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Diagnostic Accuracy of Salivary aMMP-8 Test in Infertile Women and Blood Finding Analysis

Dijagnostička točnost sline neplodnih žena dobivena testom aMMP-8 i analiza nalaza krvi

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Abstract

Introduction: The literature reviewed in this paper suggests that infertile patients present worse periodontal status, which may be causative to conception complications. This study aimed to validate an aMMP-8 point-of-care mouth rinse test in the population of women with unexplained infertility and compare it to age-matched fertile women with and without periodontitis. Furthermore, blood sampled inflammatory parameters were analyzed and compared between the two groups. It was hypothesized that the infertile women would present worse periodontal status and a greater number of positive aMMP-8 tests than fertile women, and they would have increased inflammatory blood parameters. **Material and methods:** The study included 50 healthy norm-ovulatory females aged 25-45 years with strictly defined unexplained (idiopathic) infertility, and 50 healthy norm-ovulatory women of the same age who had conceived and delivered naturally. **Results:** The sensitivity and specificity of the test for detecting periodontitis was 84% and 72% in the group of infertile patients, 88% and 68% in the group of fertile patients and 86% and 70% in the overall patient population. Infertile patients with periodontitis had less advanced periodontitis than the control group although this difference was not statistically significant. Blood inflammatory markers were significantly higher in infertile than in fertile women. **Conclusion:** This study has shown that infertile patients had better periodontal status and less advanced periodontitis than fertile women of the same age. Therefore, when interpreting the results of aMMP-8 tests for diagnosis of periodontitis, one should keep in mind the periodontal status of the examined population.

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Introduction

Periodontitis, a chronic inflammatory disease of the tooth's supporting tissues, has been previously linked with adverse pregnancy outcomes (1), in particular with lower birth weight, pre-term birth and an increased risk of preeclampsia in pregnant women (2-5). It is, however, important to highlight that the strength of this association is limited, and further research on the matter is needed. The available literature data also link periodontitis to later conception and problems with male and female fertility (1, 6, 7). Some authors have reported worse

Uvod

Parodontitis, kronična upalna bolest potpornoga tkiva zuba, prije je bila povezana s nepovoljnim ishodima trudnoće (1), posebice manjom porođajnom težinom, prijevremenim porođajem i povećanim rizikom od preeklampsije u trudnoći (2 – 5). No, važno je istaknuti da je snaga te povezanosti ograničena pa su potrebna daljnja istraživanja. Literaturni podatci također povezuju parodontitis s kasnijim začećem i problemima s muškom i ženskom plodnošću (1, 6, 7). Neki su autori izvijestili o lošijem parodontnom statusu neplod-

periodontal status in infertile patients and suspected that this is a factor that complicates conception (5, 8-12).

One of the hypothesized mechanisms on the association between periodontitis, fertility and adverse pregnancy outcomes is the effect of inflammatory mediators associated with periodontitis at the level of the endometrium and embryo implantation site. Furthermore, the systemic inflammatory response that develops in some women with periodontitis may be causative to recurrent miscarriages and infertility (1).

Periodontal infection-induced cytokines and proteolytic enzymes destroy the gingival tissues and cause persistent silent systemic inflammation. This is reflected by the activation of matrix metalloproteinase-8 (MMP-8), the salivary enzyme responsible for the destruction of periodontal collagen fibers and is directly associated with periodontal inflammation (13, 14). The clinical periodontal diagnostic procedure may present as time-consuming, as it includes comprehensive clinical periodontal charting with measurement of various indices and is complemented with the radiographic examination. Point-of-care (PoC) chair-side tests were developed to rapidly detect elevated aMMP-8 in a sampled mouth rinse or gingival sulcus fluid to discriminate periodontally healthy from periodontally diseased individuals (15, 16). With a high sensitivity of 90% and specificity of 70-85% (16, 17), the results of PoC tests are comparable with more expensive and complicated tests such as immunofluorometric assay (IFMNA) and Dento ELISA (Enzyme-linked immunosorbent assay). All these tests selectively identify the activated form of MMP-8 (aMMP-8) using the same aMMP-8 antibody and correlate well with each other. A study by Nwhator et al. (7) has shown a significant association between poor oral hygiene and/or periodontitis and subnormal sperm count. Furthermore, they have demonstrated that the use of MMP-8 PoC chair-side test overcame deficiencies of CPITN index (Community Periodontal Index of Treatment Needs) with reported 96% sensitivity for poor oral hygiene, 95% sensitivity for chronic periodontitis and 82.6% sensitivity for bleeding on probing. However, these numbers were decreased in patients with better oral hygiene or periodontal status. Recently published studies have also shown that the aMMP-8 test has certain limitations and that its sensitivity and specificity increases with the level of periodontitis (18, 19).

Subclinical chronic systemic inflammation ("low-grade chronic inflammation") is a condition characterized by elevated levels of inflammatory markers such as C-reactive protein (CRP), tumor necrosis factor α (TNF- α), and various interleukins (IL) (20). It is considered one of the etiopathogenetic mechanisms of idiopathic infertility (1). Several studies have investigated the role of hematological parameters such as complete blood count (CBC), leukocyte count (L), neutrophils (N), neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR) and mean platelet volume (MPV) in the etiopathogenesis of infertility of unknown cause, and their impact on the results of IVF procedures (21-23). It seems that parameters such as lymphocytes, PLR and MPV could play a role in predicting the success of IVF procedures. Thus, several studies that included subjects treated for idiopathic infertility presented lymphocytes as positive pre-

nih pacijenata i posumnjali da je to čimbenik koji otežava začeće (5, 8 – 11).

Jedan od pretpostavljenih mehanizama o povezanosti parodontitisa, plodnosti i nepovoljnih ishoda trudnoće jest učinak upalnih medijatora povezanih s parodontitisom na razini endometrija i mjesta implantacije embrija. Nadalje, sustavni upalni odgovor koji se pojavljuje u nekih žena s parodontitisom može biti uzrok ponavljajućih pobačaja i neplodnosti (1).

Citokini i proteolitički enzimi izazvani parodontnom infekcijom uništavaju tkivo gingive i uzrok su trajne tihe sistemske upale. To se odražava aktivacijom matriksne metaloproteinaze-8 (MMP-8), enzima sline odgovornoga za uništavanje parodontnih kolagenih vlakana i izravno povezanoga s parodontnom upalom (14). Klinički parodontološki dijagnostički postupak može biti dugotrajan jer uključuje sveobuhvatan klinički parodontološki dijagram s mjerenjem različitih indeksa i nadopunjen je radiološkim pregledom. Point-of-care (PoC) testovi na mjestu skrbi o pacijentici razvijeni su za brzo otkrivanje povišenoga aMMP-8 u ispirku usne šupljine ili tekućini gingivalnoga sulkusa kako bi se razlikovalo pojedince koji su parodontno zdravi od parodontno bolesnih (15, 16). Uz visoku osjetljivost od 90 % i specifičnost od 70 do 85 % (16, 17), rezultati PoC testova mogu se usporediti sa skupljim i kompliciranijim testovima kao što su imunofluorometrijski test (IFMNA) i Dento ELISA (enzimski imunosorbentni test). Svi ti testovi selektivno identificiraju aktivirani oblik MMP-8 (aMMP-8) korištenjem istoga aMMP-8 antitijela i uzajamno dobro koreliraju. Istraživanje Nwhatora i suradnika (7) pokazalo je značajnu povezanost između loše oralne higijene i/ili parodontitisa i subnormalnoga broja spermija. Nadalje, pokazali su da je korištenje MMP-8 PoC testa na mjestu skrbi o pacijentici prevladalo nedostatak CPITN indeksa (engl. *Community Parodontal Index of Treatment Needs*) s opaženom 96-postotnom osjetljivošću za lošu oralnu higijenu, 95-postotnom osjetljivošću za kronični parodontitis i 82,6-postotnom osjetljivošću za krvarenje pri sondiranju. Međutim, ti su brojevi bili niži u pacijentica s boljom oralnom higijenom ili parodontnim statusom. U nedavno objavljenim studijama također se ističe da test aMMP-8 ima određena ograničenja te da se njegova osjetljivost i specifičnost povećavaju s težinom parodontitisa (18, 19).

Supklinička kronična sistemska upala (*kronična upala niskoga stupnja*) stanje je koje karakteriziraju povišene razine upalnih biljega (markera) kao što su C-reaktivni protein (CRP), faktor tumorske nekroze α (TNF- α) i različiti interleukini (IL) (20). Smatra se jednim od etiopatogenetskih mehanizama idiopatske neplodnosti (1). Autori nekoliko studija istraživali su ulogu hematoloških parametara kao što su kompletna krvna slika (KKS), broj leukocita (Leu), neutrofil (Neu), omjer neutrofila i limfocita (NLR), omjer trombocita i limfocita (PLR) i srednji volumen trombocita (MPV) u etiopatogenezi neplodnosti nepoznata uzroka i njihov utjecaj na rezultate postupaka fertilizacije *in vitro* (engl. *in vitro fertilization – IVF*) (21 – 23). Čini se da bi parametri kao što su limfociti, PLR i MPV mogli biti važni u predviđanju uspjeha IVF postupaka. Zato su u nekoliko studija s ispitanicima liječenima od idiopatske neplodnosti limfociti istaknuti kao po-

dictive markers for fertilization and PLR as negative markers for implantation rates (22, 23). Platelet-to-lymphocyte ratio (PLR) and neutrophil-to-lymphocyte ratio (NLR) are cheap and reliable biomarkers of inflammation that have been used in many different diagnoses (24, 25).

This study aimed to validate an aMMP-8 point-of-care mouth rinse test in a population of women with idiopathic infertility compared to age-matched fertile women with and without periodontitis. In addition, inflammatory parameters in the sampled blood of infertile and fertile women were compared. It was hypothesized that infertile women would have worse periodontal status and a greater number of positive MMP-8 tests than fertile women, and that they would have increased inflammatory parameters in the blood.

Material and methods

This cross-sectional study was part of a doctoral dissertation study, approved by the Ethics Committee, School of Dentistry, University of Zagreb, Croatia (approval number: 05-PA-15-11/2017.) and institutional review board of "Sestre milosrdnice" University Hospital, Zagreb. The research was registered at the U.S. National Institutes of Health (clinicaltrials.gov) (trial identifier: NCT03542630). Each patient signed informed consent according to the Declaration of Helsinki. The study included 50 healthy norm-ovulatory females aged 25-45 years with strictly defined unexplained infertility attending the reproduction unit of "Sestre milosrdnice" University Hospital, Zagreb, Croatia, and 50 healthy norm-ovulatory women of the same age who conceived and delivered naturally. All subjects attended the Department of Oral Medicine, School of Dental Medicine, University of Zagreb, for clinical periodontal evaluation and aMMP-8 testing between February 2018 and December 2020. The sample size was determined based on the research of Lorenz et al. (26). According to the results of their study which have shown the percentage of positive samples of 8.6% (3/35) in the healthy group and 40% (14/35) in the group with periodontitis, it was calculated that 25 samples per each group of subjects were needed to provide an 81% power. The research plan was delivered in oral and written form, and informed consent was received from each participant before the enrolment. Medical and dental histories were obtained from each participant.

Inclusion criteria for women with unexplained infertility were: 1) age between 25 and 45 years; 2) normal ovulatory function; 3) tubal patency; 4) normal semen analysis of a male partner; 5) AMH (Anti-Müllerian hormone) values between 15 and 48 pmol/l and 6) attempt to conception for the duration of at least one year. Exclusion criteria were specified according to conditions mentioned above and on evidence of autoimmune, metabolic diseases and diabetes since these diseases may increase MMP-8 values. Inclusion criteria for the control group were: 1) age between 25 and 45 years; 2) having conceived and delivered naturally; 3) without evidence of autoimmune, metabolic diseases and diabetes. None of the participants was prescribed or was taking medications such as contraceptives, steroid hormones, insulin-sensitizing

zivni prediktivni biljezi (markeri) za oplodnju i PLR-i kao negativni biljezi za stope implantacije (22, 23). Omjer trombocita i limfocita (PLR) te omjer neutrofila i limfocita (NLR) jeftini su i pouzdani biomarkeri upale koji se koriste u mnogim različitim dijagnozama (24, 25).

Svrha ove studije bila je validirati aMMP-8 test iz ispirka usne šupljine u populaciji žena s idiopatskom neplodnošću u usporedbi s plodnim ženama odgovarajuće dobi s parodontitisom i bez njega. Uz to, uspoređeni su upalni parametri iz krvi neplodnih i plodnih žena. Pretpostavljali smo da će neplodne žene imati lošiji parodontni status i veći broj pozitivnih testova MMP-8 od plodnih žena i povišene upalne parametre u krvi.

Materijal i metode

Ovo presječno istraživanje dio je doktorske disertacije i odobrili su ga Etički odbor Stomatološkog fakulteta Sveučilišta u Zagrebu (broj odobrenja: 05-PA-15-11/2017.) i Etičko povjerenstvo Kliničkoga bolničkoga centra Sestre milosrdnice u Zagrebu. Istraživanje je registrirano u američkome Nacionalnom institutu za zdravlje (clinicaltrials.gov) (identifikator istraživanja: NCT03542630). Svaka pacijentica potpisala je informirani pristanak prema Helsinškoj deklaraciji. Istraživanjem je bilo obuhvaćeno 50 zdravih žena s urednim ovulacijskim ciklusom u dobi od 25 do 45 godina i strogo definiranom neobjašnjivom neplodnošću na Odjelu za reprodukciju KBC-a Sestre milosrdnice i 50 zdravih žena u istoj dobi s urednim ovulacijskim ciklusom koje su zatrudnjele i rodile prirodnim putem. Sve su ispitanice od veljače 2018. godine do prosinca 2020. dolazile u Zavod za oralnu medicinu Stomatološkog fakulteta Sveučilišta u Zagrebu radi kliničke parodontološke evaluacije i testiranja aMMP-8. Veličina uzorka temelji se na radu Lorenza i suradnika (26). Prema rezultatima njihove studije, pozitivan nalaz imalo je 8,6 % (3/35) ispitanica u zdravoj skupini i 40 % (14/35) u skupini s parodontitisom te je izračunato da je za 81 % snage potrebno 25 uzoraka iz svake skupine. Plan istraživanja objašnjen je ispitnicama u usmenom i pisanom obliku, a prije uključivanja svaka je potpisala informirani pristanak. Također je od svih zatražena i dobivena medicinska i stomatološka anamneza.

Kriteriji za uključivanje u studiju o neobjašnjivoj neplodnosti bili su: 1) dob između 25 i 45 godina; 2) uredni ovulacijski ciklusi; 3) uredna prohodnost jajovoda; 4) uredan nalaz spermograma; 5) vrijednost anti-Müllerova hormona (AMH) između 15 i 48 pmol/L; 6) nezaštićeni odnosi dulje od godinu dana bez postizanja trudnoće.

Kriteriji za nesudjelovanje definirani su prema gore navedenim značajkama i prisutnošću metaboličkih i autoimunih bolesti te dijabetesa zato što navedena stanja mogu povišiti razinu MMP-8.

Kriteriji za kontrolnu skupinu su: 1) dob između 25 i 45 godina; 2) u anamnezi uredna spontana trudnoća i porođaj; 3) nepostojanje metaboličkih i autoimunih bolesti te dijabetesa.

Nijedna sudionica studije nije uzimala kontraceptive, steroidne hormone, inzulin-senzitivirajuće lijekove, antibiotike

drugs, antibiotics and anti-inflammatory drugs that could affect the periodontal status within six months before the inclusion in the study.

Upon confirmation of enrolment eligibility in the study, all participants were screened for periodontitis during a clinical examination. Periodontal examination was performed by two mutually calibrated periodontists (L.M. and I.P.) Plaque index (PI, calculated as full-mouth plaque score – FMPS), bleeding on probing (BOP, calculated as full-mouth bleeding score – FMBS), gingival recession (REC) and periodontal probing depth (PPD) were determined at six sites per tooth, excluding third molars. All parameters were determined using a standard periodontal probe (PCP UNC15, Hu-Friedy Chicago, IL, USA). The staging of periodontitis was determined based on the latest 2018 classification of periodontal diseases and conditions (27). Periodontal Inflamed Surface Area (PISA), as an indicator of the systemic inflammatory burden of periodontitis (28, 29), was calculated for all patients using a calculator within an Excel spreadsheet, available on the website www.parsprototo.info for research use. The calculation was done using CAL (clinical attachment loss), REC (gingival recession) and BOP values for each tooth, as previously described (28). A total PISA was calculated through the sum of PISAs of each individual tooth.

Clinical parameters were compared for all infertile and fertile patients with positive and negative aMMP-8 test results to perform test validation. Half of the participants were diagnosed with periodontitis in both groups, while the other half of them were diagnosed as those without periodontitis. A matrix metalloproteinase 8 (MMP-8) point-of-care (PoC) chair-side test (Periosafe, Dentognostics GmbH, Jena, Germany) was done from mouth rinse samples. After they had received specific rinsing instructions as specified by the manufacturer, the mouth rinse from participants was filtered into a test cassette and examined after 5 minutes. One line on the test device indicated that the test was performed successfully, and the result was negative. The result was positive if two lines were observed, indicating an elevated risk for periodontitis. The results were observed as positive irrespective of whether the line was light (low-risk), or dark (high risk) in color (30).

Biochemical findings from infertile and fertile patients, i.e. complete blood count, differential blood count, blood glucose, erythrocyte sedimentation rate, C-reactive protein, fibrinogen and liver tests, were compared to determine the inter-group differences. Infertile women had their findings done due to processing procedures for infertility. For blood findings, the fertile women were referred to a private laboratory, free of charge.

Statistical analysis

Data were collected in MS Excel spreadsheets and statistical analysis was done in Medcalc (v11.4) program. Data were summarized with a mean (\pm standard deviation SD) or median and interquartile range (IQR) depending on the normality of the distribution. The normality of the distribution of the collected data was determined by the Kolmogorov-Smirnov test with or without logarithmic data

i protuupalne lijekove posljednjih šest mjeseci prije uključivanja u studiju, zbog mogućega utjecaja na stanje parodonta.

Nakon što su zadovoljeni svi uvjeti za uključivanje u istraživanje, ispitanice su obavile klinički pregled da bi se ustanovilo postoje li znakovi parodontne bolesti. Parodontološki pregled obavila su dva međusobno kalibrirana parodontologa (L. M. i I. P.). Indeks plaka (engl. *plaque indeks* – PI, izračunat kao iznos plaka na razini cijelih usta), krvarenje pri sondiranju (engl. *bleeding on probing* – BOP izračunato kao iznos krvarenja na razini cijelih usta; engl. *full mouth bleeding score* – FMBS) i klinička dubina sondiranja (engl. *probing depth* – PD) ispitivali su se na šest mjesta na svakom zubu, isključujući treće kutnjake. Svi parametri određivani su standardnom parodontološkom sondom (PCP UNC15, Hu-Friedy Chicago, IL, SAD). Gradacija parodontitisa određena je na temelju najnovije klasifikacije parodontnih bolesti i stanja iz 2018. godine (27). Površina parodonta zahvaćena upalom (engl. *Periodontal Inflamed Surface Area* – PISA), kao indikator sistemskoga upalnoga opterećenja parodontitisa (28, 29), izračunata je za sve pacijentice upotrebom kalkulatora unutar Excel tablice dostupne na mrežnoj stranici www.parsprototo.info za istraživačke svrhe. Izračun je rađen s pomoću vrijednosti kliničkoga gubitka pričvrstka (engl. *clinical attachment loss* – CAL), gingivne recesije (engl. *gingival recession* – REC) i krvarenja pri sondiranju za svaki zub, kao što je već opisano (28). Ukupna PISA izračunata je na temelju zbroja PISA svih zuba.

Klinički parametri uspoređeni su za sve neplodne i plodne pacijentice s pozitivnim i negativnim rezultatom testa aMMP-8 da bi se test validirao. Polovini sudionica u obje grupe dijagnosticiran je parodontitis, a druga polovina nije ga imala. Svima u istraživanju određena je matriksna metaloproteinaza-8 (MMP-8) iz ispirka usne šupljine s pomoću brzoga a-MMP8 *point of care* (PoC) testa na mjestu skrbi (Periosafe, Dentognostics GmbH, Jena, Njemačka). Uzorak testa dobiva se prema preporukama proizvođača jednostavnim metodom filtriranja u za to predviđenoj kaseti. Rezultat se očitava poslije pet minuta. Jedna crtica na testu znači da je test ispravno primijenjen i da je negativan. Dvije crtice pokazuju da je nalaz pozitivan i označavaju povećani rizik od parodontitisa. Test se smatrao pozitivnim i u slučaju kada je druga crtica bila manjeg intenziteta od kontrolne (30).

Biokemijski nalazi neplodnih i plodnih pacijentica, tj. kompletna krvna slika, diferencijalna krvna slika, glukoza u krvi, sedimentacija eritrocita, C reaktivni protein, fibrinogen i jetreni testovi uspoređeni su da bi se odredile razlike među grupama. Neplodne žene imale su te nalaze zbog postupka obrade za neplodnost, a plodne su upućene u privatni laboratorij da ih naprave besplatno.

Statistička analiza

Podatci su prikupljeni u MS Excel tablicama, a statistička analiza obavljena je u Medcalc (v11.4) programu. Podatci su sumirano prikazani sa srednjom vrijednošću (+/- standardna devijacija SD) ili medijanom i interkvartilnim rasponom (IQR), ovisno o normalnosti distribucije. Normalnost distribucije prikupljenih podataka određena je Kolmogorov-Smirnovljevim testom s logaritamskom transformacijom podata-

transformation. Continuous variables of normal distribution were compared between the two groups by a t-test. Continuous abnormal distribution data were compared by the Mann-Whitney test between the two groups. Categorical variables were compared by the Chi-square or Fischer's test and values were summed by number and percentage. Correlations between variables were examined using the Spearman test. The level of significance was set at $p < 0.05$.

Results

The outcomes of the aMMP-8 test were compared with different clinical parameters and diagnoses. The sensitivity and specificity of the test for the detection of periodontitis in the group of infertile patients were 84%, and 72%, in the group of fertile patients 88% and 68% and 86% and 70% in the total patient population (Table 1).

The characteristics of infertile and fertile patients based on periodontal findings are shown in Table 2.

In the infertile patients' group, there were 28 positive tests, while in the group of fertile patients, there were a total of 30 positive tests.

Infertile patients with periodontitis presented with a slightly better periodontal status than fertile patients with periodontitis, i.e. they had less advanced periodontitis than the control group. Although the difference was not statistically significant, they had a smaller PISA score and a smaller number of pockets deeper than 4 and 6 mm.

A positive rapid test for MMP-8 significantly correlated with the amount of full-mouth bleeding score (FMBS), the amount of full-mouth plaque score (FMPS), the area of periodontal inflammation (PISA, periodontal inflamed surface area) and the presence of pockets equal to or deeper than 4 and 6 mm (Spearman's correlation coefficient). The correlation was strongest with FMBS, PISA score, and slightly lower with the presence of pockets ≥ 4 mm, FMPS, and the presence of pockets ≥ 6 mm.

The sensitivity and specificity of the test depending on the periodontitis stage were also calculated. The result is shown in Table 3.

When assessing patients without periodontitis and moderate [stage II] periodontitis, the test's sensitivity was the lowest, - 75%, compared to 91.30% for the advanced [stage III] periodontitis and 100% for initial [stage I] periodontitis.

The results of blood findings are shown in Table 4.

Data analysis of infertile and fertile subjects found a statistically significant difference in the values of hemoglobin, hematocrit, erythrocytes, MPV, neutrophils, leukocytes, lymphocytes, NLR and PLR. Infertile patients had significantly higher values of hemoglobin, hematocrit, erythrocytes, neutrophils, leukocytes, NLR and PLR. Fertile patients had significantly higher lymphocyte counts and mean platelet volume (MPV).

Discussion

Some authors have shown worse periodontal status in infertile patients and suspected that this is a factor that complicates conception (1, 6-8, 9, 10, 12). On the contrary, others

ka ili bez nje. Kontinuirane varijable normalne distribucije usporedene su između dviju grupa t-testom. Kontinuirani podatci nenormalne distribucije između dviju grupa uspoređeni su Mann-Whitneyjevim testom. Kategoričke varijable usporedene su hi-kvadrat ili Fischerovim testom, a vrijednosti sumirane brojem i postotkom. Korelacije među varijablama ispitane su Spearmanovim testa. Za granicu značajnosti uzeta je vrijednost $p < 0,05$.

Rezultati

Ishodi aMMP-8 testa uspoređeni su s različitim kliničkim parametrima i dijagnozama. Osjetljivost i specifičnost testa za detekciju parodontitisa u skupini neplodnih pacijentica iznosila je 84 i 72 %, u skupini plodnih pacijentica 88 i 68 % te 86 te 70 % u ukupnoj populaciji pacijentica (tablica 1.).

Karakteristike neplodnih i plodnih pacijentica na temelju parodontnoga nalaza prikazane su u tablici 2.

U skupini neplodnih pacijentica ukupno je bilo 28 pozitivnih testova, a u skupini plodnih ukupno 30.

Neplodne pacijentice s parodontitisom imale su nešto bolji parodontološki status nego plodne pacijentice s parodontitisom, odnosno imale su manje uznapredovali parodontitis nego kontrolna skupina. Imale su manju površinu parodontne upale (PISA) i manji broj džepova dubljih od 4 i 6 milimetara, iako razlika nije bila statistički značajna.

Pozitivan brzi test na MMP-8 značajno je korelirao s iznosom krvarenja na razini cijelih usta (engl. *full-mouth bleeding score* – FMBS), iznosom plaka na razini cijelih usta (engl. *full-mouth plaque score* – FMPS), površinom parodontne upale (engl., *periodontal inflamed surface area* – PISA) te prisutnošću džepova jednakih ili dubljih od 4 i 6 milimetara (Spearmanov koeficijent korelacije). Korelacija je bila najznačajnija s iznosom krvarenja na razini cijelih usta te površinom parodontne upale, a nešto niža s prisutnošću džepova jednakih ili dubljih od 4 milimetara, iznosom plaka na razini cijelih usta te džepovima jednakih ili dubljih od 6 milimetara.

Izračunate su osjetljivost i specifičnost testa MMP-8, ovisno o stupnju parodontitisa. Rezultati se nalaze u tablici 3.

Pri procjeni osoba bez parodontitisa i umjerenoga stupnja parodontitisa, osjetljivost testa pokazala se najnižom (75 % u usporedbi s 91,30 % za uznapredovali parodontitis te 100 % za početni parodontitis).

Rezultati krvnih nalaza prikazani su u tablici 4.

Analizom podataka neplodnih i plodnih pacijentica utvrđena je statistički značajna razlika u vrijednostima hemoglobina, hematokrita, eritrocita, MPV-a, neutrofila, leukocita, limfocita, NLR-a i PLR-a. Neplodne pacijentice imale su znatno više vrijednosti hemoglobina, hematokrita, eritrocita, neutrofila, leukocita, NLR-a i PLR-a. Plodne su imale znatno više limfocita i srednjeg volumena trombocita (MPV).

Rasprava

Pojedini autori istaknuli su lošiji parodontni status neplodnih pacijentica te posumnjali da je to čimbenik koji otežava začeće (1, 6 – 8, 9, 10, 12). Suprotno tomu, drugi su

Table 1 Sensitivity and specificity of MMP8 test in the group of infertile and fertile patients in relation to the presence of periodontal pockets ≥ 4 mm and ≥ 6 mm, bleeding index at the level of the mouth $\geq 15\%$, periodontal disease (gingivitis or periodontitis) and periodontitis.**Tablica 1.** Osjetljivost i specifičnost MMP-8 testa u grupi neplodnih i plodnih pacijentica u odnosu prema prisutnosti parodontnih džepova ≥ 4 mm i ≥ 6 mm, indeksu krvarenja na razini usta $\geq 15\%$, parodontnoj bolesti (gingivitis i parodontitis) i parodontitisu

Periodontal finding • Parodontni nalaz		Infertile • Neplodne (N=50)	Fertile • Plodne (N=50)	Total • Ukupno (N=100)
Periodontal pocket ≥ 4 mm • Parodontni džep ≥ 4 mm	Sensitivity • Osjetljivost Specificity • Specifičnost	65 % 100 %	61.4 % 83.3 %	63.3 % 90 %
Periodontal pocket ≥ 6 mm • Parodontni džep ≥ 6 mm	Sensitivity • Osjetljivost Specificity • Specifičnost	85.5 % 55.6 %	85 % 56.7 %	85.3 % 56 %
FMBS $\geq 15\%$	Sensitivity • Osjetljivost Specificity • Specifičnost	62.2 % 100 %	65.1 % 71.4 %	53.6 % 83.3%
Periodontal disease • Parodontna bolest	Sensitivity • Osjetljivost Specificity • Specifičnost	74.3 % 86.7%	87.1 % 84.2%	80.3 % 85.3%
Periodontitis • Parodontitis	Sensitivity • Osjetljivost Specificity • Specifičnost	84% 72%	88% 68%	86% 70%

Table 2 Characteristics of infertile and fertile patients based on periodontal findings.**Tablica 2.** Karakteristike neplodnih i plodnih pacijentica prema parodontnim nalazima

	Infertile • Neplodne (N=50) Mean value \pm SD / median (IQR) • Srednja vrijednost \pm SD / medijan (IQR)	Fertile • Plodne (N=50) Mean value \pm SD / median (IQR) • Srednja vrijednost \pm SD / medijan (IQR)	P-value • P-vrijednost
Age • Dob	36.1 \pm 3.7	37 \pm 2.9	*ns
Number of teeth • Broj zuba	27 (26-28)	27 (25-28)	*ns
FMBS	38.9 \pm 19.7	37.9 \pm 19.2	*ns
FMPS	41.6 \pm 21.5	36 \pm 19	*ns
PISA	645.0 \pm 368,0	700.9 \pm 420.3	*ns
Number of pockets ≥ 4 mm • Broj džepova ≥ 4 mm	16 (3-29)	22 (6-38)	*ns
Number of pockets ≥ 6 mm • Broj džepova ≥ 6 mm	0 (0-1)	0 (0-6)	*ns
Positive MMP8 • Pozitivan MMP-8	28 (56%)	30 (60%)	*ns
Periodontal diagnosis • Parodontna dijagnoza			*ns
Without periodontitis • Bez parodontitisa	25 (50%)	25 (50%)	
Periodontitis stage I • I. stupanj parodontitisa	7 (14%)	0 (0%)	
Periodontitis stage II • II. stupanj parodontitisa	10 (20%)	10 (20%)	
Periodontitis stage III • III. stupanj parodontitisa	8 (16%)	15 (30%)	

*ns- non significant • nije značajno

Table 3 Sensitivity and specificity of MMP8 test in distinguishing subjects without periodontitis and with different stages of periodontitis.**Tablica 3.** Osjetljivost i specifičnost MMP-8 testa u razlikovanju osoba bez parodontitisa i s različitim stadijima parodontitisa

Diagnosis • Dijagnoza	Positive aMMP-8 test • Pozitivan aMMP-8 test	Negative aMMP-8 test • Negativan aMMP-8 test	Total • Ukupno
Periodontitis stage I • I. stupanj parodontitisa	7	0	7
Without periodontitis (N=50) • Bez parodontitisa (N=50)	15	35	50
		95% CI (confidence interval • interval pouzdanosti)	
Test sensitivity • Osjetljivost testa	100.00%	59.04% - 100.00	
Test specificity • Specifičnost testa	70.00%	55.39% - 82.14%	
Periodontitis stage II • II. stupanj parodontitisa	15	5	20
Without periodontitis (N=50) • Bez parodontitisa (N=50)	15	35	50
		95% CI (confidence interval • interval pouzdanosti)	
Test sensitivity • Osjetljivost testa	75.00%	50.90% - 91.34%	
Test specificity • Specifičnost testa	70.00%	55.39% - 82.14%	
Periodontitis stage III • III. stupanj parodontitisa	21	2	23
Without periodontitis (N=50) • Bez parodontitisa (N=50)	15	35	50
		95% CI (confidence interval • interval pouzdanosti)	
Test sensitivity • Osjetljivost testa	91.30%	71.96% - 98.93%	
Test specificity • Specifičnost testa	70.00%	55.39% - 82.14%	

Table 4 Biochemical and clinical parameters of infertile and fertile patients.
Tablica 4. Biokemijski i klinički parametri neplodnih i plodnih pacijentica

	Infertile • Neplodne (N=50) Mean value ± SD /median (IQR) • Srednja vrijednost ± SD /medijan (IQR)	Fertile • Plodne (N=50) Mean value ±SD /median (IQR) • Srednja vrijednost ±SD /medijan (IQR)	P-value • P-vrijednost
Age • Dob	36.1 ± 3.7	37 ± 2.9	ns
Body mass index • Indeks tjelesne mase	21.9 (21-23.5)	22 (20.8-23)	ns
Blood glucose • Šećer u krvi	5.3 ± 0,4	5.3 ± 0,5	ns
Hemoglobin • Hemoglobin	135.4 ± 7	130.6 ± 6,7	0.0007 (t-test)
Hematocrite • Hematokrit	0.409 ± 0	0.397 ± 0	0.0079 (t-test)
Erythrocytes • Eritrociti	4.5 ± 0.3	4.4 ± 0.2	0.0285 (t-test)
MCH (mean corpuscular hemoglobin • prosječni hemoglobin u eritrocitu)	29.9 (29.4-30.7)	29.7 (28.4-30.4)	ns
MCHC (mean corpuscular hemoglobin concentration • prosječna koncentracija hemoglobina u eritrocitu)	333 ± 9.9	329.4 ± 11,5	ns
MCV (mean corpuscular volume • prosječni volume eritrocita)	90.2 ± 4.5	90 ± 3.6	ns
RDW (Red blood cell Distribution Width • raspodjela eritrocita po obujmu)	12.7 (12.4-13)	13 (12.4-13.2)	ns
Thrombocytes • Trombociti	251.4 ± 46.2	246.9 ± 45	ns
MPV (mean platelet volume • srednji volumen trombocita)	8 (7.5-8.5)	8.4 (8-9,1)	0.0155 (Mann-Whitney)
Monocytes • Monociti	5.7 ± 1.3	6.3 ± 1.5	ns
Neutrophils • Neutrofilii	58.2 ± 8.3	55.1 ± 6.5	0.0439 (t-test)
Leukocytes • Leukociti	7.1 ± 1.8	6 ± 1.2	0.0003 (t-test)
Lymphocytes • Limfociti	31.5 ± 7.2	34.2 ± 6.5	0.0478 (t-test)
Bazophils • Bazofili	1 (1-1)	1 (0.5-1)	ns
Eosinophils • Eozinofili	2.8 ± 1.6	2.9 ± 1.5	ns
NLR (neutrophil-to-lymphocyte ratio • omjer neutrofila i limfocita)	1.815 (1.500 – 2.370)	1.58 (1.370 – 1.800)	0.0121 (Mann-Whitney)
PLR (platelet-to-lymphocyte ratio • omjer trombocita i limfocita)	8.68 (7.140 – 9.830)	7.07 (6.110 – 7.700)	0.0088 (Mann-Whitney)
Alanine aminotransferase • Alanin aminotransferaza	23 (18-29)	21 (18-24)	ns
Aspartate aminotransferase • Aspartat aminotransferaza	17 (15,8-20)	17 (15-20)	ns
Fibrinogen	2.4 (2.2-2.9)	2.3 (2.2-2.4)	ns
Erythrocyte sedimentation rate • Sedimentacija eritrocita	6.4 ± 3.9	6.8 ± 4.2	ns
C reactive protein • C reaktivni protein	1.9 ± 1.3	2.7 ± 3.9	ns

have reported that the periodontal status of patients has not affected the outcome of the IVF procedure (31). Early diagnosis of periodontitis in the group of infertile patients would enable treatment on time, leading to the elimination of inflammatory stimuli. The papers published after the adoption of the new classification of periodontitis in 2018 (27) considered the rapid test for aMMP-8 from oral mouth rinse, as used in present research, a key marker for early diagnosis and monitoring disease activity consistent with the new classification (32, 33).

Our results have shown that sensitivity and specificity of the test for detection of periodontitis in the group of infertile patients was 84%, and 72%, in the group of fertile patients 88% and 68% and 86% and 70% in the total patient population. Rautava et al. (34) showed lower sensitivity and specificity of the test in patients diagnosed with Crohn's disease, compared with the control group (Crohn's disease group sensitivity 60%, specificity 75%; control group sensitivity 90%,

naveli da parodontni status nije utjecao na ishode IVF postupaka (31). Rana dijagnoza parodontitisa, kad je riječ o neplodnim pacijenticama, omogućila bi pravodobnu terapiju i eliminaciju upalnih žarišta. U radovima objavljenima nakon prihvaćanja nove klasifikacije parodontitisa 2018. godine (27) predlaže se brzi test za aMMP8 iz ispirka usne šupljine, kakav je korišten i u ovom istraživanju, kao ključni biljeg za ranu dijagnozu i praćenje aktivnosti bolesti koja je u skladu s novom klasifikacijom (32, 33).

Prema našim rezultatima, osjetljivost i specifičnost testa za detekciju parodontitisa u skupini neplodnih pacijentica iznosila je 84 i 72 %, u skupini plodnih pacijentica 88 i 68 % te 86 i 70 % u ukupnoj populaciji pacijentica. Rautava i suradnici (34) zabilježili su nižu osjetljivost i specifičnost testa u pacijentica s dijagnozom Crohnove bolesti, u usporedbi s kontrolnom skupinom (skupina s Crohnovom bolešću: osjetljivost 60 %, specifičnost 75 %; kontrolna skupina osjetljivost 90 %, specifičnost 80 %). Zaključili su da dijagnostič-

specificity 80%). They concluded that the diagnostic reliability of the test might be compromised in individuals with additional inflammatory conditions due to similar mechanisms underlying disease development. This study is the only published article that has compared the diagnostic reliability of the test in patients with other inflammatory conditions and / or impaired immune responses (34).

The results of Heikkinen et al. (35) showed slightly lower test sensitivity than ours, 76.5% in more than two sites with deep periodontal pockets while specificity was higher than ours, 96.7%. On the contrary, Deng et al. (18) have shown much lower sensitivity while specificity was similar to ours, 67.1% and 68.8%. They concluded that when performing the test, the number of teeth in the mouth should be considered because with a smaller number of teeth, the concentration of aMMP-8 decreases and the reliability of the test decreases (18 stud). In the present study, patients with a minimum of 20 teeth were included. A significant correlation of the rapid aMMP-8 test with FMBS, FMPS, PISA and the presence of pockets equal to or deeper than 4 and 6 mm was observed, which is consistent with the literature (30).

Regarding the test's sensitivity in relation to the staging of periodontitis, it would be expected that the sensitivity of the test increases with the increased stage of periodontitis, as shown by previous authors (18, 30), however, some of our results deviate from this assumption. Namely, our results showed a test sensitivity of 100% for the detection of mild (stage I) periodontitis, 75% for the detection of moderate (stage II) periodontitis and 91.30% for the detection of severe/advanced (stage III) periodontitis. The explanation probably lies in the small number of patients with mild periodontitis (seven). The results of our study coincide with the published results of Izadi-Borujeni et al. (36), who in a study of 60 patients showed a test sensitivity of 87% and a test specificity of 60% in the detection of chronic periodontitis. The same authors calculated the sensitivity and specificity of the test in relation to the severity of periodontitis, and in the case of generalized moderate periodontitis, the sensitivity was 80% and specificity 60%, while in the case of generalized advanced periodontitis, the sensitivity of the test was 93%, with the same specificity. This is consistent with our results showing a test sensitivity of 91.3% and a specificity of 70% when detecting severe periodontitis.

Our research hypothesis was that in patients with idiopathic infertility, we would have a greater number of positive tests, which will be a consequence of a) worse periodontal condition and / or b) increased inflammatory response in the body. Based on the obtained results, this hypothesis has been rejected because it was shown that patients with idiopathic infertility had milder staging of periodontitis than the control group. Thus we can explain a smaller number of positive tests. This is inconsistent with published studies that have shown poorer periodontal status in patients with idiopathic infertility (1, 8, 10, 12). A possible explanation might be that infertile women included in our study were more aware of their oral health, or that they had different economic or educational background which might also affect their oral health, which was not considered in this study. The length of

ka pouzdanost testa može biti kompromitirana u osoba s dodatnim upalnim stanjima zbog sličnih mehanizama u podlozi razvoja bolesti. To je i jedini članak koji je objavljen, a uspoređivao je dijagnostičku pouzdanost testa kod pacijentica s prisutnim drugim upalnim stanjem i/ili narušenim imunskim odgovorom (34).

Rezultati Heikkinena i suradnika (35) pokazali su nešto nižu osjetljivost testa od naše – 76,5 % u slučaju više od dva-ju mjesta s dubokim parodontnim džepovima, a specifičnost je bila viša od naše – 96,7 %. Deng i suradnici (18) uočili su, pak, znatno nižu osjetljivost, a specifičnost je bila slična našoj – 67,1 i 68,8 %. Zaključili su da pri primjeni testa treba voditi računa o broju zuba u ustima jer se s manjim brojem zuba smanjuje koncentracija aMMP-8 pa tako i pouzdanost testa (18). U ovo istraživanje bile su uključene pacijentice s minimalno 20 zuba. Uočena je značajna korelacija brzoga testa aMMP-8 s FMBS-om, FMPS-om, PISA-om i prisutnošću džepova jednakih ili dubljih od 4 i 6 milimetara, što je u skladu s literaturom (30).

Kad je riječ o osjetljivosti testa u odnosu na stupanj parodontitisa, očekivano bi bilo da njegova osjetljivost raste s porastom stupnja parodontitisa, kako pokazuju prethodni autori (18, 30), no dio naših rezultata odstupa od te pretpostavke. Naime, naši rezultati pokazali su osjetljivost testa od 100 % za detekciju blagoga stupnja parodontitisa, 75 % za detekciju umjerenoga stupnja i 91,30 % pri detekciji teškoga stupnja parodontitisa. Razlog za takve rezultate vjerojatno je u malom broju pacijentica s umjerenim stupnjem parodontitisa (sedam). Rezultati našeg istraživanja podudaraju se s objavljenim rezultatima Izadi-Borujenija i suradnika (36) koji su u istraživanju na 60 pacijenata pokazali osjetljivost testa od 87 %, te njegovu specifičnost od 60 % pri detekciji kroničnoga parodontitisa. Isti autori izračunali su i osjetljivost i specifičnost testa u odnosu prema opsegu parodontitisa te je u slučaju generaliziranoga umjerenoga parodontitisa osjetljivost iznosila 80 %, a specifičnost 60 %, a u slučaju generaliziranoga uznapredovaloga parodontitisa osjetljivost testa iznosila je 93 %, uz istu specifičnost. To je u skladu s našim rezultatima koji su pokazali osjetljivost testa od 91,3 % i specifičnost od 70 % pri detekciji uznapredovaloga parodontitisa.

Hipoteza našeg istraživanja bila je da ćemo kod pacijentica s idiopatskom neplodnošću imati veći broj pozitivnih testova što će biti posljedica:

- a) lošijega parodontnoga stanja i/ili
- b) pojačanoga upalnoga odgovora u organizmu.

Na temelju naših rezultata moramo odbaciti tu hipotezu jer su rezultati pokazali da su pacijentice s idiopatskom neplodnošću imale blaži stupanj parodontitisa nego kontrolna skupina, te time možemo objasniti manji broj pozitivnih testova. To je proturječno objavljenim istraživanjima koja su pokazala lošiji parodontni status pacijentica s idiopatskom neplodnošću (1, 8, 10, 12). Moguće objašnjenje za to može biti da su neplodne žene uključene u naše istraživanje bile svjesnije svojega oralnoga zdravlja ili da su imale različitu ekonomsku ili obrazovnu pozadinu, što bi također moglo utjecati na njihovo oralno zdravlje, no to nije uzeto u obzir u ovoj studiji. Ograničavajući čimbenik je i trajanje istraživanja

the study is also a limiting factor and it is possible that their oral status was different in the time of establishing the diagnosis of infertility.

In the present study, we have observed a few significant differences between infertile and fertile women regarding the blood findings. Infertile patients had significantly higher values of hemoglobin, hematocrit, erythrocytes, neutrophils, platelets and leukocytes. A possible explanation might be that infertile women frequently take multivitamin preparations, increasing their number of erythrocytes, hematocrit, and hemoglobin. Rudnicka et al. (37) have showed elevated leukocyte counts and CRP in patients with PCOS and concluded that the main predictive factors for elevated CRP are BMI (Body Mass Index) and IR (insulin resistance). Our results, which have shown only significantly higher leukocytes in infertile patients, but without a significant difference in CRP, are consistent with the literature (37) because all of our patients had a normal range of BMI, and significant difference in CRP between the study and control group was not observed. Fertile patients had significantly higher mean platelet volume (MPV) and lymphocyte counts. A study of Cakiroglu et al. (21) proved that MPV values negatively correlated with clinical pregnancy. This is in contrast with our results which have shown higher MPV values in fertile women. We might speculate that their MPV values had been lower before pregnancy. The results from the literature show that lymphocytes are positive predictive markers for fertilization rate (22, 23), which is in accordance with present results of significantly increased lymphocytes in fertile patients.

The present results have shown that PLR and NLR were significantly increased in infertile patients. In previous studies, NLR and PLR were found to be biomarkers of different inflammatory obstetric conditions, with conflicting results. As reported in the literature, NLR and PLR are increased in women with endometriosis (38–40) and increased NLR and estradiol-progesterone ratio can be used to predict the development of ovarian hyperstimulation syndrome in patients undergoing controlled ovarian hyperstimulation during *in vitro* fertilization cycles (41). A systematic review has shown that NLR values could be a useful biomarker for predicting preterm delivery (42). On the contrary, Yldrm et al. (43) have demonstrated lower NLR values in patients diagnosed with premature ovarian failure (POI). NLR appears to be a promising marker for POI, one of the possible unrecognized causes of idiopathic infertility, even in the early stages while there is no clinical manifestation of premature menopause yet, and thus can direct clinicians to timely therapy (43). PLR, which was significantly increased in infertile women in our study, according to the literature, seems to be a promising marker for predicting implantation failure (21).

Our research has some limitations. When including patients in the control group, we did not have a predetermined deadline when the pregnancy occurred, and it is possible that the periodontal status of our patients during the examination was different from the status before the pregnancy.

te je moguće da je njihov oralni status bio različit u vrijeme postavljanja dijagnoze neplodnosti.

Naši rezultati pokazali su nekoliko značajnih razlika u krvnim nalazima neplodnih i plodnih žena. Neplodne su imale znatno više vrijednosti hemoglobina, hematokrita, eritrocita, neutrofila, trombocita i leukocita. Moguće objašnjenje za to jest da neplodne žene često uzimaju multivitaminske preparate koji povećavaju vrijednosti eritrocita, hemoglobina i hematokrita. Rudnicka i suradnici (37) zabilježili su povišeni broj leukocita i CRP-a u pacijentica s PCOS-om i zaključili da su glavni prediktivni čimbenici za povišeni CRP indeks tjelesne mase (BMI) i inzulinska rezistencija (IR). Naši rezultati, koji pokazuju samo znatno više leukocita u neplodnih pacijentica ali bez značajne razlike u CRP-u, u skladu su s literaturom (37) jer su sve naše ispitanice imale i normalan raspon BMI-ja te značajna razlika u CRP-u između studije i kontrolne skupine nije opažena. Plodne pacijentice imale su znatno povišen broj limfocita i srednji volumen trombocita (MPV). Cakiroglu i suradnici (21), u svojem istraživanju s neplodnim pacijenticama sa sindromom policističnih jajnika, pokazali su u postupku IVF-a da vrijednosti MPV-a negativno koreliraju s kliničkom trudnoćom. To je suprotno našim rezultatima koji su pokazali više vrijednosti MPV-a u plodnih pacijentica. Mogli bismo nagađati da su njihove vrijednosti MPV-a bile niže prije trudnoće. Plodne pacijentice imale su značajno povišene limfocite, što je u skladu s objavljenim rezultatima prema kojima su limfociti pozitivan prediktivni biljeg za stopu fertilizacije (22, 23).

Naši rezultati pokazali su da su PLR i NLR značajno povišeni u neplodnih pacijentica. Ranija istraživanja pokazala su da su PLR i NLR biomarkeri u različitim upalnim ginekološkim stanjima, s proturječnim rezultatima. Kao što je objavljeno u literaturi, NLR i PLR povećani su u žena s endometriozom (38 – 40), a povećani omjer NLR-a te estradiola i progesterona može se koristiti za predviđanje pojave sindroma hiperstimulacije jajnika u pacijentica podvrgnutih kontroliranoj hiperstimulaciji jajnika tijekom ciklusa oplodnje *in vitro* (41). Sustavni pregled pokazao je da se vrijednosti NLR-a mogu koristiti kao biomarker za predviđanje prijevremenog porođaja (42). Suprotno tomu, Yldrm i suradnici (43) pronašli su niže vrijednosti NLR-a u pacijentica s prijevremenim zatajenjem jajnika. Čini se da je NLR obećavajući biljeg za prijevremeno zatajenje jajnika, jedan od mogućih neprepoznatih uzroka idiopatske neplodnosti, čak i u ranim fazama kada još nema kliničke manifestacije menopauze te zato može usmjeriti kliničare na pravodobnu terapiju (43). PLR, koji je značajno povišen u neplodnih žena u našem istraživanju, prema literaturi se čini kao obećavajući biljeg za predviđanje neuspjeha implantacije (21).

Naše istraživanje ima neka ograničenja. Pri uključivanju pacijentica u kontrolnu skupinu nismo imali unaprijed definirani rok kada je ostvarena trudnoća te je moguće da je njihov parodontni status tijekom pregleda bio drukčiji od statusa u razdoblju ostvarivanja trudnoće.

Conclusion

Our results have shown that infertile patients had better periodontal status and less advanced periodontitis than fertile women of the same age, although they had significantly increased inflammatory blood parameters. These results do not support the assumption that periodontitis-induced inflammatory response may have a role in the etiology of idiopathic infertility. When interpreting the results of aMMP-8 tests for diagnosis of periodontitis, one should consider the periodontal status of the examined population (sensitivity and specificity of the test increase with the staging of periodontitis).

Conflict of interest

None declared.

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Zaključak

Naši rezultati pokazali su da su neplodne pacijentice imale bolji parodontni status i manje uznapredovali parodontitis u usporedbi s kontrolnom skupinom plodnih žena iste dobi, iako su imale značajno povišene krvne upalne parametre. Ti rezultati ne podupiru pretpostavku da bi parodontitisom prozročeni upalni odgovor mogao imati ulogu u etiologiji idiopatske neplodnosti. Pri interpretaciji rezultata testa aMMP-8 za dijagnostiku parodontitisa treba imati na umu parodontni status ispitivane populacije (osjetljivost i specifičnost testa rastu sa stupnjem uznapredovalosti parodontitisa).

Sukob interesa

Autori nisu bili u sukobu interesa.

Doprinos autora: L. Š. – primarni klinički istraživač, prikupljanje podataka, koncept i dizajn, interpretacija, vizualizacija, pisanje, odobrenje članka za objavu; L. M. – klinički istraživač; pregled i uređivanje; I. P. – klinički istraživač; pregled i uređivanje; J. E. – formalna analiza, statistička analiza, interpretacija; I. J. – prikupljanje podataka, vizualizacija, pisanje; K. K. – nadzor, kritički osvrt i uređivanje, odobrenje članka za objavu; B. L. B. – voditelj kliničkoga istraživanja, koncept i dizajn, interpretacija, pisanje, kritički pregled i uređivanje

Sažetak

Uvod: Literaturni podatci upućuju na to da neplodne pacijentice imaju lošiji parodontni status, što može biti uzrok komplikacijama tijekom začeća. Cilj ove studije bio je validacija aMMP-8 *point-of-care* testa iz ispirka usne šupljine u populaciji žena s neobjašnjivom neplodnošću u usporedbi s plodnim ženama odgovarajuće dobi s parodontitisom i bez njega. Nadalje, analizirani su upalni parametri uzorkovane krvi i uspoređeni između dviju skupina. Pretpostavljali smo da će neplodne žene imati lošiji parodontni status i veći broj pozitivnih testova aMMP-8 u usporedbi s plodnima te da će im biti povišeni upalni parametri iz krvi. **Materijal i metode:** U istraživanje je bilo uključeno 50 zdravih žena s urednim ovulacijskim ciklusima u dobi od 25 do 45 godina i strogo definiranom neobjašnjivom (idiopatskom) neplodnošću i 50 zdravih žena iste dobi s urednim ovulacijskim ciklusima koje su zatrudnjele i rodile prirodnim putem. **Rezultati:** Osjetljivost i specifičnost testa za otkrivanje parodontitisa iznosila je 84 i 72 % u skupini neplodnih pacijentica, 88 i 68 % u skupini plodnih te 86 i 70 % u ukupnoj populaciji pacijentica. Neplodne pacijentice s parodontitisom imale su manje uznapredovali parodontitis od kontrolne skupine, iako ta razlika statistički nije bila značajna. Upalni parametri u krvi bili su značajno viši u neplodnih žena nego u plodnih. **Zaključak:** Ovo istraživanje pokazalo je da su neplodne žene imale bolji parodontni status i manje uznapredovali parodontitis od plodnih žena iste dobi. Dakle, pri tumačenju rezultata dobivenih testovima aMMP-8 za dijagnostiku parodontitisa treba imati na umu parodontni status ispitivane populacije.

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