuba (p = 0,11). Navike pušenja su tijekom provedbe studije ostale nepromijenjene. U ispitivanoj populaciji više je muškaraca bilo podložno parodontnoj bolesti u odnosu prema ženama (p = 0,02). Nisu pronađene razlike između *IL-10* skupina s obzirom na dob (p = 0,30), spol (p = 0,86), konzumaciju alkohola (p = 0,90) i broj zuba (p = 0,96), dok su nepušači vjerojatno bili osjetljiviji na parodontnu bolest (p = 0,03).

Klinički ishodi parodontološkog liječenja i genotip IL-6

Klinički parametri *IL-6* pacijenata s SCP-om i NSCP-om preoperativno i tri godine nakon parodontološke terapije nalaze se u tablici 2. Sve kliničke varijable SCP i NSCP skupina bile su slične kad je riječ o početnoj vrijednosti, bez obzira na genotip (p > 0,05), i sve su se znatno smanjile poslije trogodišnjeg nekirurškog parodontološkog liječenja u objema skupinama (SCP i NSCP) (p < 0,05), osim za postotak mjesta

Table 1 Demographic characteristics of the study population
Tablica 1. Demografske karakteristike ispitanika

	Interleukin – 6			Interleukin – 10			Total • Ukupno
Parameters • Parameteri	SCP (n=26)	NSCP (n=11)	p-value*	SCP (n=17)	NSCP (n=20)	p-value*	(n=37)
Age • Dob (mean ± SD in years • arit. sredina ± SD u godinama)	48.31 ± 9.88	46.18 ± 4.56	0.50	49.29 ± 9.18	46.30 ± 8.12	0.30	47.68 ± 8.64
Gender • Spol Male • Muški (%) Female • Ženski (%)	 16 (61.5) 10 (38.5)	 2 (18.2) 9 (81.8)	 0.02	 8 (47.1) 9 (52.9)	 10 (50) 10 (50)	 0.86	 18 (48.6) 19 (51.4)
Smoking • Pušenje Yes • Da (%) No • Ne (%)	 12 (46.2) 14 (53.8)	 6 (54.5) 5 (45.5)	 0.64	 5 (29.4) 12 (70.6)	 13 (65) 7 (35)	 0.03	 18 (48.6) 19 (51.4)
Alcohol consumption • Konzumacija alkohola Yes • Da (%) No • Ne (%)	 11 (42.3) 15 (57.7)	 6 (54.5) 5 (45.5)	 0.50	 8 (47.1) 9 (52.9)	 9 (45) 11 (55)	 0.90	 17 (45.9) 20 (54.1)
Number of teeth • Broj zuba (mean ± SD • arit. sredina ± SD)	26.23 ± 1.97	25.00 ± 2.28	0.11	25.88 ± 2.21	25.85 ± 2.08	0.96	25.87 ± 2.11

* Mann-Whitney tests were used to compare age and number of teeth between SCP and NSCP groups. Chi-square tests were utilized for comparisons between SCP and NSCP groups with respect to gender, smoking, alcohol consumption. • Mann-Whitneyev test korišten je za usporedbu dobi i broja zuba između grupa SCP i NSCP; hi-kvadrat test korišten je za usporedbu između SCP i NSCP grupa ovisno o spolu, navikama pušenja i konzumaciji alkohola
Abbreviations • Kratice: SCP: Susceptible • podložni, NSCP: Non-susceptible • neosjetljivi

Table 2 Clinical parameters of the IL-6 SCP and NSCP patients at baseline and 3 years following periodontal therapy.
Tablica 2. Klinički parametri *IL-6 SCP* i *NSCP* ispitanika preoperativno i tri godina nakon parodontološke terapije

Clinical parameters • Klinički parametri	SCP (n=26)	NSCP (n=11)	p-value**
Full-mouth PPD (mm) • Sveobuhvatno sondiranje PPD-a (mm) Baseline • Preoperativno 3 years • 3 godine p-value* • p-vrijednost*	 3.57 (2.21-4.98) 2.39 (2.00-3.71) <0.001	 3.37 (2.68-4.19) 2.21 (2.00-3.21) 0.003	 0.32 0.29
Full-mouth BOP (%) • Sveobuhvatno BOP (%) Baseline • Preoperativno 3 years • 3 godine p-value* • p-vrijednost*	 100.00 (21.43-100.00) 8.63 (0-100.00) <0.001	 100.00 (10.87-100) 6.52 (0.67-62.50) 0.003	 1.00 0.56
Full-mouth CAL (mm) • Sveobuhvatno CAL (mm) Baseline • Preoperativno 3 years • 3 godine p-value* • p-vrijednost*	 3.67 (2.21-5.18) 2.66 (2.00-5.64) 0.003	 3.55 (2.83-4.31) 2.37 (2.00-5.24) 0.021	 0.49 0.15
Percentage of sites with PPD 4-6 mm (%) • Postotak mjesta s PPD-om 4 - 6 mm (%) Baseline • Preoperativno 3 years • 3 godine p-value* • p-vrijednost*	 26.88 (1.92-49.31) 4.92 (0-34.67) <0.001	 30.36 (8.67-45.65) 2.17 (0-27.38) 0.003	 0.92 0.84
Percentage of sites with PPD ≥ 7mm (%) • Postotak mjesta s PPD-om ≥ 7mm (%) Baseline • Preoperativno 3 years • 3 godine p-value* • p-vrijednost*	 4.63 (0-23.81) 0 (0-8.64) <0.001	 1.45 (0-9.62) 0 (0-2.00) 0.017	 0.19 0.52
Percentage of sites with CAL 4-6 mm (%) • Postotak mjesta s CAL-om 4 - 6 mm (%) Baseline • Preoperativno 3 years • 3 godine p-value* • p-vrijednost*	 29.15 (1.92-52.50) 8.33 (0-87.04) 0.046	 35.71 (10.0-46.38) 2.50 (0-78.21) 0.033	 0.79 0.35
Percentage of sites with CAL ≥ 7 mm (%) • Postotak mjesta s CAL-om ≥ 7 mm (%) Baseline • Preoperativno 3 years • 3 godine p-value* • p-vrijednost*	 4.63 (0-25.00) 0.30 (0-24.07) 0.003	 1.67 (0-10.67) 0 (0-15.38) 0.097	 0.37 0.22

*Wilcoxon test: comparison between baseline and 3 years. • Wilcoxonov test: usporedba između polazne točke i kontrole nakon tri godine
**Mann-Whitney test: comparison between SCP and NSCP groups. • Mann-Whitneyev test: usporedba između SCP i NSCP skupina
Abbreviations • Kratice:
PPD: Probing pocket depth • dubina sondiranja; CAL: Clinical attachment loss • klinički gubitak gingivnog pričvrstka; BOP: Bleeding on probing • krvarenje poslije sondiranja; SCP: Susceptible • podložni; NSCP: Non-susceptible • neosjetljivi

(SCP and NSCP) (p<0.05) except for the percentage of sites with CAL ≥ 7 mm in the NSCP group (p=0.097). In addition, no significant differences were detected in any of the clinical parameters between the groups after 3 years of treatment (p>0.05).

Clinical outcomes of periodontal treatment and IL-10 genotype

The clinical parameters of the IL-10 SCP and NSCP patients at baseline and 3 years following periodontal therapy are shown in Table 3. No significant differences between the SCP and NSCP groups were detected at baseline (p>0.05), while a significant clinical improvement was observed at 3 years (p<0.05) apart from the percentage of sites with CAL 4-6 mm (p=0.057) and the percentage of sites with CAL ≥ 7 mm (p=0.167) in the NSCP group. Individuals SCP to chronic periodontitis exhibited statistically significant reductions in PPD, CAL, BOP, percentage of sites with PPD=4-6 mm, percentage of sites with PPD≥7 mm, percentage of sites with CAL=4-6 mm, and percentage of sites with CAL≥7 mm at 3 years following the treatment (p<0.03). In comparisons between groups, neither SCP nor NSCP patients showed any significant difference with respect to the examined parameters (p>0.05).

A subgroup analysis was also performed to compare the clinical parameters at baseline and 3 years following a non-

s CAL-om ≥ 7 mm u skupini s NSCP-om (p = 0,097). Istaknimo da nisu pronađene značajne razlike ni u jednom od kliničkih parametara između skupina tri godine nakon liječenja (p > 0,05).

Klinički ishodi parodontološkog liječenja i genotipa IL-10

Klinički parametri *IL-10* pacijenata s SCP-om i NSCP-om na početku i i tri godine poslije parodontološke terapije prikazani su u tablici 3. Nisu ustanovljene značajne razlike između SCP i NSCP skupina (p > 0,05), a znatno kliničko poboljšanje uočeno je nakon 3 godine (p < 0,05), osim postotka mjesta s CAL-om od 4 do 6 mm (p = 0,057) i postotka mjesta s CAL-om ≥ 7 mm (p = 0,167) u NSCP skupini. Pojedinci iz SCP skupine s kroničnim parodontitisom postigli su nakon trogodišnjeg tretmana (p < 0,03) statistički značajna smanjenja PPD-a, CAL-a, BOP-a, postotak mjesta s PPD-om = 4 do 6 mm, postotak mjesta s PPD-om ≥ 7 mm, postotak mjesta s CAL-om = 4 - 6 mm i postotak mjesta s CAL-om ≥ 7 mm. U usporedbi skupina ni pacijenti s SCP-om, ni oni s NSCP-om nisu pokazali značajnu razliku u odnosu prema ispitivanim parametrima (p > 0,05).

Obavljena je također analiza podskupine radi usporedbe kliničkih parametara preoperativno i tri godine poslije ne-

Table 3 Clinical parameters of the IL-10 SCP and NSCP patients at baseline and 3 years following periodontal therapy
Tablica 3. Klinički parametri *IL-10* SCP i NSCP ispitanika preoperativno i tri godine nakon parodontološke terapije

Clinical parameters • Klinički parametri	SCP (n=26)	NSCP (n=11)	p-value**
Full-mouth PPD (mm) • Sveobuhvatno sondiranje PPD-a (mm)			
Baseline • Preoperativno	3.18 (2.66-4.98)	3.55 (2.21-4.44)	0.35
3 years • 3 godine	2.31 (2.00-3.71)	2.50 (2.00-3.23)	0.49
p-value* • p-vrijednost*	<0.001	<0.001	
Full-mouth BOP (%) • Sveobuhvatno BOP (%)			
Baseline • Preoperativno	100.00 (10.87-100.00)	100.00 (35.26-100.00)	0.48
3 years • 3 godine	7.50 (0-100.00)	11.26 (0-62.50)	0.89
p-value* • p-vrijednost*	0.002	<0.001	
Full-mouth CAL (mm) • Sveobuhvatno CAL (mm)			
Baseline • Preoperativno	3.55 (2.84-5.18)	3.58 (2.21-5.02)	0.63
3 years • 3 godine	2.38 (2.00-4.54)	2.66 (2.00-5.64)	0.59
p-value* • p-vrijednost*	0.002	0.023	
Percentage of sites with PPD 4-6 mm (%) • Postotak mjesta s PPD-om 4 - 6 mm (%)			
Baseline • Preoperativno	23.81 (8.67-47.44)	29.77 (1.92-49.31)	0.25
3 years • 3 godine	4.76 (1.28-34.67)	3.21 (0-27.38)	0.80
p-value* • p-vrijednost*	<0.001	<0.001	
Percentage of sites with PPD ≥ 7mm (%) • Postotak mjesta s PPD-om ≥ 7mm (%)			
Baseline • Preoperativno	2.56 (0-23.81)	3.13 (0-18.75)	0.66
3 years • 3 godine	0 (0-3.33)	0 (0-8.64)	0.70
p-value* • p-vrijednost*	0.001	0.003	
Percentage of sites with CAL 4-6 mm (%) • Postotak mjesta s CAL-om 4 - 6 mm (%)			
Baseline • Preoperativno	29.17 (9.88-49.36)	33.65 (1.92-52.50)	0.77
3 years • 3 godine	8.33 (0-87.04)	5.06 (0-78.21)	0.76
p-value* • p-vrijednost*	0.028	0.057	
Percentage of sites with CAL ≥ 7 mm (%) • Postotak mjesta s CAL-om ≥ 7 mm (%)			
Baseline • Preoperativno	4.49 (0-25.00)	4.46 (0-22.22)	0.50
3 years • 3 godine	0 (0-7.74)	0 (0-24.07)	0.99
p-value* • p-vrijednost*	0.001	0.167	

*Wilcoxon test: comparison between baseline and 3 years. • Wilcoxonov test: usporedba između polazne točke i kontrole nakon tri godine
**Mann-Whitney test: comparison between SCP and NSCP groups. • Mann-Whitneyev test: usporedba između SCP i NSCP skupinama
Abbreviations • Kratice:
PPD: Probing pocket depth • dubina sondiranja; CAL: Clinical attachment loss • klinički gubitak gingivnog pričvrstka; BOP: Bleeding on probing • krvarenje poslije sondiranja; SCP: Susceptible • podložni; NSCP: Non-susceptible • neosjetljivi

surgical periodontal therapy between individuals susceptible (n=13) to chronic periodontitis carrying both the susceptible genotypes for IL-6 (IL-6 GG) and IL-10 (IL-10 CA or IL-10 AA) with those non-susceptible (n=24) carrying one or all three non-susceptible genotypes IL-6 GC, IL-6 CC, IL-10 CC. Both subgroups showed similar age (p=0.50) and gender (p=0.25) distribution. Following the treatment, significant reductions were observed for the susceptible group in regards to BOP (p=0.001), PPD (p=0.001), CAL (p=0.013), PPD=4-6 mm (p=0.001), PPD≥7 mm (p=0.001) and CAL≥7 mm (p=0.01). Sites with CAL=4-6 mm demonstrated no significant differences between baseline and 3 years in the combined IL-6 and IL-10 susceptible subgroup (p=0.13). In the IL-6 and IL-10 non-susceptible combined subgroup, all examined clinical parameters showed significant reduc-

kirurške parodontološke terapije između pojedinaca podložnih (n = 13) kroničnom parodontitisu koji su nositelji obaju podložnih genotipova za *IL-6 (IL-6 GG)* i *IL-10 (IL-10 CA* ili *IL-10 AA*) s onima otpornima (n = 24) koji nose jedan ili sva tri otporna genotipa *IL-6 GC*, *IL-6 CC* i *IL-10 CC*. Obje podskupine imale su sličan raspon u dobi (p = 0,50) i spolu (p = 0,25). Nakon tretmana uočeno je značajno smanjenje za podložnu skupinu u odnosu prema BOP-u (p = 0,001), PPD-u (p = 0,001), CAL-u (p = 0,013), PPD-u = 4 do 6 mm (p = 0,001), PPD-u ≥ 7 mm (p = 0,001) i CAL-u ≥7 mm (p = 0,01). Mjesta s CAL-om = 4 do 6 mm nisu pokazala značajne razlike između inicijalnih vrijednosti i nakon tri godine u kombiniranoj podskupini podložnoj na *IL-6* i *IL-10* (p = 0,13). U kombiniranoj podskupini s *IL-6* i *IL-10* u kojoj su bili pacijenti koji nisu podložni, svi ispitivani klinički pa-

Table 4 Multiple logistic regression analysis for the association between the IL-6 -572 G/C and IL-10 -592 C/A genotypes and the demographic and clinical variables at baseline and 3 years.
Tablica 4. Multipla logistička regresijska analiza za povezanost između IL-6 -572 G/C i IL-10 -592 C/A genotipa te demografske i kliničke varijable preoperativno i 3 godine nakon terapije

	Parameters • Parametri	OR	95%CI	p-value* • p-vrijednost*	Adjusted p-value (Bonferroni correction) • Prilagođena p-vrijednost (Bonferronijeva korekcija)
IL-6	**Baseline • Preoperativno**				
	Age • Dob	1.12	0.97-1.29	0.12	0.99
	Gender • Spol	52.09	2.41-1125.37	**0.01**	0.10
	Smoking • Pušenje	1.09	0.09-13.01	0.95	7.56
	Alcohol consumption • Konzumacija alkohola	0.42	0.04-4.78	0.49	3.86
	Number of teeth • Broj zuba	1.79	1.00-3.22	**0.05**	0.41
	Full-mouth BOP • Puni zubni niz BOP	0.97	0.93-1.02	0.21	1.66
	Full-mouth PPD • Puni zubni niz PPD	0.10	0.01-104.32	0.52	4.17
	Full-mouth CAL • Puni zubni niz CAL	35.69	0.07-18052.25	0.26	2.08
	3 years • 3 godine				
	Age • Dob	1.09	0.96-1.25	0.19	1.56
	Gender • Spol	26.86	2.00-360.16	**0.01**	0.10
	Smoking • Pušenje	1.39	0.18-10.96	0.76	6.04
	Alcohol consumption • Konzumacija alkohola	0.35	0.05-2.38	0.29	2.28
	Number of teeth • Broj zuba	1.32	0.82-2.11	0.26	2.06
	Full-mouth BOP • Puni zubni niz BOP	0.99	0.95-1.03	0.63	5.04
	Full-mouth PPD • Puni zubni niz PPD	0.28	0.01-6.11	0.42	3.34
	Full-mouth CAL • Puni zubni niz CAL	1.94	0.59-6.38	0.28	2.22
IL-10	**Baseline • Preoperativno**				
	Age • Dob	1.05	0.95-1.15	0.33	2.63
	Gender • Spol	1.07	0.20-5.78	0.94	7.49
	Smoking • Pušenje	0.18	0.03-0.93	**0.04**	0.33
	Alcohol consumption • Konzumacija alkohola	2.07	0.36-11.89	0.42	3.33
	Number of teeth • Broj zuba	1.08	0.73-1.59	0.70	5.58
	Full-mouth BOP • Puni zubni niz BOP	0.98	0.95-1.01	0.16	1.30
	Full-mouth PPD • Puni zubni niz PPD	0.46	0.01-109.19	0.78	6.25
	Full-mouth CAL • Puni zubni niz CAL	3.37	0.02-541.77	0.64	5.11
	3 years • 3 godine				
	Age • Dob	1.05	0.95-1.15	0.34	2.75
	Gender • Spol	0.61	0.11-3.50	0.57	4.59
	Smoking • Pušenje	0.18	0.30-1.05	0.06	0.45
	Alcohol consumption • Konzumacija alkohola	2.1	0.42-10.64	0.37	2.95
	Number of teeth • Broj zuba	0.80	0.51-1.26	0.34	2.68
	Full-mouth BOP • Puni zubni niz BOP	1.01	0.98-1.05	0.48	3.82
	Full-mouth PPD • Puni zubni niz PPD	1.58	0.13-19.75	0.72	5.78
	Full-mouth CAL • Puni zubni niz CAL	0.63	0.25-1.60	0.33	2.62

*p-values in bold denote statistical significance (p<0.05). • p-vrijednosti u krvi podebljane označavaju statističku značajnost (p < 0,05)
Abbreviations • Kratice:
PPD: Probing pocket depth • sondiranje parodontnih džepova; CAL: Clinical attachment loss • klinički gubitak gingivnog pričvrstka; BOP: Bleeding on probing • krvarenje poslije sondiranja

tions (p<0.04). SCP and NSCP subgroups showed similar clinical variables at baseline and 3 years (p>0.05).

Multiple logistic regression analysis

Multiple logistic regression analysis for the association between the IL-6 -572 G/C and IL-10 -592 C/A genotypes and the demographic and clinical variables at baseline and 3 years is shown in Table 4. At baseline, IL-6 genotype was significantly associated with gender (p=0.01) and the number of missing teeth (p=0.05), while IL-10 genotype and smoking status showed a significant association (p=0.04). In addition, there was a significant association between susceptibility to chronic periodontitis (as determined by the presence of the IL-6 -572GG genotype) with gender at 3 years (p=0.01). However, after the Bonferroni adjustment for multiple comparisons, these findings lost statistical significance (p>0.05). After 3 years of periodontal treatment, there was no association between any of the variables tested (p>0.05).

Discussion

Current evidence suggests that the treatment outcome following non-surgical periodontal treatment may vary between patients, teeth as well as treated sites within individuals (18,19). Each individual's genetic background may explain the variation in treatment response. Since IL-6 -572 and IL-10 -592 gene polymorphisms have been significantly associated with chronic periodontitis (10-13) and since gene variant carriage could possibly affect the response to periodontal therapy (23), we investigated, in this study, the influence of genetic susceptibility to chronic periodontitis on periodontal treatment outcome over a three-year period of time.

In contrast with our hypothesis that patients susceptible to chronic periodontitis as determined by the presence of the IL-6 -572GG genotype or the IL-10 -592A allele would have significantly worse response to non-surgical periodontal treatment than non-susceptible individuals, a similar treatment outcome was observed for both included groups. More specifically, susceptible and non-susceptible to chronic periodontitis patients showed a statistically significant improvement in the examined clinical parameters at 45 days (as shown in our previous publication, 24) which has been maintained up to 3 years following the initial treatment (as shown in the present investigation). However, this significant clinical periodontal improvement was irrespective of a patient's genetic background.

To the best of our knowledge, this is the first study that evaluated the association between gene polymorphisms and non-surgical periodontal treatment over a long term period. Previous investigations included individuals that were followed-up for a period of time between 45 days to 6 months following a non-surgical periodontal therapy (23). Our findings may differ with previous studies due to the longer period of observation. Long-term monitoring and supportive periodontal treatment are of paramount importance to achieve long-term success of periodontal treatment and to minimize the risk of tooth loss (26). In addition, long-term studies can confirm or reject an association that has been shown in rel-

rametri pokazali su značajno smanjenje (p < 0,04). Za SCP i NSCP podgrupe zabilježene su slične kliničke varijable na početku i poslije tri godine (p > 0,05).

Multipla logistička regresijska analiza

Analiza multiple logističke regresije za povezanost između genotipova *IL-6-572 G/C i IL-10 -592 C/A* te demografskih i kliničkih varijabli inicijalno i nakon tri godine prikazana je u tablici 4. Na početku je genotip *IL-6* bio značajno povezan sa spolom (p = 0,01) i brojem zuba koji nedostaju (p = 0,05), a genotip *IL-10* i pušački status pokazali su značajnu povezanost (p = 0,04). Postojala je i značajna povezanost između podložnosti za pojavu kroničnog parodontitisa (što je određeno prisutnošću genotipa *IL-6 -572GG*) sa spolom i dobi nakon tri godine (p = 0,01). No poslije Bonferronijeve prilagodbe za višestruke usporedbe ti su nalazi izgubili statističku značajnost (p> 0,05). Tri godine poslije parodontološkog liječenja nije bilo povezanosti između ni jedne od ispitivanih varijabli (p > 0,05).

Rasprava

Sadašnji dokazi upućuju na to da ishod liječenja nakon nekirurškog parodontološkog liječenja može varirati među pacijentima između zuba i liječenih mjesta (18, 19). Svaka genetska pozadina može objasniti varijacije u odgovoru na liječenje. Budući da su polimorfizmi gena *IL-6* -572 i *IL-10 -592* bili značajno povezani s kroničnim parodontitisom (10 – 13) i zato što bi prijenos genske varijante mogao utjecati na odgovor na parodontološku terapiju (23), u ovom istraživanju analizirali smo utjecaj genetske osjetljivosti na kronični parodontitis i na ishod parodontološkog liječenja tijekom trogodišnjeg razdoblja.

Za razliku od naše hipoteze da bi pacijenti osjetljivi na kronični parodontitis utvrđen prisutnošću genotipa *IL-6-572GG* ili alela *IL-10-592A* imali značajno lošiji odgovor na nekirurško parodontološko liječenje u odnosu prema osobama koje nisu osjetljive, rezultati upućuju na sličan ishod liječenja u obje uključene skupine. Konkretnije, podložni i otporni na kronični parodontitis pokazali su statistički značajno poboljšanje ispitivanih kliničkih parametara u roku od 45 dana (kao što je predstavljeno u našoj publikaciji, 24) koje se zadržalo do tri godine nakon početnog liječenja (kao što je opisano u ovom tekstu). No to znatno kliničko poboljšanje parodonta nije bilo ovisno o pacijentovu genetskom podrijetlu.

Prema našim spoznajama, ovo je prvo istraživanje u kojemu je procijenjena dugoročna povezanost polimorfizama gena i nekirurškoga parodontološkog liječenja. U dosadašnja ispitivanja bile su uključene osobe koje se pratilo od 45 dana do 6 mjeseci nakon nekirurške parodontološke terapije (23). Naši se nalazi mogu razlikovati od ostalih studija zbog duljeg razdoblja promatranja. Dugotrajno praćenje i podupiruće parodontološko liječenje iznimno su važni za postizanje dugoročnog uspjeha parodontološkog liječenja i minimiziranje rizika od gubitka zuba (26). U to, dugoročnim se istraživanjima može potvrditi ili isključiti povezanost koja se pokazala u razmjerno kratkoročnim istraživanjima. No tijekom longitudinalnih ispitivanja nije uvijek moguće održati po-

atively short-term studies. However, it is not always possible to maintain the initial sample size and preserve an equal distribution of the sample size between groups when performing longitudinal studies. The loss of recruited patients during the follow-up time can be considered as inherent limitation of our investigation. Although differences in gender distribution between IL-6 SCP and NSCP groups were observed, gender demonstrated no significant effects on treatment outcome.

Furthermore, the majority of previous studies that aimed to examine the effect of susceptibility to chronic periodontitis on the treatment outcome following non-surgical periodontal therapy have shown similar results (23). Matrix metalloproteinase (MMP)-1, MMP-13, IL-1, IL-4, IL-6, IL-8, mannose-binding lectin (MBL) and monocyte chemoattractant protein (MCP)-1 gene polymorphisms have been analyzed (23, 27). These short-term studies demonstrated no significant differences between genetic polymorphisms of the IL-8, MMP-13, MMP-1, IL-1, IL4, IL-8, MBL genes and response to non-surgical periodontal treatment (23). On the contrary, in a prospective longitudinal study by D' Aiuto et al., susceptible patients carriers of the C allele of the IL-6 –174 polymorphism with generalized severe periodontitis showed a worse periodontal treatment outcome compared to non-susceptible individuals (28). However, the inclusion of various ethnic populations and the differences in the examined variables may explain the contrasting findings. More specifically, Caucasian, African/Caribbean and Asian patients were included in the same analysis rather than a specific ethnic group and the effect of genotype on periodontal treatment outcome was determined based on the decrease in the number of pockets rather than the evaluation of any continuous periodontal parameters. In addition, a Taiwanese study demonstrated that MCP-1 -2518 A/G genotype was significantly associated with the treatment outcome of non-surgical periodontal treatment as determined by evaluation of the probing depth, gingival index and bleeding index (27). However, this gene polymorphism influenced the treatment outcome only in patients with a diagnosis of aggressive periodontitis rather than in patients with chronic periodontitis.

Although there is evidence supporting that patient-related factors influence the periodontal treatment outcome and the risk of tooth loss (18,19), genetic susceptibility to chronic periodontitis, conferred by the presence of the IL-6 -572GG genotype or the IL-10 -592A allele, was not associated with a worse treatment outcome in this study. Other factors, such as smoking, age and oral hygiene, may be stronger factors for a poorer treatment outcome than genetic factors (29). Additionally, systemic medical conditions such as diabetes mellitus may affect periodontal healing and tissue homeostasis following periodontal therapy (20). In order to minimize the potential confounding factors, systemically healthy patients were only recruited. Moreover, the lack of association between smoking status and susceptibility to chronic periodontitis after adjusting for multiple comparisons may be due to different smoking habits (light, moderate, heavy smoking) of the included population.

IL-6 is a pleotropic cytokine that promotes the evolution of chronic inflammation and bone resorption through

četnu veličinu uzorka i sačuvati jednaku raspodjelu veličine uzorka između skupina. Odlazak odabranih pacijenata tijekom praćenja može se smatrati ograničenjem našeg istraživanja. Iako su uočene razlike u rodnoj distribuciji između skupina *IL-6* SCP i NSCP, spol nije pokazao značajne učinke na ishod liječenja.

Nadalje, u većini istraživanja, kojima je bila svrha ispitati učinak osjetljivosti na kronični parodontitis kad je riječ o ishodu liječenja nakon nekirurške parodontološke terapije, dobiveni su slični rezultati (23). Analiziran je polimorfizam gena metaloproteinaze matriksa (*MMP*)-1, *MMP-13, IL-1, IL-4, IL-6, IL-8*, lektin koji veže manozu (MBL) i monocitni kemoatraktantni protein (MCP)-1 (23, 27). U nekim kratkoročnim istraživanjima nisu istaknute značajne razlike između genetskih polimorfizama gena *IL-8, MMP-13, MMP-1, IL-1, IL4, IL-8, MBL* i odgovora na nekirurško parodontološko liječenje (23). Suprotno tomu, u prospektivnoj longitudinalnoj studiji D 'Aiuta i suradnika, podložni pacijenti koji nose alel C polimorfizma *IL-6-174* s generaliziranim teškim parodontitisom, pokazali su lošiji ishod parodontološkog liječenja u usporedbi s genetski otpornim pojedincima (28). No uključivanje različitih etničkih populacija i razlike u ispitivanim varijablama mogu objasniti takve suprotne nalaze. Preciznije, kavkaski, afrički/karipski i azijski pacijenti bili su uključeni u istu analizu, a ne specifična etnička skupina, pa je utjecaj genotipa na ishod parodontološkog liječenja određen na temelju smanjenja broja džepova, a ne procjene bilo kojeg kontinuiranog parodontološkg parametra. Uz to, u tajvanskom je istraživanju istaknuto da je *MCP-1-2518 A/G* genotip značajno povezan s ishodom nekirurškoga parodontološkog liječenja utvrđenog procjenom dubine sondiranja, indeksa gingive i indeksa krvarenja (27). No taj polimorfizam gena utjecao je na ishod liječenja samo kod bolesnika s dijagnozom agresivnog parodontitisa, a ne i onih s kroničnim parodontitisom.

Premda postoje dokazi koji potvrđuju da čimbenici vezani za pacijenta utječu na rezultat parodonološkog liječenja i rizik od gubitka zuba (18, 19), genetska primljivost za kronični parodontitis, podijeljena prisutnošću genotipa *IL-6 -572GG* ili alela *IL-10-592*, u ovom istraživanju nije bila povezana s lošijim ishodom liječenja. Ostali čimbenici, poput pušenja, dobi i oralne higijene, mogu biti važniji u slučaju lošeg ishoda liječenja od onih genetskih (29). Uz to, sistemsko zdravstveno stanje, poput dijabetesa melitusa može utjecati na zarastanje parodonata i homeostazu tkiva nakon parodontološke terapije (20). Kako bi se smanjili mogući loši čimbenici, birani su samo sustavno zdravi pacijenti. Nadalje, nedostatak povezanosti između statusa i osjetljivosti na kronični parodontitis nakon prilagođavanja višestrukim usporedbama, može biti posljedica različitih navika pri pušenju (lagano, umjereno, teško pušenje) uključene populacije.

IL-6 je pleotropni citokin koji složenim interakcijama potiče razvoj kronične upale i resorpciju kosti (31). Budući da su polimorfizmi *IL-6* povezani s pojačanim upalnim odgovorom ponajprije u prisutnosti parodontnih patogena, gen *IL-6* može biti važan u patogenezi parodontitisa (6, 7). *IL-10* smatra se protuupalnim citokinom koji suzbija imunosni i upalni odgovor koji može zaštititi od koštane resorpcije

complex interactions (31). Since IL-6 polymorphisms are associated with an increased inflammatory response primarily in the presence of periodontal pathogens, IL-6 gene may play an important role in the pathogenesis of periodontitis (6,7). IL-10 is considered an anti-inflammatory cytokine that suppresses immune and inflammatory responses which can protect against bone resorption (32). Polymorphisms in the IL-10 promoter -592 region are associated with a decrease in IL-10 production, which may result in the development of periodontal diseases (9). Although both polymorphisms are associated with an increased inflammatory response and therefore play an important role in disease onset and progression, their effect may not influence the healing process after periodontal treatment. IL-6 and IL-10, as shown in this study, may have no effect on the resolution of periodontal inflammation.

Future studies encompassing larger samples with different characteristics including smoking habits, various bacterial strains and systemic conditions from different populations and ethnicities are required to further examine the possible effect of gene polymorphisms on periodontal treatment outcome over a long period of time. In addition, further studies should include a greater number of patients with a generalized form of chronic periodontitis (>30% of sites) to determine whether the patients with a greater extent of periodontitis demonstrate significant differences with respect to treatment outcome. Due to the inclusion criteria of this study (interproximal CAL ≥ 3 mm, PPD ≥ 5 mm and BOP in two or more non-adjacent sites), the individuals with localized and generalized periodontitis were included in the analysis.

Conflict of interest

The authors declare that there is no potential conflict of interest regarding this article.

Contribution to the paper

G.S.C. concept and design, analysis and interpretation of data, drafting the article, revising it critically, final approval of the version to be published, agreement for publication; **A.E.D.** concept and design, acquisition of data, analysis and interpretation of data, revising it critically, final approval of the version to be published, agreement for publication; **S.F.** analysis and interpretation of data, revising it critically, final approval of the version to be published, agreement for publication; M.A. analysis and interpretation of data, revising it critically, final approval of the version to be published, agreement for publication; **A.K.** concept and design, acquisition of data, analysis and interpretation of data, revising it critically, final approval of the version to be published, agreement for publication.

(32). Polimorfizmi u regiji promotora *IL-10-592* povezani su sa smanjenjem proizvodnje *IL-10*, što može rezultirati pojavom parodontonih bolesti (9). Iako su oba polimorfizma povezana s povećanim upalnim odgovorom i zato su važni u nastanku i progresiji bolesti, njihov učinak možda neće utjecati na proces ozdravljenja nakon parodontološkog liječenja. *IL-6* i *IL-10,* kao što je istaknuto u ovom istraživanju, ne mogu utjecati na rješavanje parodontne upale.

Buduća istraživanja na većem uzorku s različitim karakteristikama sudionika, uključujući naviku pušenja, različite bakterijske sojeve i sistemska stanja, iz različitih populacija i etničkih skupina potrebna su radi daljnjeg ispitivanja mogućeg utjecaja genskih polimorfizama na rezultat dugotrajnog liječenja parodonta. Istaknimo da bi u ta buduća ispitivanja trebalo uključiti veći broj pacijenata s generaliziranim oblikom kroničnog parodontitisa (> 30 % mjesta) kako bi se utvrdilo pokazuju li oni s većim opsegom parodontitisa značajne razlike s obzirom na ishod liječenja. Zbog kriterija uključivanja u ovu studiju (interproksimalni CAL ≥ 3 mm, PPD ≥ 5 mm i BOP na dva ili više susjednih mjesta) u analizu su bili uključeni pojedinci s lokaliziranim i generaliziranim parodontitisom.

Sukob interesa

Autori izjavljuju da nisu u sukobu interesa.

Doprinosi članku

G S. C. – koncept i nacrt, analiza i interpretacija podataka, nacrt članka, kritičko pregledavanje teksta, odobravanje krajnje verzije za objavljivanje, suglasnost za objavljivanje; **A. E. D.** – koncept i nacrt, prikupljanje podataka, analiza i interpretacija podataka, kritičko pregledavanje članka, odobravanje krajnje verzije za objavljivanje, suglasnost za objavljivanje; **S. F.** – analiza i interpretacija podataka, kritičko pregledavanje članka, odobravanje krajnje verzije za objavljivanje, suglasnost za objavljivanje; **M. A.** – analiza i interpretacija podataka, kritičko pregledavanje članka, odobravanje krajnje verzije za objavljivanje, suglasnost za objavljivanje; **A. K.** – koncept i nacrt, prikupljanje podataka, analiza i interpretacija podataka, kritičko pregledavanje članka, odobravanje krajnje verzije za objavljivanje, suglasnost za objavljivanje.

Sažetak

Cilj: Istraživalo se utječe li genetska osjetljivost na kronični parodontitis, potvrđena genotipom *IL-6-572GG* ili alelom *IL-10-592A*, na rezultate nekirurške parodontne terapije (NSPT) nakon duljeg vremena. **Materijal i metode:** U dvije skupine raspoređeno je 37 pacijenata s kroničnim parodontitisom prema genotipu i to kao podložni (SCP) i otporni (NSCP). Svi ispitanici klinički su procijenjeni na početku i tri godine poslije NSPT-a. Uzorci krvi za određivanje polaznih vrijednosti prikupljeni su od onih koji su ispunili uvjete za sudjelovanje. Svi su primili NSPT od jednog specijalista parodontologije koji nije znao genotip statusa pacijenata. Provedena je statistička analiza usporedbom varijabli za skupine u kojima se koristio Mann-Whitneyjev U-test, između početne i treće godine za svaku skupinu upotrebljavajući Wilcoxov test. **Rezultati:** Prosječna dob ispitanika bila je 47,68 ± 8,64 godine, a sudjelovalo je 51,4 % žena, 48,6 % pušača i 45,9 % konzumenata alkohola. Nakon genetske analize zaključeno je da su 70,3 % pacijenata homozigotni nositelji *IL-6-572G* (*IL-6* SCP), a njih 46,0 % bili su nositelji alela *IL-10 592A* (*IL-10* SCP). NSPT je smanjio sve ispitivane parametre ispitanicima (dubina sondiranja, gubitak epitelnog pričvrstka, krvarenje poslije sondiranja, postotak mjesta sa džepovima od 4 do 6 mm i ≥ 7 mm i gubitak epitelnog pričvrstka), ali ishod liječenja nije bio povezan s genotipom. Osobe s SCP-om i NSCP-om imale su slične kliničke parametre na početku tretmana i poslije tri godine. **Zaključak:** Unutar ograničenja ove trogodišnje kohortne studije u kojoj su sudjelovali bijelci s dijagnosticiranim kroničnim parodontitisom, pojedincima podložnima parodontitisu, kako je određeno prisutnošću genotipa *IL-6 -572GG* ili alela *IL-10 -592A*, nakon NSPT-a rezultat liječenja bio je sličan.

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Ključne riječi
kronični parodontitis; interleukin-6; interleukin-10; terapija; genetski polimorfizam

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