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# ODNOS IZMEĐU NIVOVA PERIODONTALNE DŽEPNE DUBINE I TEŽINE GINGIVALNE CERVİKALNE TEČNOSTI KORIŠĆENJEM ANALITIČKOG BALANSA KAO POKAZATELJA PERIODONTALNE UPALE TKIVA

## THE RELATIONSHIP BETWEEN THE LEVEL OF PERIODONTAL POCKET DEPTH WITH GINGIVAL CREVICULAR FLUID WEIGHT USING ANALYTICAL BALANCE AS AN INDICATOR OF PERIODONTAL TISSUE INFLAMMATION

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### Sažetak

**Uvod:** Ova studija ima za cilj utvrđivanje odnosa između dubine parodontalnog džepa i težine gingivalne crevicularne tečnosti (GCT) pomoću analitičke vage.

**Materijal i metode:** Radi se o opservaciono-analitičkoj studiji preseka. Nivo dubine parodontalnog džepa analiziran je sondiranjem. Dvadeset sedam ispitanika podjeljeno je u tri grupe, i to na sledeći način: grupa 1 (sa normalnom dubinom džepa 0 mm – 3,5 mm), grupa 2 (dubina džepa 3,6 mm – 5,5 mm) i grupa 3 (dubina džepa veća od 5,5 mm). Uzorkovanje GCT-a sprovedeno je metodom pomoću papirnih tačaka, a merenje njegove težine pomoću analitičke vage. Podaci su analizirani jednosmernom analizom varijanse praćene post hoc Tukey testom, kako bi se utvrdilo poređenje težine GCT-a između grupa. Korišćen je Pearsonov test korelacije.

**Rezultati:** Prosečna težina GCT-a dobijena u svakoj grupi bila je 0,352 mcg ± 0,09 mcg, 0,598 mcg ± 0,10 mcg i 0,899 mcg ± 0,06 mcg. Utvrđena je značajna razlika ( $p < 0,001$ ) između poređenih grupa, pri čemu je najveća težina GCT-a pronađena u grupi sa dubinom džepa > 5,5 mm. Pearsonov test korelacije pokazao je da postoji pozitivna korelacija između dubine parodontalnog džepa i težine GCT-a.

**Zaključak:** Nivo dubine parodontalnog džepa povezan je sa težinom GCT-a.

**Cljučne reči:** gingivalna crevicularna tečnost, upala, parodontalni džep

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### Abstract

**Background:** The present study aims to determine the relationship between the level of periodontal pocket depth with the weight of gingival crevicular fluid (GCF) using an analytical balance.

**Material and Methods:** This is an observational analytic study with a cross-sectional approach. The level of periodontal pocket depth was analyzed by probing. Twenty-seven subjects were divided into three groups, namely, group 1 (with normal pocket depth: 0–3.5 mm), group 2 (pocket depth of 3.6–5.5 mm), and group 3 (pocket depth of more than 5.5 mm). GCF sampling was conducted by the infraclavicular method using paper points and its weight measurement with an analytical balance. Data were analyzed by one-way analysis of variance followed by post hoc Tukey test to determine the comparison of GCF's weight between groups. The correlation was determined using the Pearson correlation test.

**Results:** The average of GCF's weight obtained respectively in each group was 0.352 ± 0.09 mcg, 0.598 ± 0.10 mcg, and 0.899 ± 0.06 mcg. There was a significant difference ( $p < 0.001$ ) with the highest GCF's weight was found in the pocket depth group > 5.5 mm. The Pearson correlation test showed that there was a positive correlation between the depth of the periodontal pocket and the weight of GCF.

**Conclusion:** The level of periodontal pocket depth is related to the weight of GCF.

**Key words:** gingival crevicular fluid, inflammation, periodontal pocket.

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## Uvod

Parodontopatije su rasprostranjene i u razvijenim zemljama i u zemljama u razvoju i pogađaju oko 20% – 50% svetske populacije<sup>1</sup>. Preovlađujuća statistika parodontnih bolesti varira zbog pristrasnosti, pogrešne klasifikacije slučajeva i broja zuba i pregledanih mesta<sup>2</sup>. Na osnovu podataka iz Osnovnog zdravstvenog istraživanja (Riskesdas) Ministarstva zdravlja Republike Indonezije 2018. godine pokazalo se da je parodontno tkivo uobičajen problem oralnog zdravlja u Indoneziji, sa 96,58% prevalencije u svim starosnim grupama<sup>3</sup>. Parodontne bolesti tkiva, koje se obično nalaze jesu gingivitis i parodontitis. Gingivitis je upala gingive, koja se karakteriše kao crvenom do plavkastom bojom, sa uvećanim konturama gingive usled edema i lako krvari kada se stimuliše, na primer prilikom pranja zuba. Parodontopatija je upala potpornih tkiva zuba, uzrokovana mikroorganizmima, a karakteriše je uništavanje parodontalnog ligamenta i alveolarne kosti sa povećanjem dubine džepa, recesijom ili oboma. Klinički simptom po kome se mogu razlikovati gingivitis i parodontopatija jeste gubitak rubnog pripoja gingive kod pacijenata koji pate od parodontopatije<sup>4,5</sup>.

Bolest parodontalnog tkiva započinje upalom gingivalnog ruba, koja napreduje ka parodontopatiji, sa labavljenjem pripojnog epitela, koji može kontinuirano produbljivati gingivalni sulkus sve dok se ne formiraju parodontalni džepovi. Parodontalni džepovi nastaju usled migracije pripojnog epitela do apikalnom foramenu, kada dolazi do pojave ulceracije. Bakterije zarobljene u parodontalnim džepovima brzo se množe, sa povećanjem broja neutrofila, koji prelaze u pripojni epitel, prekidajući ga<sup>6,7</sup>. U sve težoj situaciji, dolazi do patološke resorpcije kosti, sve dok luksacija zuba na kraju ne dovede do ispadanja zuba. Ovo ima uticaja na gubitak zuba i funkcije usne duplje od žvakanja, preko fonetike, do estetike lica<sup>5</sup>. Prema Svetskoj Zdravstvenoj Organizaciji iz 2017. godine, parodontopatija je definisana na osnovu stepenovanja i ocenjivanja<sup>8</sup>. Međutim, ovim metodama, rezultati su ograničeni samo na istoriju bolesti, a ne na trenutno stanje bolesti, koje se može proceniti<sup>9</sup>. Napredak u dijagnostici istraživanja oralnih i parodontnih bolesti kreće se ka metodama, pomoću kojih se parodontalni rizik može identifikovati i kvantifikovati.

## Introduction

Periodontal diseases are prevalent in both developed and developing countries and affect about 20%–50% of the global population<sup>1</sup>. The prevalent and incident statistics of periodontal diseases vary because of bias, case misclassification, and the number of teeth and the sites examined<sup>2</sup>. Based on data from the Basic Health Research (Riskesdas) by the Republic of Indonesia Ministry of Health in 2018, periodontal tissue disease is a common oral health problem in Indonesia with a 96.58% prevalence in all age groups<sup>3</sup>. Periodontal tissue diseases commonly found are gingivitis and periodontitis. Gingivitis is an inflammation of the gingiva, which is characterized as red to bluish with enlarged gingival contours due to edema and easily bleeds when stimulated, such as when brushing teeth. Periodontitis is an inflammation of the tooth-supporting tissue caused by microorganisms, which is characterized by the destruction of the periodontal ligament and alveolar bone with increasing pocket depth, recession, or both. The clinical symptom in which gingivitis and periodontitis can be distinguished is the attachment loss (loss of attachment) of gingiva in patients suffering from periodontitis<sup>4,5</sup>.

Periodontal tissue disease begins with the inflammation of the gingival margin, which then becomes more severe than periodontitis, with the loosening of junctional epithelium attachment, which can continually deepen the gingival sulcus until periodontal pockets are formed. Periodontal pockets are formed due to the junctional epithelium migration to the apical foramen at the border of the tooth where ulceration occurs. Bacteria trapped in periodontal pockets multiply rapidly and increases the number of neutrophils that transmigrate to the junctional epithelium and pocket epithelium, interrupting the barrier epithelium periodontally<sup>6,7</sup>. In an increasingly severe situation, there will be pathological resorption in the bone, until the oscillation of the teeth eventually causes the teeth to fall out. This has an impact on the functional loss of teeth and oral cavity health further leading to problems ranging from mastication and phonetics to facial aesthetics<sup>5</sup>. Based on the 2017 World Workshop, periodontitis has been characterized based on staging and grading system<sup>8</sup>. However, with these methods, the results will be limited only to the disease history, not current disease status, which can be assessed<sup>9</sup>. Advances in oral and periodontal disease diagnostic research are moving toward methods whereby periodontal risk can be identified and quantified.

Određivanje inflamatornog statusa i stadijuma bolesti parodontalnog tkiva zahteva precizniji pregled, na primer procenom inflamatornog biomarkera dostupnog u usnoj šupljini, kao što je GCT. GCT je eksudatna tečnost, koja se nalazi u sulkusu ili parodontalnom džepu, između zuba i gingivalnog pripoja. Ova tečnost dobija se iz gingivalnog pleksusa krvnih sudova, koji se nalazi subepitelu između kosti i zuba<sup>10</sup>. Komponenta GCT-a dolazi iz krvi, tkiva i subgingivalnog plaka. Molekuli GCT-a uključuju elektrolite, organske molekule, proteine, citokine, specifična antitela, bakterijske agense i enzime koji potiču iz tela i bakterije prisutne u tom području. Supstanca derivata domaćina sadržana u GCT-u uključuje antitela, enzime, proizvode razgradnje tkiva i neke proinflamatorne citokine<sup>11</sup>.

U normalnoj gingivi, volumen GCT-a, koji se nalazi u gingivalnom sulkusu veoma je mali, što se može smatrati zanemarljivim<sup>4</sup>. Međutim, u parodontalnim džepovima pacijenata koji pate od parodontopatije povećanje volumena GCT-a i tri proporcije pokazuje ozbiljnost upale. U inflamatornim stanjima, zapaljenjski eksudat se povećava skoro pet puta<sup>12</sup>. Ovo ukazuje na to da se povećanje težine GCT-a može koristiti kao jednostavan pokazatelj za određivanje statusa upale parodontalnog tkiva, jer sadrži nekoliko biohemijskih faktora koji su blisko povezani sa statusom parodontalne bolesti i sredstvo za praćenje odgovora na lečenje<sup>13</sup>.

Budući da je GCF upalni eksudat, koji odražava tekuće događaje u parodontnim tkivima koja ga proizvode, opsežno se tragalo za komponentama GCT-a koje bi mogle poslužiti kao potencijalni dijagnostički ili prognostički markeri za progresiju parodontopatije<sup>14</sup>. Većina studija koristila je papirne trake za koje se smatralo da su efikasnije u prikupljanju GCT-a, jer su se mogle lako umetnuti u gingivalni sulkus ili parodontalne džepove i apsorbovati tečnost<sup>10</sup>. Studija koju su sprovedli Guentsch i sar. pokazala je da su nivoi citokina bili veći kada su se koristile papirne trake<sup>15</sup>. Papirne trake se češće koriste za prikupljanje subgingivalnih plakova u mikrobiološkoj analizi. Drugi način prikupljanja GCT-a jeste pranje, ali takva tehnologija nije bila uobičajena zbog zehničkih smetnji. Štaviše, postojala je velika stopa kontaminacije krvi zbog povećane mogućnosti iritacije gingive<sup>10</sup>.

Determining the inflammatory status and the stage of a periodontal tissue disease necessitates a more accurate examination, for example, by assessing an inflammatory biomarker available in the oral cavity, such as GCF. GCF is an exudate fluid found in the sulcus or periodontal pocket that lies between the teeth and the gingival margin. This fluid is obtained from the gingival plexus of a blood vessel in the gingival cavity located under the epithelium between the bone and the teeth<sup>10</sup>. The GCF's component comes from blood, tissue, and subgingival plaque. GCF's molecules include electrolytes, organic molecules, proteins, cytokines, specific antibodies, bacterial agents, and enzymes that originate in the body and bacteria present in the area. The substance of host derivatives contained in GCF includes antibodies, enzymes, tissue degradation products, and some proinflammatory cytokines<sup>11</sup>.

In normal gingiva, the volume of GCF found in the gingival sulcus is very small, which can be considered to be negligible<sup>4</sup>. However, in periodontal pockets of periodontitis sufferers, an increase in GCF's volume and three proportions shows the severity of the inflammation that occurs. In inflammatory conditions, inflammatory exudate increases almost fivefold<sup>12</sup>. This indicates that an increase in GCF's weight can be used as a simple indicator to determine the status of periodontal tissue inflammation because it contains several biochemical factors that are closely related to periodontal disease status and a means of monitoring response to treatment<sup>13</sup>.

Since GCF is an inflammatory exudate that reflects ongoing events in the periodontal tissues that produce it, an extensive search has been made for GCF components that might serve as potential diagnostic or prognostic markers for the progression of periodontitis<sup>14</sup>. The majority of the studies used paper strips that were considered to be more efficient in GCF collection because they could be inserted easily into gingival sulcus or periodontal pockets and could absorb fluids<sup>10</sup>. A study done by Guentsch et al. indicated that cytokine levels were higher when paper strips were used<sup>15</sup>. Paper points are more commonly used for subgingival plaque collection in microbiological analysis. Another method to collect GCF is washing, but such technology was not common because of technique-sensitive difficulties. Moreover, there was a high rate of blood contamination due to the increased possibility of gingival irritation<sup>10</sup>. Indirect measurement of GCF's weight by measuring weight using an analytical balance has not been explored.

Indirektno merenje težine GCT-a pomoću analitičke vage nije istraživano. Na ovoj osnovi, u našoj studiji ispituje odnos između nivoa dubine parodontalnih džepova i težine GCT-a, kao pokazatelja upale parodontalnog tkiva.

## **Materijali i metode**

### *Izbor pacijenata za istraživanje*

Ovo je analitička opservaciona studija sa pristupom preseku. Korišćena je metoda uzastopnog uzorkovanja, a pacijenti koji su ispunili kriterijume studije bili su pacijenti Doma zdravlja Janti u Malangu. Ovo istraživanje trajalo je tri meseca (od novembra 2019. do januara 2020. godine). Pacijenti su bili stari od 18 do 45 godina, dobrovoljno su učestvovali ovoj studiji i dali su pisanu saglasnost. Oni koji su ranije imali sistemske bolesti (dijabetes melitus, HIV/AIDS, kardiovaskularne bolesti, hipertenziju i hematološke poremećaje), ili koristili lekove (analgetici, antibiotici, antihipertenzivi, antihistaminici, antiholinergici, hipnotici i sedativi), trudnice, dojilje, pušači, kao i pacijenti koji su bili na parodontalnom lečenju u poslednjih šest meseci, bili su isključeni. Ova studija je prošla etički komitet sa nekoliko etičkih odobrenja br. 598/KEPK-POLKESMA/2020. Za sve tri grupe lečenja bilo je ukupno dvadeset sedam pacijenata (Tabela 1). Za stratifikaciju pacijenata sa parodontopatijom, klinički parodontalni parametri predela prvog molara bukalne strane maksile procenjeni su pri prvom pregledu srednjom dubinom sondiranja (DS) i krvarenje pri sondiranju (KPS). Prema kriterijumima, uzorke smo podelili u tri grupe: zdravi, blaga i umerena parodontopatija, na osnovu dubine sondiranja (DS). Zdravi ispitanici sa DS < 3,5 mm, sa blagim parodontitisom sa DS od 3,6 do 5 mm i sa umerenim parodontitisom sa DS > 5,5 mm.

On this basis, the present study examines the relationship between the depth level of periodontal pockets and the weight of GCF as an indicator of inflammation of periodontal tissue.

## **Materials and Methods**

### *Research subject recruitment*

This is an analytic observational study with a cross-sectional approach. The consecutive sampling method was used, and the patients who met the study criteria were from the Janti Health Center, Malang. This research was conducted for 3 months (November 2019 to January 2020). The patients were 18–45 years, willing to be respondents in the present study, and provided informed consent. Those who had a history of systemic diseases (diabetes mellitus, HIV/AIDS, cardiovascular, hypertension, and hematological disorders); were taking drugs (analgesics, antibiotics, antihypertensives, antihistamines, anticholinergics, hypnotics, and sedatives); were pregnant or nursing, smokers; and those who received periodontal treatment within the last six months were excluded. The present study has passed an ethical review with several ethical approval letters no. 598/KEPK-POLKESMA/2020. There were a total of 27 patients divided into three treatment groups (Table 1). To stratify chronic periodontitis patients, the clinical periodontal parameters from the buccal side maxilla first molar were assessed at the initial examination for mean probing depth (PD) and bleeding on probing (BOP). According to the criteria, we divided the samples into 3 groups, healthy, mild, and moderate periodontitis based on Probing Depth (PD). Healthy subjects with PD <3.5mm, mild periodontitis with PD 3.6-5 mm, and moderate periodontitis with PD >5.5 mm.

**Tabela 1.** Predmetne grupe istraživanja  
**Table 1.** Research subject groups

Groupa/ Group	Dubina parodontalnog džepa/ Periodontal pocket depth	Ukupan broj/Total number
1 (kontrola) 1 (control)	0 mm – 3,5 mm	9
2	3,6 mm – 5,5 mm	9
3	Više od 5.5 mm/More than 5.5 mm	9
Ukupno predmeta/Total subjects		27

### Uzimanje uzoraka gingivalne crevicularne tečnosti

Za uzimanje GCT uzorka pacijenata korišćene su papirne tačke. Korišćena je veličina papira broj 25. Plak je očišćen na mestu gde je trebalo uzeti GCT i osušen izolacijom navedenih uzorka. GCT uzorkovanje je izvršeno na zubima 16 ili 26 sa bukalne strane. Papirne tačke ubacuju se u gingivalni sulkus bukalnog dela zuba koji je određen (uzorci su uzeti sa vestibularne strane maksilarnog prvog molara, dok se GCT ne apsorbuje 30 sekundi). Papirne tačke ne bi trebalo da su kontaminirane pljuvačkom ili krvlju. Ako je došlo do kontaminacije, potrebno je ponovno uzorkovanje nakon jednog minuta. Papirne tačke su uklonjene iz gingivalnog sulkusa i stavljene u Eppendorfovu epruvetu, koja sadrži 250  $\mu$ l fiziološkog rastvora puferisanog fosfatom, zatim su obeležene, stavljene u kutiju za skladištenje i skladištene u frižideru na  $-40^{\circ}\text{C}$ .

### Merenje težine GCT-a

Korišćena je veličina tačke papira broj 25. Tačke papira merene su pomoću analitičke vage. Utvrđeno je da je težina papirnih tačaka 42,5 mg, (obeleženo  $v_p$ ). Eppendorfove epruvete, koje sadrže fiziološki rastvor puferisan fosfatom 250  $\mu$ l izmerene su pomoću analitičke vage i čuvane u frižideru na  $-40^{\circ}\text{C}$ . Težina Eppendorfovih epruveta, koje sadrže fiziološki rastvor puferisan fosfatom označena je kao  $v_1$ . U daljem tekstu, težina Eppendorfove epruvete koja sadrži fiziološki rastvor puferisan fosfatom i papirna tačka iz prikupljanja uzoraka GCT-a, koji su umetnuti u sulkus pacijenta, mereni su pomoću analitičke vage, a zatim su označeni kao  $v_2$ . Konačno, merenje težina GCT-a ( $v_{\text{GCF}}$ ) uzoraka pomoću analitičke vage izračunato je pomoću sledeće formule:

$$v_{\text{GCF}} = v_2 - v_1 - v_p \quad (1)$$

Gde je:

$v_{\text{GCF}}$  = težina apsorbovanog GCT-a od pacijenta

$v_2$  = težina Eppendorfa koji sadrži rastvor fiziološkog rastvora puferisanog fosfatom, papirne tačke i GCT-a

$v_1$  = težina Eppendorfa koji sadrži 250  $\mu$ l fiziološkog rastvora puferisanog fosfatom

$v_p$  = težina vrha papira (42,5 mg)

### Merenje dubine parodontalnog džepa

Dubina parodontalnog džepa merena je sondom na maksilarnim prvim molarima (zubi 16 i 26). Na osnovu dubine parodontopatije, određene su grupe ispitanika.

### Gingival crevicular fluid sampling

We took the GCF sample of patients by using paper points (size 25). The plaque was cleaned in the area where GCF was to be taken and dried by isolating the specified sample teeth. GCF sampling is preferred for tooth 16 or 26 on the buccal side. Paper points were inserted in the gingival sulcus of the buccal part of the determined tooth (samples were taken from the vestibular side of the maxillary molar 1 tooth until the GCF was absorbed for 30 s). Paper points should not be contaminated with saliva or blood. If contaminated, then re-sampling should be done after 1 min. Paper points were removed from the gingival sulcus and put into an Eppendorf tube containing phosphate-buffered saline solution 250  $\mu$ l, labeled, placed in a storage box, and then stored in a refrigerator at  $-40^{\circ}\text{C}$ .

### Measurement of GCF's weight

The paper's point size used was number 25. Paper points were weighed using an analytical balance. We found that the weight of paper points was 42.5 mg, referred to as  $w_p$ . The Eppendorf tubes containing phosphate-buffered saline 250  $\mu$ l were weighed using an analytical balance and is stored in the refrigerator at  $-40^{\circ}\text{C}$ . The weight of the Eppendorf tubes containing phosphate-buffered saline was referred to as  $w_1$ . Hereafter, the weight of the Eppendorf tube containing phosphate-buffered saline solution and the paper point from collecting GCF samples that had been inserted in the patient's sulcus were measured using an analytical balance and then referred to as  $w_2$ . Finally, the measurement of GCF's weights ( $w_{\text{GCF}}$ ) of samples using an analytical balance were calculated using the following formula:

$$w_{\text{GCF}} = w_2 - w_1 - w_p \quad (1)$$

Where:

$w_{\text{GCF}}$  = the weight of absorbed GCF from the patient

$w_2$  = the weight of the Eppendorf containing a solution of phosphate-buffered saline, paper points, and GCF

$w_1$  = the weight of the Eppendorf containing a 250  $\mu$ l phosphate-buffered saline

$w_p$  = the weight of paper point (42.5 mg)

### Measurement of periodontal pocket depth

The depth of the periodontal pocket was measured using a probe on the maxillary first molars (teeth 16 and 26). Based on the depth of periodontal pocket, the subject groups were determined.

### Statistička analiza

Svi dobijeni podaci zatim se analiziraju pomoću programa za analizu SPSS sa nivoom značajnosti od 95% (nivo pouzdanosti). Statističkim testovima prethodili su Shapiro–Wilkov test normalnosti i Leveneov test za ispitivanje homogenosti, nakon čega su usledili jednosmerna analiza varijanse i post hoc Tukey testovi za utvrđivanje razlika u prosečnoj masi GCT-a u tri grupe. Sprovedena je Spearmanova analiza korelacije, kako bi se utvrdila jačina odnosa između dubine džepa i zapremine GCT-a.

### Rezultati

Proučavali smo dubinu parodonta i težinu GCT-a kod dvadeset sedam pacijenata. Podaci o dubinama parodontalnih džepova (mm) i težinama GCT-a (mcg) prikazani su u Tabeli 2. Rezultati pokazuju da postoji povećanje prosečnih težina GCT-a od najniže do najveće dubine džepa (grupe 1, 2 i 3, respektivno). Kao što je pokazala Pearsonova korelaciona analiza, postoji jaka pozitivna korelacija između dubine parodontalnog džepa i težine GCT-a ( $r = 0,949$ ,  $p = 0,0001$ ).

### Statistical analysis

All obtained data were then analyzed using the SPSS analysis program with a 95% significance level (confidence level). Statistical tests were preceded by using Shapiro–Wilk normality test and Levene's test to examine the homogeneity, followed by one-way analysis of variance and post hoc Tukey tests to determine differences in the average mass of GCF in the three groups. Then, the Spearman correlation analysis was conducted to determine the strength of the relationship between pocket depth and the volume of GCF.

### Results

We studied the periodontal depths and GCF's weights of 27 patients. The data of periodontal pocket depths (mm) and GCF's weights (mcg) are shown in Table 2. The results show that there was an increase in the average weights of GCF from the lowest to the highest depth group (groups 1, 2, and 3, respectively). As shown by Pearson correlation analysis, there was a strong positive correlation between periodontal depth and GCF's weight ( $r = 0.949$ ,  $p = 0.0001$ ).

**Tabela 2.** Dubine parodontalnog džepa i težine GCF -a  
**Table 2.** The periodontal pocket depths and GCF's weights

No.	Grupa 1* (dubina parodontalnog džepa 0 mm – 3,5 mm)/ Group 1* (periodontal pocket depth 0 mm – 3.5 mm)		Grupa 2* (dubina parodontalnog džepa 3.6 mm – 5.5 mm)/ Group 2* (periodontal pocket depth 3.6 mm – 5.5 mm)		Grupa 3* (dubina parodontalnog džepa >5.5 mm)/ Group 3* (periodontal pocket depth >5.5 mm)		p*
	Dubina džepa(mm)/ Pocket depth (mm)	Težina GCF (mcg)/ GCF weight (mcg)	Dubina džepa (mm)/ Pocket depth (mm)	Težina GCF (mcg)/ GCF weight (mcg)	Dubina džepa (mm)/ Pocket depth (mm)	Težina GCF(mcg)/ GCF weight (mcg)	
1	3	0.512	5	0.751	6	0.975	0.000 1
2	2	0.414	4	0.580	6	0.766	
3	3	0.392	4	0.551	6	0.913	
4	3	0.372	5	0.570	6	0.879	
5	1.5	0.223	4	0.391	7	0.964	
6	2	0.350	5	0.694	6	0.896	
7	3	0.374	4	0.683	6	0.877	
8	0.5	0.215	4	0.644	6	0.899	
9	2	0.314	4	0.517	7	0.927	
Srednja vrednost ± SD/ Mean ± SD	2.222 ± 0.87	0.352 ± 0.09	4.333 ± 0.50	0.598 ± 0.10	6.222 ± 0.44	0.899 ± 0.06	

GCT = gingivalna crevicular fluid

p\*: srednja razlika u značaju na nivou <0,001

GCF = gingival crevicular fluid

p\*: the mean difference in significant at the <0.001 level



## Diskusija

GCT je eksudatna tečnost koja se nalazi u sulkusu ili parodontalnom džepu, između zuba i gingivalnog ruba. Ova tečnost dobija se iz subepitelno gingivalnog pleksusa krvnih sudova, koji se nalazi između kosti i zuba<sup>10</sup>. U ovoj studiji, uzorci GCT-a uzeti su iz regiona prvih maksilarnih molara. Prema Attar i sar., zapremina i sastav GCT-a razlikovali su se za svaku lokaciju uzorkovanja<sup>9</sup>. Zapremina GCT-a u zadnjoj regiji veća je od one u prednjoj, donja vilica je veća od gornje, a zadnja interproksimalna veća je od prednje labijalne<sup>9</sup>. Kako bismo odredili lokaciju uzorkovanja, uzeli smo u obzir molarne zube, zube sa najvećom površinom, i, kako bismo smanjili kontaminaciju pljuvačkom, izabrana je gornja vilica. Osim toga, uzorke bi prilikom uzimanja trebalo izolovati pomoću vate, kako bi se smanjio rizik od kontaminacije pljuvačkom i krvlju<sup>4</sup>. Pacijenti koji su u istoriji imali sistemske bolesti (dijabetes melitus, HIV/AIDS, kardiovaskularne bolesti, hipertenziju i hematološke poremećaje), oni koji su uzimali lekove (analgetici, antibiotici, antihipertenzivi, antihistaminici, anti-chistergici, hipnotici i sedativi), trudnice, dojilje, pušači, kao i pacijenti koji su bili na parodontalnom lečenju u poslednjih šest meseci, bili su kriterijumi za isključenje, budući da ova stanja mogu izazvati parodontalnu upalu.

Na osnovu rezultata sprovedenih istraživanja, težina GCT-a dobijena, u grupi 1, kao kontrolnoj grupi, lakša je nego u grupama 2 i 3, kao što je prikazano u Tabeli 2. Najveće povećanje težine GCT-a zabeleženo je u grupi 3. Kao što pokazuju rezultati testova korelacije u ovoj studiji, može se zaključiti da postoji pozitivna korelacija između dubine parodontalnih džepova sa povećanjem mase GCT-a. Rezultati takođe pokazuju da će sve veća dubina parodontalnog džepa povećati težinu GCT-a. U pogledu stepena korelacije, veličina koeficijenta korelacije je veoma je jaka. Ovo je preliminarna studija čiji je cilj bio indirektno utvrđivanje težine tečnosti GCT-a pomoću analitičke vage, povezana sa dubinom parodontalnog džepa. Dubina parodontalnog džepa odražava ozbiljnost upalnih stanja u parodontalnom tkivu<sup>16</sup>. Parodontni džep uzrokovan je upalom vezivnog tkiva gingivalnog sulkusa, tako da ćelije i zapaljenskog eksudata i vezivnog tkiva i gingivalna vlakna degenerišu. Dolazi do destrukcije pripojnog epitela vezivnog tkiva, koje se zamenjuje ćelijama inflamacije i edemom. Dolazi do gubitka pripojnog koronarnog epitela, sa gubitakom veze i produbljanje gingivalnog sulkusa<sup>15</sup>.

## Discussion

GCF is an exudate fluid found in the sulcus or periodontal pocket that is located between the teeth and the gingival margin. This fluid is obtained from the gingival plexus of a blood vessel in the gingival cavity located under the epithelium between the bone and the teeth<sup>10</sup>. In the present study, GCF samples were taken in the maxillary molar 1 region. According to Attar et al., the volume and composition of the GCF differed at each sampling location<sup>9</sup>. The volume of the GCF in the posterior region is greater than that in the anterior, the lower jaw is greater than the upper jaw, and the posterior interproximal is greater than the anterior labial<sup>9</sup>. To determine the sampling location, we considered the molar teeth, which are teeth with the largest surface area, and to minimize saliva contamination, the upper jaw has been considered instead of the lower jaw. Additionally, in taking samples, they should be isolated by using a cotton roll to minimize the risk of saliva and blood contamination<sup>4</sup>. Patients who had a history of systemic diseases (diabetes mellitus, HIV/AIDS, cardiovascular, hypertension, and hematological disorders), were taking drugs (analgesics, antibiotics, antihypertensives, antihistamines, anti-chistergics, hypnotics, and sedatives), were pregnant or nursing, smokers, and patients who received periodontal treatment within the last 6 months, met exclusion criteria because these conditions could cause periodontal inflammation.

Based on the results of research conducted, the weight of GCF obtained among others in the pocket 1 depth group as control is lighter than in groups 2 and 3, as shown in Table 2. The highest increase in GCF's weight is found in the pocket 3 depth group. As correlation test results in the present study show, it can be concluded that there is a positive correlation between the depth of periodontal pockets with an increase in the mass of GCF. The results also show that the increasing depth of the periodontal pocket will increase the weight of GCF. In the range of the strength of the relationship, the magnitude of the correlation coefficient obtained into the range is very strong. This is a preliminary study to determine whether the analysis of GCF indirectly through simple measurement of GCF fluid weight using the analytical balance is related to the depth level of the periodontal pocket. The depth of the periodontal pocket reflects the severity of the inflammatory conditions in the periodontal tissue<sup>16</sup>. Periodontal pocketing is caused by

Gingivalna crevikularna tečnost normalno se nalazi u gingivalnom sulkusu, kao i u patološkim stanjima. U normalnim okolnostima, količina GCT-a manja je nego u patološkim stanjima<sup>12</sup>. GCT pronađen u zdravom parodontalnom tkivu pokazuje transudatnu tečnost iz gingivalnog tkiva i nastalu tokom osmoze. ČGT se povećava u zapremini, ako dođe do upale i povećane propustljivosti kapilara. Upalne ćelije prelaze iz perifernih krvnih sudova u gingivalni sulkus. Ovo pokazuje da GCT može pokazati imunološke i inflamatorne reakcije, koje se javljaju kod parodontalne bolesti<sup>17</sup>. Do danas su iz GCT-a izolovani različiti upalni faktori, uključujući citokine, fosfatazu, proteinazu, lokalne proizvode razgradnje tkiva i proteine<sup>18</sup>. Prijavljeno je da su ovi faktori mogući dijagnostički markeri kod parodontopatije. GCT ne samo da može biti buduće dijagnostičko oruđe u identifikaciji parodontitisa, već može pomoći i u otkrivanju progresije ove bolesti. Rano otkrivanje progresije parodontopatije može biti klinički korisno, jer može pružiti bolju kontrolu aktivnosti bolesti i poboljšati praćenje pacijenata<sup>19</sup>.

Protok i sastav GCT-a služe kao merač ili barometar intenziteta upale. Ova tečnost sadrži sve proteine plazme, kao i ćelijske elemente, kao što su polimorfonukleari kod blage upale. Sastav GCT-a karakteriše pojava bakterijskih produkata, produkata razgradnje imunološkog sistema domaćina, posrednika upale i nusproizvoda imunološkog sistema domaćina. Dodatno, prema „Alfanovoj teoriji“, ovo povećanje koncentracije može biti posledica modulacije obimom eksudacije proteina plazme<sup>20</sup>. To se dešava zato što, kada se parodontalno tkivo upali, vaskularna propustljivost i ulceracija iz upaljenog epitela postaju veće. Shodno tome, proinflammatory citokini i proteini do 619 vrsta nalaze se u GCT-ima, u zavisnosti od težine upale i dubine parodontološkog džepa<sup>18</sup>. Neki proinflammatory protein pronađeni u GCT-u uključuju aktin, keratin, histon, aneksin, albumin, makrofage, matriks metaloproteinaze, kao što su imunoglobulin G, imunoglobulin A, imunoglobulin M, faktor nekroze tumora-alfa (TNF-a), interleukin-6 (IL-6) i prostaglandinE-2 (PGE-2)<sup>4,18</sup>. Osim upale, nekoliko faktora rizika uzrokuje povećanje volumena GCT-a, uključujući pušenje, cirkadijalnu periodičnost, polne hormone, oralne kontraceptive, ortodontske i parodontne tretmane<sup>4,21</sup>. Pretpostavlja se da lučenje upalnih proteina pronađeno u GCT-u uzrokuje povećanje težine GCT-a izmereno u ovoj studiji, ali to treba dokazati merenjem specifičnih proteinskih masa sadržanih u GCT-u.

inflammation of the gingival sulcus connective tissue so that cells and exudate inflammation and connective tissue and gingival fibers degenerate. This destroys collagen tissue under the junctional epithelium, replaced by inflammatory cells and edema. Consequently, there will be a loss of coronal attachment of the junctional epithelium cell, causing intraepithelial cleft and deepening of the gingival sulcus<sup>15</sup>.

The gingival crevicular fluid is found in the gingival sulcus normally as well as in pathological conditions. In normal circumstances, the amount of GCF volume is lower than in pathological conditions<sup>12</sup>. GCF found in healthy periodontal tissue shows transudate fluid from gingival tissue produced from osmotic conditions that will increase in volume if inflammation and increased capillary permeability occur. Inflammatory cells will move from peripheral blood vessels to the gingival sulcus. This shows that GCF can show immune and inflammatory reactions that occur in periodontal disease<sup>17</sup>. To date, various inflammatory factors have been isolated from GCF, including cytokines, phosphatase, proteinase, local tissue degradation products, and proteins<sup>18</sup>. These factors have been reported to be possible diagnostic markers in periodontitis. GCF not only can be a future diagnostic tool in the identification of periodontitis but also can aid in the detection of the progression of this disease. Early detection of periodontitis progression can be clinically useful as it can provide better control of disease activity and can enhance patient monitoring<sup>19</sup>.

The flow and composition of GCF serve as a gauge or barometer of the intensity of inflammation. This fluid contains all the plasma proteins as well as cellular elements such as polymorphonuclears in mild inflammation, and the composition of GCF is characterized by the appearance of bacterial products, degradation products of the host immune system, mediator of inflammation, and by-products of the host immune system and by-products of connective tissue breakdown in severe inflammation. Additionally, according to “Alfano’s theory” this increase in concentration may be due to the modulation by the extent of plasma protein exudation<sup>20</sup>. This occurred because when the periodontal tissue becomes inflamed, vascular permeability and ulceration from the inflamed epithelium become greater. Consequently, proinflammatory cytokines and proteins of up to 619 species are found in GCFs depending on the severity of inflammation and the depth of the periodontic pocket<sup>18</sup>.



## **Zaključak**

Na osnovu rezultata dobijenih istraživanjem može se zaključiti da dubina parodontalnog džepa utiče na težinu GCT-a, i da su oni međusobno povezani. Što je parodontalni džep dublji, veća je težina dobijenog GCT-a. Težina GCT-a može se koristiti kao alternativni pokazatelj upale parodontalnog tkiva. Sa GCT-om možemo videti patološke promene tokom formiranja džepa i proceniti efekte parodontalnog tretmana neinvazivnim metodama, što bi trebalo dokazati merenjem specifičnih proteinskih masa sadržanih u GCT-u.

## **Zahvalnice**

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ome proinflammatory cytokines found in GCF include actin, keratin, histone, annexin, albumin, macrophages, matrix metalloproteinases, and some cytokines such as Immunoglobulin G, Immunoglobulin A, Immunoglobulin M, tumor necrosis factor-alpha (TNF- $\alpha$ ), interleukin-6 (IL-6), and prostaglandinE-2 (PGE-2)<sup>4,18</sup>. Apart from inflammation, several risk factors cause an increase in the volume of GCF, including smoking, circadian periodicity, sex hormones, oral contraceptives, orthodontic and periodontal treatments<sup>4,21</sup>. The secretion of inflammatory proteins found in GCF is assumed to cause an increase in the weight of GCF measured in the present study. But this still needs to be proven by measuring specific protein masses contained in GCF.

## **Conclusion**

Based on the results of the research that has been obtained, it can be concluded that the depth of the periodontal pocket affects the weight of the GCF, and the two are interrelated. The deeper the periodontal pocket, the greater the weight of GCF obtained. Thus, it can be concluded that the hypothesis of the present study can be accepted. Hence, the weight of GCF can be used as an alternative indicator of periodontal tissue inflammation. With GCF, we can see pathological changes during pocket formation and evaluate the effects of periodontal treatment with non-invasive methods. But this still needs to be proven by measuring specific protein masses contained in GCF.

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