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 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.

Received: February 12, 2022
Accepted: April 21, 2022
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MeSH terms: Periodontitis; Female Infertility; Matrix Metalloproteinase 8; Inflammation

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 pre eclampsia 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 potrebne daljnja istraživanja. Literaturni podaci također povezuju parodontitis s kasnijim začećem i problemima s muškom i ženskom plodnošću (1, 6, 7). Neki su autori izvijestili o lošijem parodontnom statusu neplodnih žena dobivena testom aMMP-8 i analiza nalaza krvi.
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 (IFMA) 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 Nwahor 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 predictors of IVF success and posnjih pacijenata i posumnjali da je to čimbenik koji otežava začeće (5, 8 – 11).

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

Citokini i proteolitički enzimi izazvani periodontnom infekcijom unističavaju tkivo gingive i uzrok su traje ti sistematske upale. To se odražava aktivacijom matriksne metaproteinaze-8 (MMP-8), enzima sline odgovornoga za unističavanje periodontnih kolagenih vlakana i izravno povezanoga s periodontnom upalom (14). Klinički periodontološki dijagnostički postupak može biti dugotrajnij tj. uključuje sveobuhvatna klinički periodontološki dijagram s mjerenjem različitih indikera 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 periodontno zdravi od periodontno 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 (IFMA) i Dento ELISA (enzimski imunosorbentni test). Svi ti testovi selektivno identificiraju aktivirani oblik MMP-8 (aMMP-8) kod variranog istoga aMMP-8 antijela i uzajamno dobro koreliraju. Istraživanje Nwhatora i suradnika (7) pokazalo je značajnu povezanost između loše oralne higijene i/ili periodontitisa i subnormalnoga broja spermija. Nadalje, pokazali su da je korističenje MMP-8 PoC testa na mjestu skrbi o pacijentici prevladalo nedostatke CPITN indeksa (engl. Community Periodontal Index of Treatment Needs) s opaženom 96-postotnom osjetljivostišću za lošu oralnu higijenu, 95-postotnom osjetljivostišću za kronični periodontitis i 82,6-postotnom osjetljivostišću za kravjenje pri sondiranju. Međutim, ti su brojevi bili niži u pacijentica s boljim oralnom higijenom ili periodontnim 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 periodontitisa (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 nekrozite (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 krivna slika (KKS), broj leukocita (Leu), neutrofili (Neu), omjer neutrofila i limfocita (NLR), omjer trombocita i limfocita (PLR) i srednji volumen trombocita (MPV) u etiopatogenesne neplodnosti nepoznate uzroka i njihov utjecaj na rezultate postupaka fertilitacije 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 uspeha IVF postupaka. Zato su u nekoliko studija s ispitnicama liječenima od idiopatske neplodnosti limfociti istaknuti kao po-
ditive 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 milosrdnica” 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 milosrdnica” 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 48pmol/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

Material i metode

Ovo presjечно 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 Kliničkog 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 ovulačijom ciklusom u dobi od 25 do 45 godina i strogog definiranog neobjašnjivom neplodnošću na Odjelu za reprodukciju KBC-a Sestre milosrdnice i 50 zdravih žena u istoj dobi s urednim ovulačijom ciklusom koje su zatrudnjuje i rodile prirodnim putom. 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 imao je 8,6 % (3/35) ispitnica u zdravoj skupini i 40 % (14/35) u skupini s parodontitom te je izračunato da je za 81 % snage potrebno 25 uzoraka iz svake skupine. Plan istraživanja objašnjeno 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 anamnezna.

Kriteriji za uključivanje u studiju o neobjašnjivoj neplodnosti bili su: 1) dob između 25 i 45 godina; 2) uredni ovulačiji ciklus; 3) uredna prohodnost jajovoda; 4) uredan način životnja ili načina života; 5) norma hormona plodnosti. Kriteriji za uključivanje u kontrolnu skupinu bili su: 1) dob između 25 i 45 godina; 2) norma hormona plodnosti; 3) norma hormona plodnosti; 4) norma hormona plodnosti; 5) norma hormona plodnosti; 6) norma hormona plodnosti; 7) norma hormona plodnosti; 8) norma hormona plodnosti; 9) norma hormona plodnosti; 10) norma hormona plodnosti; 11) norma hormona plodnosti; 12) norma hormona plodnosti; 13) norma hormona plodnosti; 14) norma hormona plodnosti; 15) norma hormona plodnosti; 16) norma hormona plodnosti; 17) norma hormona plodnosti; 18) norma hormona plodnosti; 19) norma hormona plodnosti; 20) norma hormona plodnosti; 21) norma hormona plodnosti; 22) norma hormona plodnosti; 23) norma hormona plodnosti; 24) norma hormona plodnosti; 25) norma hormona plodnosti; 26) norma hormona plodnosti; 27) norma hormona plodnosti; 28) norma hormona plodnosti; 29) norma hormona plodnosti; 30) norma hormona plodnosti; 31) norma hormona plodnosti; 32) norma hormona plodnosti; 33) norma hormona plodnosti; 34) norma hormona plodnosti; 35) norma hormona plodnosti; 36) norma hormona plodnosti; 37) norma hormona plodnosti; 38) norma hormona plodnosti; 39) norma hormona plodnosti; 40) norma hormona plodnosti; 41) norma hormona plodnosti; 42) norma hormona plodnosti; 43) norma hormona plodnosti; 44) norma hormona plodnosti; 45) norma hormona plodnosti; 46) norma hormona plodnosti; 47) norma hormona plodnosti; 48) norma hormona plodnosti; 49) norma hormona plodnosti; 50) norma hormona plodnosti; 51) norma hormona plodnosti; 52) norma hormona plodnosti; 53) norma hormona plodnosti; 54) norma hormona plodnosti; 55) norma hormona plodnosti; 56) norma hormona plodnosti; 57) norma hormona plodnosti; 58) norma hormona plodnosti; 59) norma hormona plodnosti; 60) norma hormona plodnosti; 61) norma hormona plodnosti; 62) norma hormona plodnosti; 63) norma hormona plodnosti; 64) norma hormona plodnosti; 65) norma hormona plodnosti; 66) norma hormona plodnosti; 67) norma hormona plodnosti; 68) norma hormona plodnosti; 69) norma hormona plodnosti; 70) norma hormona plodnosti; 71) norma hormona plodnosti; 72) norma hormona plodnosti; 73) norma hormona plodnosti; 74) norma hormona plodnosti; 75) norma hormona plodnosti; 76) norma hormona plodnosti; 77) norma hormona plodnosti; 78) norma hormona plodnosti; 79) norma hormona plodnosti; 80) norma hormona plodnosti; 81) norma hormona plodnosti; 82) norma hormona plodnosti; 83) norma hormona plodnosti; 84) norma hormona plodnosti; 85) norma hormona plodnosti; 86) norma hormona plodnosti; 87) norma hormona plodnosti; 88) norma hormona plodnosti; 89) norma hormona plodnosti; 90) norma hormona plodnosti; 91) norma hormona plodnosti; 92) norma hormona plodnosti; 93) norma hormona plodnosti; 94) norma hormona plodnosti; 95) norma hormona plodnosti; 96) norma hormona plodnosti; 97) norma hormona plodnosti; 98) norma hormona plodnosti; 99) norma hormona plodnosti; 100) norma hormona plodnosti.
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 Inflammed 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.parsproto.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 (± 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 and tukey’s test for normality. Data were normalized using the logarithmic transformation.

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Istakači 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 jednostavnom metodom filtriranja u za to predviđenom kaseti. Rezultat se očitava poslije pet minuta. Jedna crtica na testu znači da je test ispravno primijenjen i da je negativan. Dvije crticte 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-Smirnovijevim testom s logaritamskom transformacijom podata-
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 uspoređene su između dviju grupa t-testom. Kontinuirani podatci nenormalne distribucije između dviju grupa uspoředeni su Mann-Whitneyjevim testom. Kategoričke varijable uspoređene su hi-kvadrat ili Fisherovim testom, a vrijednosti sumirane brojem i postotkom. Korelacije među varijablama ispitane su Spearmanovim testom. 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 iznosi 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 parodontitosom 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 brži test na MMP-8 značajno je korelirao s iznosom krvenja 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 (Spearman koečijent korelacije). Korelacija je bila najjača s iznosom krvenja 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.

Pри процени осoba без парodontитиса и умереногa степена parodontitisa, osjetljivost testa pokazala se najnižom (75 % u usporedbi s 91,30 % za uznapredovali parodontitis 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 hemoglobin-a, hematokrit, eritrocita, MPV-a, neutrofila, leukocita, limfocita, NLR-a i PLR-a. Neplodne pacijentice imale su znatno više vrijednosti hemoglobin-a, hematokrit, 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 ≥4mm and ≥6mm, bleeding index at the level of the mouth ≥15%, periodontal disease (gingivitis or periodontitis) and periodontitis.

| 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 65 % 100 % | 61.4 % 83.3 % | 63.3 % 90 % |
| Periodontal pocket ≥6 mm • Parodontni džep ≥ 6 mm | Sensitivity • Osjetljivost 85.5 % 55.6 % | 85 % 56.7 % | 85.3 % 56 % |
| FMBS ≥ 15% | Sensitivity • Osjetljivost 62.2 % 100 % | 65.1 % 71.4 % | 53.6 % 83.3 % |
| Periodontal disease • Parodontrna bolest | Sensitivity • Osjetljivost 74.3 % 86.7% | 87.1 % 84.2% | 80.3 % 85.3 % |
| Periodontitis • Parodontitis | Sensitivity • Osjetljivost 84% 72% | 88% 68% | 86% 70% |

Table 2. Characteristics of infertile and fertile patients based on periodontal findings.

| Diagnosis • Dijagnoza | Infertile • Neplodne (N=50) | Fertile • Plodne (N=50) | Total • Ukupno |
|-----------------------|-----------------------------|--------------------------|----------------|
| Age • Dob             | 36.1 ± 3.7 37 ± 2.9         | *ns                        |
| Number of teeth • Broj zuba | 27 (26-28) 27 (25-28)  | *ns                        |
| FMBS                  | 38.9 ± 19.7 37.9 ± 19.2    | *ns                        |
| FMPS                  | 41.6 ± 21.5 36 ± 19        | *ns                        |
| PISA                  | 645.0 ± 368.0 700.9 ± 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                        |
| Periodontitis stage I • I. stupanj parodontitisa | 7 (14%) 0 (0%) | *ns                        |
| Periodontitis stage II • II. stupanj parodontitisa | 10 (20%) 10 (20%) | *ns                        |
| Periodontitis stage III • III. stupanj parodontitisa | 8 (16%) 15 (30%) | *ns                        |

*ns - non significant • nije značajno

Table 3. Sensitivity and specificity of MMP8 test in distinguishing subjects without periodontitis and with different stages of periodontitis.

| 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 |
| 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 |
| 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 |
| Test sensitivity • Osjetljivost testa | 91.30% | 71.96% - 98.93% |
| Test specificity • Specifičnost testa | 70.00% | 55.39% - 82.14% |

95% CI (confidence interval • interval pouzdanosti)
| Parameter                                      | Infertile • Neplodne (N=50) | Fertile • Plodne (N=50) | 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 • Hemoglobolin                    | 135.4 ± 7                   | 130.6 ± 6.7             | 0.0007 (t-test)         |
| Hematocrit • 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 objimu) | 12.7 (12.4-13.1)            | 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 • Neutrofili                     | 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 neutrofili 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 pacijentcima, 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–
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 do-darnim upalnim stanjima zbog sličnih mehanizama u podlozi razvoja bolesti. To je i jedini članak koji je objavljen, a uspo-ređivao je dijagnošćku pouzdanost testa kod pacijentica s prisutnim drugim upalnim stanjem i/ili narušenim imuno-snim odgovorom (34).

Rezultati Heikkinena i suradnika (35) pokazali su nešto nižu osjetljivost testa od naše – 76,5 % u slučaju više od dva-mjesta s dubokim parodontalnim 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 na-šoj – 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 te-sta aMMP-8 s FMBS-om, FMPS-om, PISA-om i prisutno-šću 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 stapanje parodontitisa, očekivano bilo bi da njegova osjetljivost raste s porastom stupnja parodontititisa, kako pokazuju prethodni autori (18, 30), no dio naših rezultata odtupa od te pret-postavke. Naime, naši rezultati pokazali su osjetljivost testa od 100 % za detekciju blagoga stupnja parodontitisa, 75 % za detekciju umjerenoga stupnja i 91,30 % pri detekciji teš-koga stupnja parodontitisa. Razlog za takve rezultate vjero-jatno je u malom broju pacijentica s umjerenim stupnjem parodontitisa (sedam). Rezultati našeg istraživanja poduda-raju se s objavljenim rezultatima Izadi-Borujenija i suradni-kaka (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 uznapredovalo-loga parodontitisa.

Hipoteza našeg istraživanja bila je da ćemo kod pacijen-tica s idiopatskom neplodnošću imati veći broj pozitivnih te-stova što će biti posljedica: a) lošijega parodontnoga stanja i/ili b) pojačanog upalnoga odgovora u organizmu.

Na temelju naših rezultata moramo odbaciti tu hipote-zu jer su rezultati pokazali da su pacijentice s idiopatskom neplodnošću imale blagi stupanj parodontitisa nego kontrol-nu skupinu, 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 bati da su neplodne žene uključene u naše istraživanje bi-le svjesnije svojega oralnoga zdravlja ili da su imale različi-tu ekonomsku ili obrazovnu pozadinsku, što bi također moglo utjecati na njihovo oralno zdravlje, no to nije uzeto u obzir u ovog 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.

However, we have found that the periodontal status in infertile women was different from the status before the pregnancy.

The results from the literature show that lymphocytes, neutrophils, and CRP are biomarkers of different inflammatory conditions, with conflicting results. On the contrary, PLR and NLR have been found to be biomarkers of different inflammatory conditions, with conflicting results. Moreover, the MPV values negatively correlated with clinical pregnancy 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. A systematic review has shown that NLR values could be a useful biomarker for predicting preterm delivery. On the contrary, Yldrm et al. have demonstrated lower NLR values in patients diagnosed with premature ovarian failure. 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. 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.

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. However, we have found that the periodontal status in infertile women was different from the status before the pregnancy.
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 parodontitis oprošteni upalni odgovor mogao imati ulogu u etiologiji idioptatske neplodnosti. Pri interpretaciji rezultata testa aMMP-8 za dijagnostiku parodontitisa treba imati na umu parodontni status ispitivane populacije (osjetljivost i specifičnost testa rašte sa stupnjem uznapredovalosti parodontitisa).

Conflict of interest

None declared.

Sukob interesa

Autori nisu bili u sukobu interesa.

References

1. Hart R, Doherty DA, Pennell CE, Newham IA, Newham JP. Periodontal disease: a potential modifiable risk factor limiting conception. Hum Reprod. 2012 May;27(5):1332-42.
2. Goldenberg RL, Culhane JF. Pre-term birth and periodontal disease. N Engl J Med. 2006;355(18):1925-7.
3. Gibbs RS. The relationship between infections and adverse pregnancy outcomes: an overview. Ann Periodontol. 2001;6:53-63.
4. Offenbacher S, Katz V, Fertik D, Collins J, Boyd D, Maynor G, et al. Periodontal Infecion as a Possible Risk Factor for Pre-term Low Birth Weight. J Periodontol. 1996 Oct;67(10 Suppl):1103-13.
5. Pretorius C, Jagatt A, Lamont RF. The relationship between periodontal disease, bacterial vaginosis, and pre-term birth. J Perinat Med. 2007;35(2):93-9.
6. Klinger A, Hain B, Yaffe H, Schonberger O. Periodontal status of males attending an in vitro fertilization clinic. J Clin Periodontol. 2011 Jun;38(6):542-6.
7. Nwhorah SO, Umeizudike KA, Ayanbadejo PO, Opeodu OL, Olumija JO, Sorsa T. Another reason for impeccable oral hygiene: oral hygiene-sperm count link. Int J Contemp Dent Pract. 2013;15(3):352-8.
8. Kavoussi SK, West BT, Taylor GW, Lebovic D. Periodontal disease and endometriosis: analysis of the National Health and Nutrition Examination Survey. Fertil Steril. 2009;91(2):335-42.
9. Paju S, Oittinen J, Haapala H, Asikainen S, Paavonen J PP. Porphyromonas gingivalis may interfere with conception in women. J Oral Microbiol. 2017 Jun 12;9(1):130644.
10. Telatar GY, Gürlek B, Telatar BC. Periodontal and caries status in unexplained female infertility: A case-control study. J Periodontol. 2020 Mar;92(3):446-454.
11. Bieniek KW, Riedel HH. Bacterial foci in the teeth, oral cavity, and jaw–secondary effects (remote action) of bacterial colonies with respect to bacteriospermia and subfertility in males. Andrology. 1993; 25(3):159-62.
12. Machado V, Botelho J, Proença L, Mendes JJ. Comparisons of periodontal status between females referenced for fertility treatment and fertile counterparts: A pilot case–control study. Int J Environ Res Public Health. 2020;17(15):5281.
13. Marcaccini AM, Novaes AB Jr, Meschiari CA, Souza SL, Palito DB, Sorgi CA, et al. Circulating matrix metalloproteinase-8 (MMP-8) and MMP-9 are increased in chronic periodontal disease and decrease after non-surgical periodontal therapy. Clin Chim Acta. 2009; 409(1-2):117-22.
14. Sorsa T, Tjäderhane L, Konttinen YT, Lauhio A, Salo T, Lee HM, et al. Matrix Metalloproteinases: Contribution to Pathogenesis, Diagnosis and Treatment of Periodontal Inflammation. Ann Med. 2006; 38(5):306-21.
15. Johnson N, Ebersole JL, Kryscio RJ, Danaher RJ, Dawson D, Al-Sabbagh M, et al. Rapid Assessment of Salivary MMP-8 and Periodontal Disease Using Lateral Flow Immunoassay. Oral Dis. 2016 Oct;22(7):681-7

16. Sorsa T, Giesseimann D, Arweiler NB, Hernández M. A Quantitative Point-of-Care Test for Quantitative Periodontal and Dental Peri-Implant Diseases. Nat Rev Dis Primers. 2017;14:3;17069.

17. Sorsa T, Hernández M, Leppilahti J, Munjal S, Netuschil L, Mäntylä P. Detection of Gingival Crevicular Fluid MMP-8 Levels With Different Laboratory and Chair-Side Methods. Oral Dis. 2010;16(1):39-45.

18. Deng K, Pekelos G, Jin L, Tonetti MS. Diagnostic accuracy of a point-of-care aMMP-8 test in the discrimination of periodontal health and disease. J Clin Periodontol. 2021 Aug;48(8):1051-1065.

19. Sorsa T, Sahni V, Buduneli N, Gupta S, Räisänen IT, Golub LM, et al. The association between in vitro fertilization outcome and the inflammatory markers in polycystic ovary syndrome: association with obesity and IVF outcomes. J Endocrinol Invest. 2016;39(8):899-907.

20. Klinger A, Hain B, Yaffe H, Schonberger O. Periodontal status of males attending an in vitro fertilization clinic. J Clin Periodontol. 2011;38(6):542-6.

21. Çakıroğlu Y, Vural F, Vural B. The inflammatory markers in polycystic ovary syndrome: association with obesity and IVF outcomes. J Endocrinol Invest. 2016;39(8):899-907.

22. Tola EN. The association between in vitro fertilization outcome and the inflammatory markers of complete blood count among nonobese unexplained infertile couples. Taiwan J Obstet Gynecol. 2018 Apr;57(2):289-294.

23. Ozgu-Erdinc AS, Coskun B, Yonganci A, Hancerliogullari N, Yilmaz N, Engin-Ustun Y. The Role of Inflammatory Hematological Markers in Predicting IVF Success. JBR Assis Reprod. 2021;25(1):71-5.

24. Gasparyan AV, Ayvazyan L, Mukanova U, Yessirkepov M, Kitas GD. The Platelet-to-Lymphocyte Ratio as an Inflammatory Marker in Rheumatic Diseases. Ann Lab Med. 2019;39(4):345-57.

25. Ye GL, Chen Q, Chen X, Liu YY, Yin TT, Meng QH, et al. The prognostic role of platelet-to-lymphocyte ratio in patients with acute heart failure: A cohort study. Sci Rep. 2019;9(1):10639.

26. Lorenz K, Keller T, Noack B, Freitag A, Netuschil L, Hoffman T. Evaluation of a novel point-of-care test for active matrix metalloproteinase-8: agreement between qualitative and quantitative measurements and relation to periodontal infection. J Periodontal Res. 2017 Apr;52(2):277-284.

27. Tonetti MS, Greenwell H, Komman KS. Staging and grading of periodontitis: Framework and proposal of a new classification and case definition. J Periodontol. 2018;89(1):159-72.

28. Nesse W, Abbas F, van der Ploeg I, Spijkervert FKL, Dijkstra PU, Vissink A. Periodontal inflamed surface area: quantifying inflammatory burden. J Clin Periodontol. 2008;35(8):668-73.

29. Musić L, Par M, Peručić J, Badovinac A, Piančak D, Puhar I. Relationship Between Halitosis and Periodontitis: a Pilot Study. Acta Stomatol Croat. 2021 Jun;55(2):198-206.

30. Nwhator SO, Ayanbadejo PO, Umeizudike KA, Opeodu OI, Agbelusi GA, Olamijulo JA, et al. Clinical correlates of a lateral-flow immunoassay oral risk indicator. J Periodontol. 2014;85:188-94.

31. Khalife F, Khalil A, Itani MN, Khalifeh F, Faour S, Salame A, et al. No association between the presence of periodontal disease and poor IVF outcomes: a pilot study. Int J Womens Health. 2019 Jun 10;11:363-370.

32. Sorsa T, Alassiri S, Grigoriadis A, Räisänen IT, Pärnänen P, Nwhator SO, et al. Active MMP-8 (aMMP-8) as a Grading and Staging Biomarker in the Periodontitis Classification. Diagnostics (Basel). 2020;10(2):61.

33. Sorsa T, Grigoriadis A, Sakellari D, Gupta S, Sahni V, Tervahartiala T, et al. On the accuracy, sensitivity, and grading of mouthrinse active matrix metalloproteinase-8 (aMMP-8) point-of-care testing (POCT). J Clin Periodontol. 2021 Nov;48(11):1495-1498.

34. Rautava J, Gürsoy UK, Kulström A, Könnönen E, Sorsa T, Tervahartiala T et al. An Oral Rinse Active Matrix Metalloproteinase-8 Point-of-Care Immunotest May Be Less Accurate in Patients with Crohn’s Disease. Biomolecules. 2020;10(3):395.

35. Heikkinen AM, Nwhator SO, Rathnayake N, Mäntylä P, Vatanen P, Sorsa T, Pilots Study on Oral Health Status as Assessed by an Active Matrix Metalloproteinase-8 Chairside Mouthrinse Test in Adolescents. J Periodontol. 2016 Jan;87(1):36-40.

36. Izadi Borujeni S, Mayer M, Eckholz P. Activated matrix metalloproteinase-8 in saliva as diagnostic test for periodontal disease? A case-control study. Med Microbiol Immunol. 2015 Dec;204(6):665-72.

37. Rudnicka E, Kunicki M, Suchta K, Machura P, Grymowicz M, Smolareczk R. Inflammatory Markers in Women with Polycystic Ovary Syndrome. Biomed Res Int. 2020 Mar 4;2020:4092470..

38. Zondervan KT, Becker CM, Koga K, Missmer SA, Taylor RN, Viganò P. Endometriosis. Nat Rev Dis Primers. 2018;4(1):9.

39. Tokmak A, Yildirim G, Öztə E, Akar S, Erkenekli K, Gül en P, et al. Use of Neutrophil-to-Lymphocyte Ratio Combined With CA-125 to Distinguish Endometriomas From Other Benign Ovarian Cysts. Reprod Sci. 2016 Jun;23(6):795-802.

40. Jing X, Li C, Sun J, Peng J, Dou Y, Xu X, et al. Systemic Inflammatory Response Markers Associated with Infertility and Endometrioma or Uterine Leiomyoma in Endometriosis. Ther Clin Risk Manag. 2020 May 11;16:403-412.

41. Baser E, Kirmizi DA, Ozelci R, Aldemir O, Dilbaz B, Dilbaz S, et al. Neutrophil:lymphocyte and estradiol:progesterone ratios as predictive markers for ovarian hyperstimulation syndrome (OHSS). Reprod Fertil Dev. 2022 Feb;34(3):343-349.

42. Vakili S, Torabian M, Tabrizi R, Shojazadeh A, Asadi N, Hessa HA, et al. Association between some inflammatory markers and primary ovarian insufficiency. Menopause. 2015 Sep;22(9):1000-5.