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UTICAJ ESTROGENA NA ZDRAVLJE GINGIVE KOD DEVOJČICA

THE INFLUENCE OF ESTROGEN ON THE GINGIVAL HEALTH OF GIRLS

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Sažetak

Uvod. Inflamacija gingive je veoma česta u detinjstvu i pubertetu.

Cilj istraživanja bio je odrediti indekse gingive i vrednosti 17β estradiola u pljuvački i serumu i njegov uticaj na zdravlje gingive kod tinejdžerki.

Pacijenti i metode. Studija je obuhvatila 30 devojčica (starosti od 11 do 14 godina) sa inflamacijom gingive i 30 devojčica kontrolne grupe koje nemaju inflamaciju gingive. Step en zdravlja gingive je procenjen kliničkim merenjem indeksa gingive. Koncentracija 17β estradiola u serumu i pljuvački određena je DRG Estradiol ELISA (EIA-2693) i DRG Salivary Estradiol ELISA (SLV-4188) metodom.

Rezultati. Vrednosti indeksa gingive jasno ukazuju na prisustvo inflamacije gingive. Analiza vrednosti nivoa estrogena u serumu i pljuvački ukazuju na pozitivnu korelaciju indeksa, posebno ističući uticaj 17β estradiola na inflamaciju i krvarenje gingive.

Zaključak. Dobijene vrednosti koncentracije hormona u serumu i pljuvački pokazuju u obe grupe njegov potencijalni uticaj na zdravlje gingive, što ističe ulogu stomatologa u preventivi i terapiji parodontopatije u pubertetu.

Ključne reči: pubertetski gingivitis, indeks gingive, estrogenu hormoni, zdravlje gingive

Abstract

Introduction. Gingival inflammation during childhood and pubertal maturation increases remarkably.

The aim of the study was to determine the values of the gingival indices among teenage girls, salivary and serum values of the dominant female sex hormone 17β estradiol and influence of estrogen hormone on gingival health.

Patients and method. The study included 30 girls (aged 11 to 14 years) with diagnosed gingival inflammation and 30 girls with no gingivitis as a control group. Gingival health was evaluated through clinical examination of gingival indices. Serum and salivary concentrations of 17β estradiol were evaluated with DRG Estradiol ELISA (EIA-2693) and DRG Salivary Estradiol ELISA (SLV-4188) methods.

Results. Gingival index values clearly indicate the presence of gingival inflammation. Analyses of correlative values comparing serum and salivary levels of estrogen hormone with indices of gingival status indicate a positive correlation with all index values, particularly emphasizing the impact of 17 β estradiol on gingival inflammation and gingival bleeding.

Conclusion. The obtained values of the hormone concentrations in serum and saliva, in both groups, suggest their potential impact on the gingival health. This emphasizes the role of dentists in preventive and treatment modalities in patients during the period of puberty.

Key words: puberty gingivitis, gingival index, estrogen hormones, gingival health

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Uvod

Postoji veliki broj novih istraživanja koja nastoje da u potpunosti rasvetle uticaj seksualnih hormona u različitim fiziološkim uslovima i periodima ljudskog života na oralno i parodontalno zdravlje. Smatra se da pubertet može uticati na pojavu inflamacije i uvećanja gingive kod osoba oba pola, sa većom prevalencijom kod devojčica. Različiti rezultati su dobijeni u istraživanju gingivitisa u pubertetu, jedni, za razliku od drugih, jasno potvrđuju povećanje gingivitisa^{1,2}.

Smatra se da su promene u nivou estrogena i progesterona kod devojčica prolazne i da se posle puberteta vraćaju na normalan nivo. Kod većine devojčica dobrog opšteg i gingivalnog zdravlja neće se razviti značajnije promene parodonticijuma izazvane pubertetom ili menstrualnim ciklusom, za razliku od devojčica koje imaju inflamaciju gingive, kod kojih se razvijaju parodontalne promene, čak i kod male akumulacije oralnog biofilma³. Hiperplazije gingive su često prisutne kod osoba sa pubertetskim gingivitisom i predstavljaju direktnu potvrdu da endokrini poremećaji ili varijacije hormona utiču na tkivo gingive menjajući odgovor gingive na lokalne faktore, koji favorizuju akumulaciju oralnog biofilma i progresiju parodontalne bolesti^{4,5}. Smatra se da nakon puberteta, uz normalizaciju hormonskih varijacija, spontano dolazi do smanjenja uvećane gingive, ali samo u odsustvu oralnog biofilma i kamenca⁶.

Cilj istraživanja

Uzimajući u obzir učešće polnih hormona u brojnim fiziološkim procesima u organizmu i održavanju gingivalnog zdravlja, cilj istraživanja bio je utvrditi:

- nivo 17β estradiola u serumu i pljuvački devojčica sa i bez inflamacije gingive u pubertetu;
- gingivalni status devojčica u pubertetu uz pomoć gingivalnih indeksa;
- uticaj estrogena na zdravlje gingive kod devojčica u pubertetu.

Introduction

There is an extensive body of research which seeks to fully elucidate the events associated with fluctuating levels of sex steroid hormones in different physiological conditions and periods of human life, and their impact on oral and periodontal health. Puberty, which is the beginning of sexual maturation of individuals, involves reproductive changes. It is considered that this period may influence the occurrence of gingival inflammation and gingival enlargement in both sexes, with greater prevalence among girls. Pubertal gingivitis is a topic on which conflicting results may be found, some of which clearly confirm the trend of increased signs of gingival disease, and others their absence^{1,2}.

In girls, it is considered that changes in levels of estrogen and progesterone are transient and at postpuberty they return to normal levels. Most of the girls who are in good general and gingival health will not develop significant periodontal changes due to puberty or the menstrual cycle, unlike those with initial signs of gingival inflammation with even relatively small accumulation of dental plaque³. Gingival enlargement can often be found in the population with puberty gingivitis and represents direct confirmation that endocrine disorders or hormonal variations affect the gingival tissue modifying the tissue response to local factors, which may favor plaque accumulation and progression of disease^{4,5}. It is thought that after puberty, with normalization of hormonal variations, there is a spontaneous reduction of gingival increase, but only in the absence of dental plaque and dental calculus⁶.

Aims

Taking into consideration the involvement of sex hormones in numerous physiological processes in the body and maintaining gingival health, the aim of the study was to determine:

- serum and salivary levels of 17β estradiol in girls with and without gingival inflammation at puberty;
- the gingival status among teenage girls using gingival index values;
- the possible influence of estrogen hormone on gingival health in girls in puberty.

Pacijenti i metode

Istraživanje je obavljeno na Klinici za oralnu patologiju i parodontologiju u Skoplju, koje je uključilo dve grupe devojčica u pubertetu. Prva grupa se sastojala od 30 pacijentkinja, uzrasta od 11 do 14 godina sa dijagnozom pubertetski gingivitis, a druga (kontrolna) grupa, koja se sastojala od jednakog broja devojčica, bez znakova inflamacije gingive. Sve ispitanice morale su ispuniti sledeće kriterijume kako bi učestvovala u istraživanju: odsustvo opštih bolesti, da nisu primale antibiotsku terapiju u poslednja tri meseca, da ne primaju hormonsku terapiju.

Zdravlje gingive je procenjeno uz pomoć indeksa oralne higijene: plak indeks (PI) (Silness-Loe)⁷, indeks zubnog kamenca (CI) (Silness-Loe)⁷ i gingivalnih indeksa: gingivalni indeks (GI) (Silness-Loe)⁸, indeks krvarenja gingive (GBI) (Ainamo i Bay)⁹, indeks uvećanja gingive (GE)⁵. Svi pacijenti su bili informisani o prirodi istraživanja i dali pisani pristanak za učestvovanje.

Laboratorijski testovi

Da bi se odredio nivo steroidnog hormona 17 β -estradiola u serumu, kod svih pacijenata uzeto je 5 ccm krvi venepunkcijom kubitalne vene. Nakon centrifugiranja na 3000 rpm 10 minuta, izdvojen je serum u epruvetama koje su zamrznute na -20°C i čuvane do dana analize. U jutarnjim časovima je kod svih ispitanika sakupljana nestimulisana pljuvačka radi određivanja nivoa 17 β -estradiola u pljuvački. Epruvete sa pljuvačkom zamrznute su na -20°C do dana ispitivanja. Nakon odmrzavanja, pljuvačka je ultracentrifugirana, a izdvojeni supernatant ekstrakt analiziran.

Serumski i pljuvačni nivo 17 β estradiola je određen uz pomoć ELISA metode na Klinici za ginekologiju i akušerstvo Medicinskog fakulteta u Skoplju. Za određivanje serumskog nivoa 17 β estradiola korišćen je ELISA DRG Estradiol (EIA-2693) komplet. Metoda se zasniva na principu kompetitivnog vezivanja poliklonalnih zečjih antitela za antigenska mesta na molekulu estradiola. Endogeni estradiol bolesnika je kompetitivan sa estradiolom konjugovanim peroksidazom za vezivanje za antitela. Posle inkubacije, nevezani konjugat se ispere. Količina konjugata

Patients and methods

The investigation was performed at the Clinic for Oral Pathology and Periodontology in Skopje, including two groups of puberty girls. The first group consisted of 30 female patients, aged 11 to 14 years with diagnosed puberty gingivitis, and the second, control group, consisted of an equal number of girls with no signs of gingival inflammation. All patients met the following inclusion criteria: absence of general diseases, not receiving antibiotic therapy in the previous three months, and not receiving hormone therapy.

Gingival health was evaluated using indices of oral hygiene: Index of dental plaque (PI) (Silness-Loe)⁷, Calculus index (CI) (Silness-Loe)⁷, and indices of gingival status: Index of gingival inflammation (GI) (Loe-Silness)⁸, Gingival bleeding index (GBI) (Ainamo & Bay)⁹, Index of gingival enlargement (GE)⁵. All patients were informed about the nature of the research and gave written consent.

Laboratory tests

To determine the levels of steroid hormone 17 β -estradiol, 5 ccm of blood was taken from all patients by venepuncture of the cubitalis vein. After spinning at 3000 rpm for 10 minutes, separated serum was dragged into test tubes and frozen at -20°C until the day of analysis. To determine the salivary levels of 17 β -estradiol, in the morning, unstimulated saliva was collected by a simple extraction, in all the subjects. Tubes with saliva were frozen at -20 ° C until the day of treatment. Saliva after thawing was undergone to ultracentrifugation and the supernatant extract was analyzed with the appropriate method. Serum and salivary 17 β estradiol levels were assessed using the ELISA method, at the Department of Obstetrics and Gynecology, Medical Faculty in Skopje. For the determination of serum 17 β estradiol, ELISA DRG Estradiol (EIA-2693) kit was used. The method is based on the principle of competitive binding of a polyclonal rabbit antibody directed to the antigenic sites of the estradiol molecule. Endogenous estradiol from patients' samples is in competition with peroxidase conjugated estradiol for binding to the antibodies. After

sa vezanom peroksidazom je obrnuto proporcionalna koncentraciji estradiola u uzorku. Posle dodavanja supstrata rastvoru, intenzitet razvijene boje bio je obrnuto proporcionalan koncentraciji estradiola u uzorku pacijenata. Nivo 17β estradiola u pljuvački određen je uz pomoć DRG pljuvačnog estradiol ELISA (SLV-4188) kita na osnovu kompetitivnosti i razdvajanja uzoraka na mikropločici. Nepoznata količina estradiola u uzorku i poznata količina estradiola u peroksidaznom konjugatu kompetitivna su za mesta vezivanja u estradiolnom poliklonalnom antiserumu. Posle dva sata inkubacije, mikro ploča je isprana kako bi se prekinula kompetitivna reakcija. Posle dodavanja rastvora supstrata, koncentracija 17β estradiola obrnuto je proporcionalna izmernoj optičkoj gustini.

Statistički metod

Prikupljeni podaci su statistički obrađeni uz pomoć kompjuterskog programa "Statistika 6" opisnim i inferencijalnim statističkim metodama.

Rezultati

Dobijene vrednosti ukazuju na prisutnu inflamaciju gingive (GI 2,07) praćenu krvarenjem i uvećanjem gingive (GB 1,73 i GE 1,93) (Tabela 1).

Pirsonov koeficijent korelacije (r) ukazuje na snažnu pozitivnu korelaciju između indeksa plaka i inflamacije gingive (0,68), kao i između indeksa zubnog kamenca i krvarenja gingive (Tabela 2).

Prosečne vrednosti 17β estradiola u eksperimentalnoj grupi kretale su se od 4,15 pg/ml u pljuvački do 56,73 pg/ml u serumu, dok su se kod devojčica bez znakova pubertetskog gingivitisa kretale od 3,31 pg/ml u pljuvački do 46,20 pg/ml u serumu (Tabela 3).

Pirsonov koeficijent korelacije (r) ukazuje na snažnu pozitivnu korelaciju između serumskih vrednosti 17β estradiola i inflamacije gingive i krvarenja, kao i između pljuvačnih vrednosti estradiola i krvarenja gingive (Tabela 4).

incubation, unbound conjugate was washed away. The amount of peroxidase-linked conjugate was inversely proportional to the concentration of estradiol in the sample. After adding the substrate solution, intensity of developed color was inversely proportional to the concentration of estradiol in patient sample. Determination of salivary 17β estradiol level was done using DRG Salivary Estradiol ELISA (SLV-4188) kit. It was based on the principle of competition and separation on a microplate. An unknown amount of estradiol present in the sample and a particular amount of estradiol peroxidase conjugate competed for binding sites of estradiol polyclonal antiserum. After two-hour incubation, microplate was washed to stop the competitive reaction. After adding the substrate solution, concentration of 17β estradiol was inversely proportional to the measured optical density.

Statistical method

The collected data were statistically processed by the computer program "Statistika" 6 using descriptive and inferential statistical methods.

Results

The obtained values indicate significant gingival inflammation (GI 2.07) followed by gingival enlargement and gingival bleeding (GB 1.73 and GE 1.93) (Table 1).

Pearson's coefficient of correlation (r) indicates a strong positive correlation between the plaque index and gingival inflammation (0,68), and between the calculus index and gingival bleeding (Table 2).

The average values of 17β estradiol in experimental group range from 4.15 pg / ml in the saliva to 56.73 pg / ml in serum, whereas in girls with no signs of puberty gingivitis they range from 3.31 pg / ml in saliva to 46.20 pg / ml in serum (Table 3).

Pearson's coefficient of correlation (r) indicates a strong positive correlation between serum values of 17β estradiol and gingival inflammation and gingival bleeding, and between the salivary values of estradiol and gingival bleeding (Table 4).

Tabela 1. Srednje vrednosti indeksa oralne higijene i gingivalnih indeksa kod devojčica sa pubertetskim gingivitisom**Table 1.** Mean values of indices of oral hygiene and gingival indices in girls with puberty gingivitis

<i>Index</i>	<i>AVERAGE</i>	<i>SD</i>	<i>min</i>	<i>max</i>
Plak I /Plaque I	1.13	0.35	1.0	2.0
Kamenac I /Calculus I	0.73	0.45	0.0	1.0
Inflamacija gingive /Gingival inflammation	2.07	0.26	2.0	3.0
Uvećanje gingive /Gingival enlargement	1.93	0.26	1.0	2.0
Krvarenje gingive /Gingival bleeding	1.73	0.46	1.0	2.0

Tabela 2. Pearsonov koeficijent korelacije (r) između vrednosti gingivalnih indeksa i indeksa oralne higijene kod djevojčica sa pubertetskim gingivitisom**Table 2.** Pearson's coefficient of correlation (r) between the values of gingival indices and indices of oral hygiene in girls with puberty gingivitis

	GI	GE	GB
	r		
PI	0.68	0.10	0.24
CI	0.16	0.44	1

Tabela 3. Srednje vrednosti 17 β estradiola (E) u serumu i pljuvački kod devojčica sa i bez gingivitisa**Table 3.** Mean values of 17 β estradiol(E) in serum and saliva in female children with and without gingivitis

Parameter pg/ml	sa gingivitisom/with gingivitis		bez gingivitisa/without gingivitis	
	average	SD	average	SD
E u serumu/E in serum	56.73	14.32	46.20	8.03
E u pljuvački/E in saliva	4.15	0.66	3.31	0.52

Tabela 4. Pearsonov koeficijent korelacije (r) između vrednosti 17 β estradiola (E) u serumu i pljuvački i gingivalnih indeksa kod devojčica sa pubertetskim gingivitisom**Table 4.** Pearson's coefficient of correlation(r) between the values of 17 β estradiol(E) in serum and saliva and gingival indices in female children with puberty gingivitis

17 β estradiol	GI	GE	GB
	r		
serum/serum	0.64	0.52	0.62
pljuvačka/saliva	0.44	0.48	0.69

Postoji jaka pozitivna korelacija ($r=0,64$) između serumskih vrednosti 17β estradiola i inflamacije gingive (Grafikon 1).

Postoji jaka pozitivna korelacija ($r=0,62$) između serumskih vrednosti 17β estradiola i vrednosti gingivalnog krvarenja (Grafikon 2).

Postoji jaka pozitivna korelacija ($r=0,69$) između vrednosti 17β estradiola u pljuvački i krvarenja gingive (Grafikon 3).

Analiza primenom Mann-Whitney U testa pokazala je da postoje statistički značajne razlike u srednjim vrednostima 17β estradiola u serumu između devojčica sa i bez gingivitisa ($Z=-2,447$, $p=0,0144$) (Grafikon 4).

Analiza primenom Mann-Whitney U testa pokazala je da postoje statistički značajne razlike u srednjim vrednostima 17β estradiola u pljuvački kod devojčica sa i bez gingivitisa ($Z=-3,152$, $p=0,0016$) (Grafikon 5).

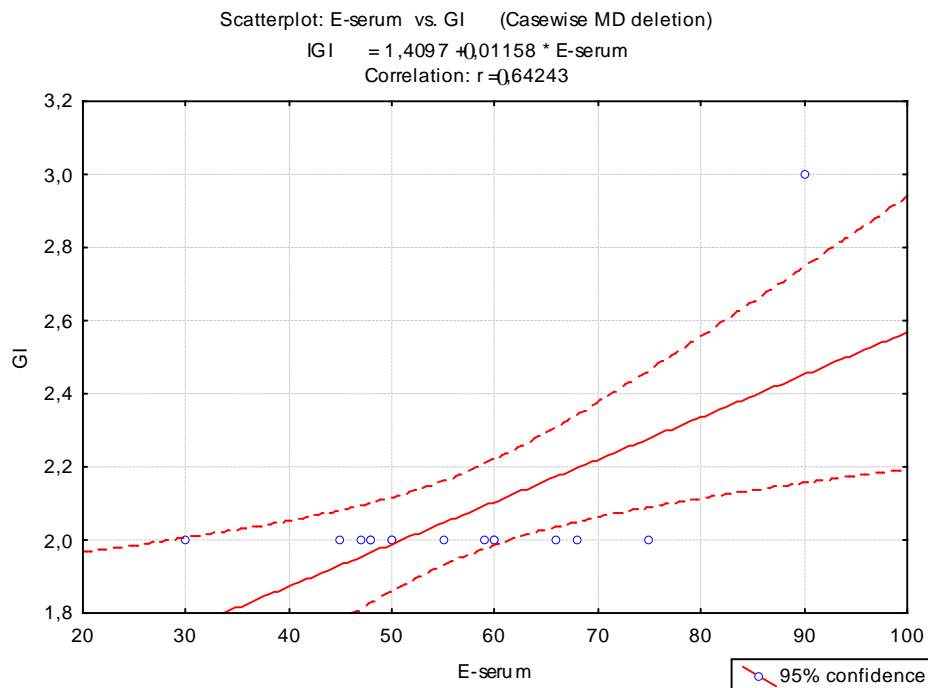
There is a strong positive correlation ($r=0,64$) between serum 17β estradiol values and gingival inflammation (Figure 1).

There is a strong positive correlation ($r=0,62$) between serum 17β estradiol values and gingival bleeding (Figure 2).

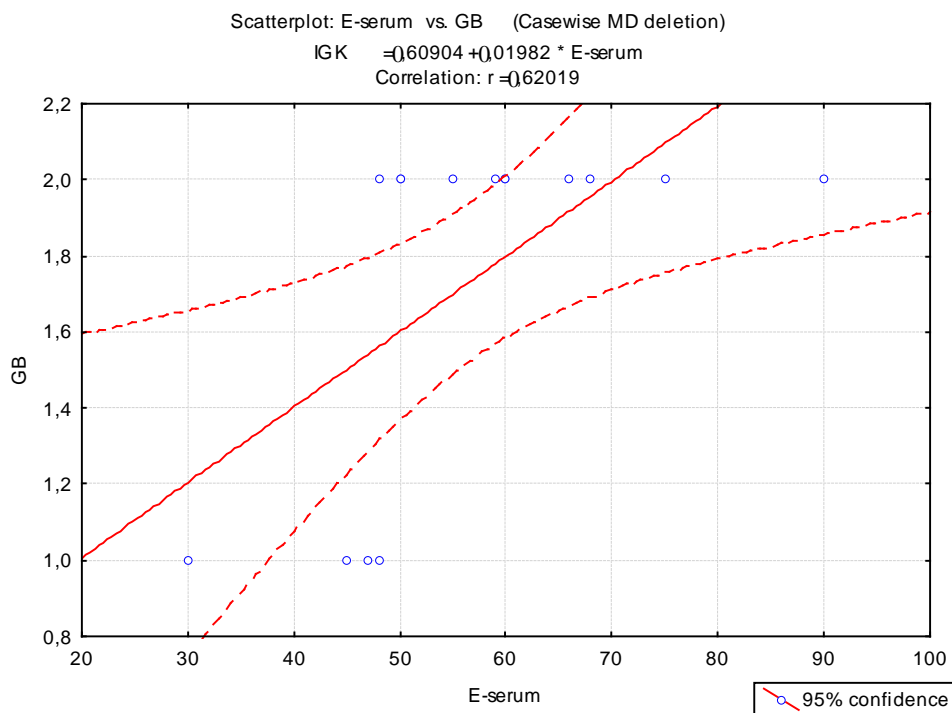
There is a strong positive correlation ($r=0,69$) between 17β estradiol values in saliva and gingival bleeding (Figure 3).

Analysis using Mann-Whitney U tests shows that there are statistically significant differences in mean values of serum 17β estradiol values between girls with and without gingivitis ($Z=-2.447$, $p=0.0144$) (Figure 4).

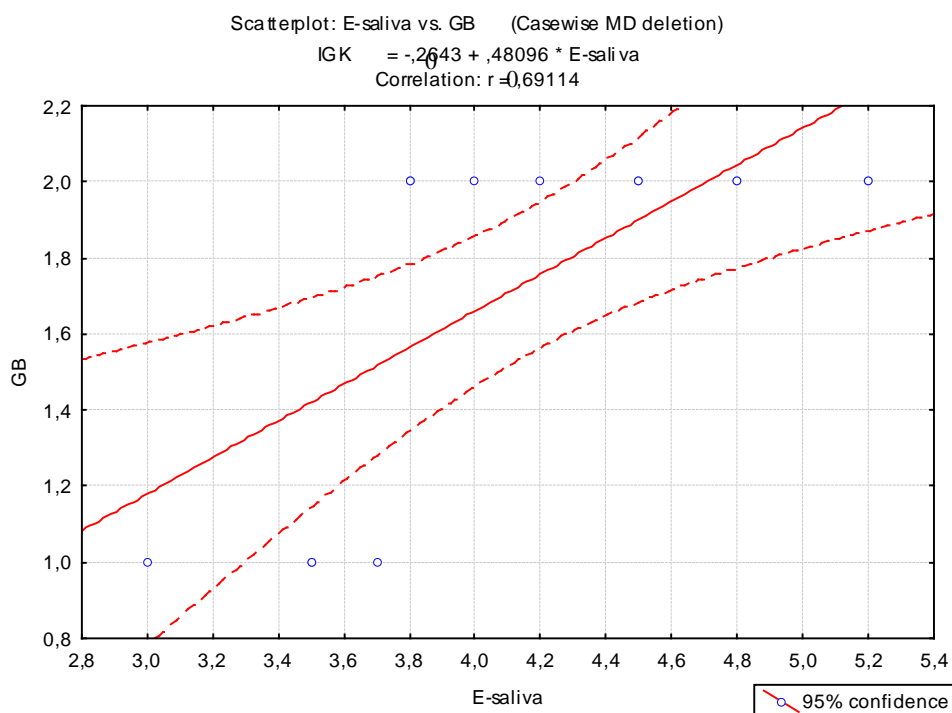
Analysis using Mann-Whitney U test shows that there are statistically significant differences in mean values of salivary 17β estradiol between girls with and without gingivitis ($Z=-3.152$, $p=0.0016$) (Figure 5).



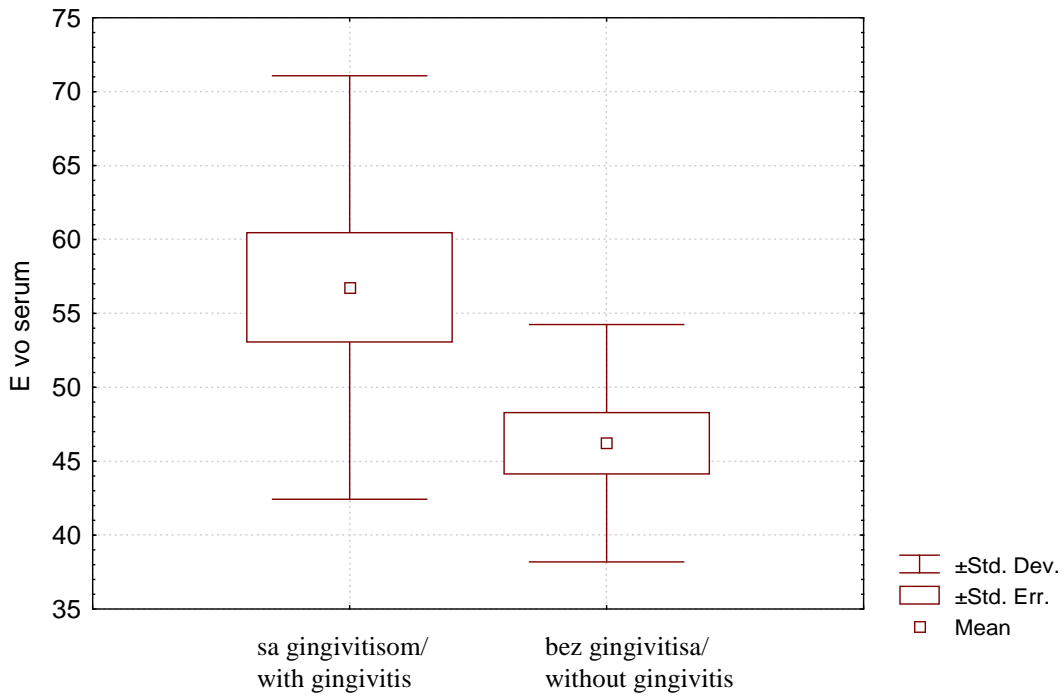
Grafikon 1. Korelacija između serumskih vrednosti u 17β estradiola i inflamacije gingive (GI)
Figure 1. Correlation between serum 17β estradiol values and gingival inflammation (GI)



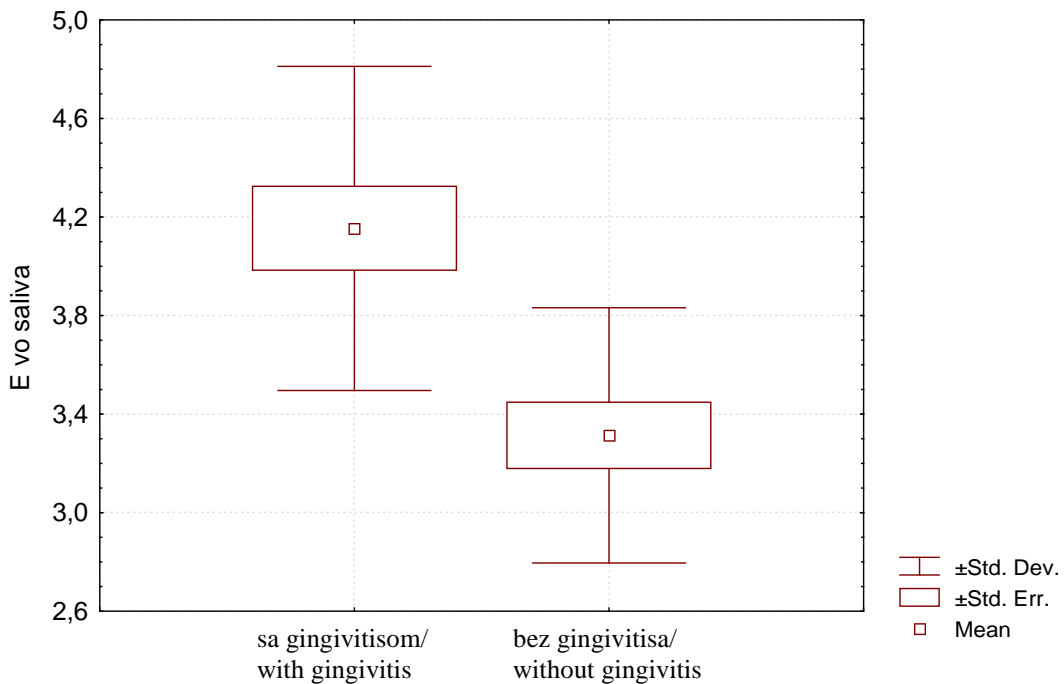
Grafikon 2. Korelacija između serumskih vrednosti 17β estradiola i gingivalnog krvarenja (GB)
Figure 2. Correlation between serum 17β estradiol values and gingival bleeding (GB)



Grafikon 3. Odnos između vrednosti 17β estradiola u pljuvački i krvarenja gingive (GB)
Figure 3. Correlation between 17β estradiol in saliva and gingival bleeding (GB)



Grafikon 4. Srednje vrednosti 17β estradiola u serumu kod devojčica sa i bez gingivitisa
Figure 4. Mean values of serum 17β estradiol values in girls with and without gingivitis



Grafikon 5. Srednje vrednosti 17β estradiola u pljuvački kod devojčica sa i bez gingivitisa
Figure 5. Mean values of salivary 17β estradiol values in girls with and without gingivitis

Diskusija

Posljednjih godina poboljšano je razumevanje uticaja seksualnih steroidnih hormona na zdravlje. Iako nema sumnje o značaju polnih hormona u reproduktivnoj endokrinologiji, postoje mnogobrojni dokazi koji ukazuju na njihovu širu ulogu u ljudskom organizmu^{10,11}. Smatra se da su estrogenski hormoni direktno ili indirektno uključeni u rad različitih tkiva, kao što su mozak, bubreg, srce, jetra, koža i parodontalni kompleks. Iako su u cirkulaciji prisutni u izuzetno niskim koncentracijama, oni su u stanju da regulišu diferencijaciju i rast ciljnih tkiva udaljenih od mesta sekrecije hormona.

Veliki broj istraživanja sugeriraju da reakcija parodonta zavisi od aktivnosti androgenih hormona, estrogena i progesterona. Ističe se da parodont predstavlja ciljno mesto dejstva seksualnih hormona^{12,13}, što se manifestuje kroz kliničke promene parodonta u toku puberteta. Ovi nalazi potvrđuju učestalost oboljenja gingive u slučaju promene nivoa polnih hormona, čak i u uslovima kada je oralna higijena nepromenjena¹⁴.

Zbog individualnih razlika u vremenu nastanka i trajanja puberteta, svi klinički, mikrobiološki i hormonski parametri treba da budu u skladu sa starošću pacijenata i parametrima koji opisuju sazrevanje u toku puberteta. Dakle, vrednosti gingivalnih indeksa koje su povećane u pubertetu zavise i od pravilnog izbora parametara i primenjenih analitičkih metoda^{15,16}.

Neki autori¹⁷ su primetili da svi faktori koji mogu da menjaju zapaljenski odgovor moraju biti eliminisani kako bi se procenilo dejstvo hormona na zdravlje gingive. Iz ovih razloga, veruje se da su tinejdžeri najadekvatnija starosna grupa, jer je koncentracija polnih hormona i učestalost gingivitisa povećana u ovom periodu života. Nastanak gingivitisa, koji je uočen kod dece i adolescenata, može biti povezan i sa sastavom oralnog biofilma, odgovorom ćelija na inflamaciju, hormonskim promenama, morfološkim razlikama i nicanjem zuba^{18,19}. Predeo gingivalnog sulkusa snažno odražava endokrini status osobe. Istraživanja kod dece u pubertetu opisuju iznenadno i prolazno povećanje inflamacije gingive koja nije praćena

Discussion

Recent years have dramatically enhanced the perception of action of sex steroid hormones in terms of health. While there is no doubt about the importance of sex hormones in reproductive endocrinology, there is growing evidence that suggests much broader role of gonadal hormones in the human organism^{10,11}. It is thought that estrogen hormones are directly or indirectly involved in the regulation of various tissues such as brain, kidney, heart, liver, skin, and periodontal tissue complex. Although the blood circulating sex hormones exist in extremely low concentrations, they are able to regulate differentiation and growth in selected tissues distant from the site of secretion.

The growing body of evidence suggests that periodontal tissue reactions are modulated by the action of androgens, estrogens and progestins. Some of them imply that periodontal tissues are target tissues of sex hormone^{12,13}, referring to the clinical manifestations during periods of hormonal changes. These clinical observations confirm the prevalence of gingival disease with fluctuating levels of sex hormones, even in conditions when oral hygiene remains unchanged¹⁴.

Because the period of starting and duration of puberty shows individual variations, all clinical, microbiological and hormonal parameters should be in line with chronological age and the parameters that describe pubertal maturation. Hence, demonstration of gingival index values which increase during puberty strongly depends on the proper selection of parameters and analytical methods^{15,16}.

Some authors¹⁷ noticed that all additional factors that could modify the inflammatory response must be eliminated in order to explore the effects of hormone action on gingival health. For these reasons, it is believed that children of teenage group are most adequate, since the concentration of sex hormones and the incidence of gingivitis are increased at that period of life. The onset of gingivitis, which is evident in children and adolescents, may be associated with the composition of dental plaque, inflammatory cell response, hormonal changes,

kvantitativnim promenama oralnog biofilma²⁰⁻²⁴. Mombelli i sar.²⁵ su u longitudinalnom istraživanju utvrdili da je prosečna vrednost indeksa krvarenja papile (PBI) u vezi sa razvojem sekundarnih polnih karakteristika dece u pubertetu. Vrednosti ovog indeksa su dostigle svoj vrhunac 1,5 godinu od početka puberteta kod 35% osoba, ali ukupan plak indeks u toku godine nije bio u porastu. Nasuprot ovome, druga istraživanja nisu otkrila značajnu povezanost između ranog puberteta i gingivalnih promena kod devojčica²⁶.

Ova disproporcija u nalazima može biti posledica različitih faktora, kao što su nivo oralne higijene ili sam način istraživanja. Prosečni uzrast u kome se dostiže maksimalni stepen gingivalne inflamacije bio je 12 godina i 10 meseci za devojčice i 13 godina i 10 meseci za dečake, dok prema drugoj obimnijoj studiji, inflamacija gingive počinje u 11. godini kod oba pola, pri čemu nivo oralnog biofilma ostaje konstantan u svim starosnim grupama²⁷. Povećanje inflamacije gingive može se delimično objasniti različitom odbranom domaćina²⁸. Značajna veza je uočena između nivoa antitela IgG u serumu i antitela usmerenih na *P. Intermedia*, sa nivoom polnih hormona kod osoba oba pola sa inflamacijom gingive, a odnos IgM antitela usmerenih na sojeve *P. intermediae* i polnih hormona je značajan samo kod dečaka sa izraženom inflamacijom gingive²⁹.

Zbog nedostatka adekvatnih istraživanja, koja će istražiti uticaj polnih hormona na zdravlje gingive, u ovom istraživanju tačno je određen nivo polnog hormona estradiola 17 β u serumu i pljuvački i njegov odnos sa gingivalnim indeksom. Rezultati eksperimentalne grupe pokazali su prisutnu inflamaciju gingive, koja je u skladu sa nalazima drugih autora koji ukazuju na inflamaciju gingive kod devojčica u pubertetu^{2,15,25}, koja zavisi od promenjenih nivoa polnih hormona, starosti, bakterijskog sastava oralnog biofilma, inflamatornog odgovora ćelija, vaskularne reakcije, morfološke razlike itd^{30,31}. Istraživači ističu da je u pubertetu povećan gingivalni indeks, koji značajno raste sa razvitkom puberteta, a njegov pad je uočen posle 14 godina kod devojčica^{15,25}.

morphological differences, as well eruption of teeth^{18,19}. Sulcus ecological milieu is a dynamic medium that is in line with the process of pubertal maturation and strongly reflects the chronological and endocrine parameters. Several researches performed in children in puberty describe sudden and transient amplification of gingival inflammation which is not accompanied by quantitative changes in plaque²⁰⁻²⁴. Mombelli et al.²⁵ in longitudinal research determined that the average index value of papillary bleeding (PBI) is consistent with the development of secondary sexual characteristics of children in puberty. These values reached their peak after 1.5 years from the start of puberty in 35% of individuals, but total plaque index recorded annually did not display significant trend of increase. In contrast, other studies failed to note a significant association between early puberty and gingival changes in girls on the verge of puberty²⁶.

This disproportion could be due to factors such as status of oral hygiene or the design of the study. The average age at which girls and boys reach the maximum degree of gingival inflammation was 12 years and 10 months and 13 years and 10 months respectively, while according to another more extensive study it is estimated that gingival inflammation starts at 11 years of age in both sexes, whereby the level of plaque remains constant across all age groups²⁷. Growth of gingival inflammatory reaction, in part, can be explained by the factors of host defenses²⁸. Significant proportion occurs between serum IgG antibodies directed to *P. intermedia* and the level of sex hormones in both sexes noted in gingival inflammation, while the ratio between IgM antibodies directed to the strains of *P. intermedia* and sex hormones is significant only in boys with marked gingival reaction²⁹.

Due to the lack of more subtle studies, which would elaborate the influence of sex steroid hormones on gingival health, in our research we made an accurate determination of serum and salivary values of sex hormone 17 β estradiol and its correlation with gingival indices. The results from the experimental group expressed the presence of gingival inflammation. The obtained results are consistent with the

U cilju određivanja uticaja lokalnih etioloških faktora na zdravlje gingive, u ovom istraživanju određen je odnos između indeksa zubnog plaka, indeksa zubnog kamenca, indeksa uvećanja gingive i krvarenja gingive. Dobijeni rezultati pokazuju snažnu pozitivnu vezu između plak indeksa i indeksa gingive, koji je $r=0,68$; dok odnos indeksa zubnog kamenca i uvećanja gingive u rasponu od slabog ($r=0,10$) do umerenog ($r=0,24$) u odnosu na indeks krvarenja gingive. Ovi nalazi naglašavaju ulogu oralnog biofilma u nastanku inflamacije gingive i ukazuju na uticaj drugih faktora u njihovoj ekspresiji.

Što se tiče uticaja oralnog biofilma kao lokalnog iritirajućeg faktora uočena je snažna veza sa indeksom krvarenja gingive ($r=1$), umereno pozitivna ($r=0,44$) sa indeksom uvećanja gingive i blago pozitivna ($r=0,16$) sa inflamacijom gingive. Prema rezultatima longitudinalnog istraživanja Yanover i Ellena³² postoji pozitivna veza između gingivalnog indeksa i indeksa zubnog plaka kod devojčica u pubertetu. U istraživanju Nakagawe i sar.²⁹ uočeno je statistički značajno povećanje inflamacije gingive kod devojčica u pubertetu u odnosu na prepubertetski period, bez značajne razlike u vrednostima indeksa zubnog plaka i kamenca. Suprotno ovome, Tianen i sar.³³ su uočili statistički značajnu vezu između oralnog biofilma i stepena krvarenja gingive na početku i na kraju puberteta, čime ukazuju na važnost oralne higijene u održanju zdravlja gingive, uprkos oscilacijama u nivou polnih hormona.

Pored procene kliničkih parametara gingivalnog statusa kod devojčica u pubertetu, u ovom istraživanju određene su i serumske i koncentracije 17β estradiola u pljuvački, koje su se u eksperimentalnoj grupi kretale od 4,15 pg/ml u pljuvački do 56,73 pg/ml u serumu, a kod devojčica bez znakova pubertetskog gingivitisa od 3,31 pg/ml u pljuvački do 46,20 pg/ml u serumu.

Kako bi se procenio uticaj 17β estradiola na zdravlje gingive u eksperimentalnoj grupi devojčica, određen je nivo estradiola u serumu i gingivalni indeks i uočena jaka međusobna veza. Prema ovome, odnos vrednosti 17β estradiol u serumu i inflamacije gingive je

the findings of several authors who suggest an increased trend of gingival disorder in girls during puberty maturation^{2,15,25}, which depends on the fluctuating levels of sex hormones, age, bacterial composition of dental plaque, inflammatory cell response, vascular reactions, morphological differences, etc^{30,31}. During puberty, an increased gingival bleeding index significantly grows with development of puberty, while significant downward trend is seen after 14 years of age for girls¹⁵.

In order to determine the influence of local etiological factors on gingival health, the ratio between the index of dental plaque, index of calculus, index of gingival inflammation, index of gingival enlargement and gingival bleeding was established. The obtained results demonstrated a strong positive correlation between the plaque index and gingival inflammation ($r=0,68$), while the ratio between dental plaque and gingival enlargement ranged from weak insignificant ($r=0,10$) to moderately positive ratio ($r=0,24$), with regard to the index of gingival bleeding. These findings emphasize the supreme role of dental plaque in the development of gingival inflammation and suggest the involvement of additional factors for their expression.

Regarding the impact of dental plaque as a local irritating factor, a strong correlation with gingival bleeding index ($r=1$) was obtained, as well as a moderate positive correlation ($r=0,44$) with the index of gingival enlargement and slight positive correlation ($r=0,16$) with gingival inflammation. According to the results from this study, Yanover and Ellen's³² longitudinal study on girls who normally go through puberty indicates a positive correlation observed between gingival index and index of dental plaque. In a longitudinal study, Nakagawa et al.²⁹ presented statistically significant increase in gingival inflammation in girls at the age of puberty, unlike the prepubertal age, without noting significant differences in indices of dental plaque and calculus. Contrary, Tianen et al.³³ report statistically high significant correlation between dental plaque and the degree of gingival bleeding at the beginning and at the end of puberty, suggesting the dominant importance of oral hygiene on

=0,64, uvećanja gingive i krvarenja gingive $r=0,52$ i $r=0,62$, za svaki pojedinačno.

Rezultati ovog istraživanja su u skladu sa nalazima drugih longitudinalnih istraživanja³⁴ koja su pokazala vezu između visokog nivoa 17β estradiola u serumu i gingivalnog indeksa kod devojčica sa pubertetskim gingivitisom. Uočena je pozitivna veza povećanog nivoa estradiola u plazmi i prisustva sojeva *Bacteroides* kod devojčica u ranom pubertetu³². Smatra se da nivo hormona bezuslovno ne podržava kolonizaciju patogenih sojeva predominantnih kod parodontopatije odraslih. Serumski nivo estradiola kod devojčica u pubertetu u pozitivnoj je korelaciji sa nivoom *P. intermedia* i *P. nigrescens*, dok je nivo serumskih IgG antitela usmerenih na ove sojeve značajno povećan i zavisen od nivoa ženskih polnih hormona²⁹.

Analiza vrednosti 17β estradiola u pljuvački i gingivalnog indeksa kod devojčica sa pubertetskim gingivitisom otkriva jaku pozitivnu vezu $r=0,69$ sa indeksom krvarenja gingive, umereno zavisnu vezu sa gingivalnim indeksom $r=0,44$ i sa uvećanjem gingive $r=0,49$. U ovom istraživanju, pored određivanja koncentracija estradiola u serumu i pljuvački devojčica sa pubertetskim gingivitisom, nivo odgovarajućeg hormona određen je i kod devojčica bez znakova gingivitisa. Upoređujući dobijene vrednosti 17β estradiola u serumu obe grupe, Mann-Whitney U test pokazao je statistički značajnu razliku u prosečnim vrednostima serumskog estradiola u obe grupe. Takođe, analiza prosečnih vrednosti estradiola u pljuvački, uz pomoć Mann-Whitney U testa, pokazala je da postoje statistički značajne razlike u nivou estradiola između devojčica sa gingivitisom i onih bez gingivitisa.

Nova istraživanja³⁴⁻³⁶ su svakako još jedna direktna potvrda uticaja polnih hormona u nastanku gingivitisa kod devojčica u pubertetu.

gingival condition despite fluctuations in the level of sex hormones.

Besides the evaluation of clinical parameters of gingival status in girls during puberty, in our study, serum and salivary concentrations of ovarian hormone 17β estradiol in experimental group ranged from 4.15pg/ml in saliva to 56.73pg/ml in serum, whereas in girls with no signs of puberty gingivitis ranged from 3.31pg/ml in saliva to 46.20pg/ml in serum. In order to determine the impact of 17β estradiol on gingival health in the experimental group of girls, correlations between serum levels of estradiol and indices of gingival status were established and strong positive correlation with each of them was obtained. Thus, the correlation of serum 17β estradiol with gingival inflammation was $r=0.64$, while gingival enlargement and gingival bleeding were $r=0.52$ and $r=0.62$, respectively. The results from our study are consistent with findings from longitudinal study³⁴ presenting statistically high significant correlation between increased serum levels of 17β estradiol and gingival index in girls with puberty gingivitis. Other authors³² observed a positive ratio with the increased plasma levels of estradiol and the presence of black pigmented strains of *Bacteroides* in girls in early puberty. They suggest that the hormonal events do not unconditionally support the colonization of pathogenic strains prevalent in adult periodontal disease. Serum levels of estradiol in girls at puberty were positively correlated with the level of *P. Intermedia* and *P. Nigrescens* while the levels of serum IgG antibodies directed to these strains are significantly increased, which also shows a significant positive correlation with levels of female sex hormones²⁹.

Analysis of the ratio between salivary values of 17β estradiol and gingival indices in girls with puberty gingivitis reveals a strong positive correlation $r=0.69$ with the index of gingival bleeding and moderate positive relative value of gingival inflammation $r = 0.44$ and gingival enlargement $r = 0.49$. In our study, despite the determination of serum and salivary concentrations of estradiol in pubertal girls with gingivitis, the level of the appropriate hormone was determined in girls without signs of gingival disease. Comparing

Zaključak

1. Vrednosti 17β estradiola u serumu ukazuju na jaku pozitivnu vezu sa gingivalnim indeksom i indeksom krvarenja gingive i umerenu sa indeksom uvećanja gingive. Vrednosti 17β estradiola u pljuvački ukazuju na jaku pozitivnu vezu sa krvarenjem gingive i umerenu sa gingivalnim indeksom i indeksom uvećanjem gingive. Takođe, i u serumu i u pljuvački uočena je jaka pozitivna veza sa koncentracijom 17β estradiola.

2. Indeksi gingivalnog statusa u eksperimentalnoj grupi devojčica u pubertetu pokazuju izraženo prisustvo inflamacije gingive.

3. Dobijene vrednosti koncentracija hormona u oba medijuma, u obe grupe, sugerišu njihov potencijalni uticaj na zdravlje gingive. Ovaj nalaz ističe ulogu stomatologa u preventivi i terapiji gingivitisa kod pacijenata u pubertetu.

the obtained values of serum 17β estradiol in both groups of girls, the analysis using the Mann-Whitney U test showed a statistically significant difference in average values of serum estradiol in both groups. Also, the analysis of average salivary values of estradiol, using the Mann-Whitney U test, showed that there are statistically significant differences in the level of estradiol in girls with apparent gingival inflammation and in girls without gingivitis.

Recent findings³⁴⁻³⁶ are certainly another direct confirmation of involvement of sex hormones in the development of gingival alterations among girls in puberty.

Conclusions

1. Serum 17β estradiol values indicated a strong positive correlation with the index of gingival inflammation and gingival bleeding and moderate positive correlation with the index of gingival enlargement. Salivary 17β estradiol values indicated a strong positive correlation with gingival bleeding, and moderate positive correlation with the index of gingival inflammation and gingival enlargement. Also, in both media - serum and saliva, a strong positive correlation between the concentrations of 17β estradiol was found.

2. The indices of gingival status in the experimental group of girls at the age of puberty reveal considerable presence of gingival inflammation.

3. The obtained values of the hormone concentrations in both media and in both groups suggest their potential influence on the gingival health. This emphasizes the role of dentists in preventive and treatment modalities in patients with gingivitis in puberty.

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