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KOMPARATIVNA ANALIZA KLINIČKOG I PATOHISTOLOŠKOG NALAZA U GINGIVI KOD DECE

COMPARATIVE ANALYSIS OF THE CLINICAL AND PATHOHISTOLOGIC GINGIVAL STATUS IN CHILDREN

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Kratak sadržaj

U kliničkoj praksi se često susrećemo sa izmenjenom gingivom, u smislu kataralnog početnog ili hroničnog gingivitisa koji može dovesti do parodontitisa. Imunocitohemijska zbivanja inflamirane gingive se reperkutuju na anatomo-morfološke osobenosti gingive. Radi adekvatne dijagnostike, rađena su patohistoloska i imunocitohemijska istraživanja izmenjene gingive, kako bi se utvrdilo da li su dobijeni rezultati u korelaciji sa kliničkim nalazom.

Cilj istraživanja je bio da se kliničkim indeksima (PMA-indeks po Schouru i Masleru) proceni stanje gingive, utvrdi priroda inflamiranog infiltrata izmenjene gingive, identifikuje imunofenotipski profil celularnog infiltrata, izvrši subtipizacija T i B limfocita i utvrdi da li se nađeno stanje gingive poklapa sa patohistološkim nalazom.

Za patohistološku i imunocitohemijsku analizu uzeti su biopsijski uzorci gingive od 52 ispitanika kod dece koja su imala jasnu ili izraženu inflamaciju na gingivi, a kod kojih je bila indikovana ekstrakcija zuba. Kontrolnu grupu činili su gingivalni uzorci uzeti od 10 ispitanika, koji su pripadali ukupnom uzorku od 86 ispitanika, a kod kojih je gingiva bila bez elemenata zapaljenja (zdrava).

Na osnovu dobijenih rezultata došlo se do zaključka da je nađeno stanje gingive u korelaciji sa patohistološkim i imunocitohemijskim nalazom, što je i bio glavni cilj istraživanja.

Ključne reči: *gingivitis, parodontitis, patohistološka analiza, imunocitohemijska analiza*

Abstract

In our clinical practice we have very often observed gums affected by catarrhal-initial or chronic-developed gingivitis that may lead, alongside with the disease progression, to periodontitis. The immunocytochemical changes of the inflamed gingival reverberate in the anatomical and morphological gingival characteristics. With the aim to make an adequate diagnosis and thus administer suitable treatment, immunocytochemical research of the affected gums was conducted in order to determine whether the pathohistologic findings coincide with the clinical findings.

The aim of the research was to determine the condition of the changed gingiva by the clinical indexes (PMA-index according to the method of Schour and Massler), to determine the nature of gingival inflammatory infiltrate, to identify the immuno-phenotypical profile of cellular by means of monoclonal antibodies of the affected gingiva, and to subtypify T and B lymphocytes in the affected gingiva.

For pathohistologic and immunocytochemical analysis they took gingival samples for biopsy from 52 subjects, from the children with clear or expressed inflammation of the gingival where tooth extraction was indicated. The control group consisted of the gingival samples taken from 10 subjects who belonged to the total sample of 86 subjects and who had the healthy gingiva.

The achieved results confirmed that the clinical gingival status corresponds to the pathohistologic and immunocytochemical findings, which was the main goal of the research.

Key words**:** *gingivitis, parodontitis, pathohistologic analysis, immunocytochemical analysis*

Uvod

Zapaljenjski procesi na gingivi nastaju kao posledica dejstva primarnog etiološkog faktora dentalnog plaka, koji dužim dejstvom na jednom mestu uz odgovarajuću bakterijsku floru, menja gingivu u anatomo-morfološkom pogledu. Pod hemijskim i toksičnim dejstvom mikroflore dentalnog plaka kao i pod dejstvom

Introduction

Gingival inflammatory processes originate primarily from a poor oral hygiene, which may be clinically confirmed by the presence of dental plaque and by the anatomically and morphologically changed gingivae. The gingival changes are reflected in its color, volume, shape, consis-

lokalne imunološke reakcije domacina kao posledica homeostatskih mehanizama, može doći do nepovratnog oštećenja ćelija što vodi ispoljavanju bolesti na gingivi i ostalim delovima parodonta.

Mnogobrojne sistemske bolesti kao sto su infekcije sa herpes virusom, krvne diskrazije, autoimune bolesti (pemfigus), bolesti metabolizma se mogu manifestovati na gingivi sa karakterističnim promenama koje prate gingivitis. Pojedini lekovi, kao antiholinergici, antihistaminici mogu izazvati lezije na gingivi.[1]

Nelečeni gingivitis predstavlja dobru podlogu i put za prodiranje patogena u ostala tkiva parodoncijuma. Sa pripojne gingive inflamacija se širi i dovodi do ogoljavanja korena zuba i formiranja parodontalnih džepova. Na alveolarnoj kosti promene su u vidu resorpcije. Konačan ishod bolesti je labavljenje zuba, migracija i ispadanje zuba.[2]

Još nije razjašnjeno koliko je destrukcija parodontalnih tkiva posledica direktnog dejstva mikrobnog agensa, a koliko izraz indirektnih efekata kao reakcija domaćina na patogen.[3]

Opservacije da su T i B limfociti prisutni kod juvenilne parodontopatije a da ih nema u zdravoj gingivi, bile su povod proučavanja njihove uloge u parodontalnim bolestima.[4] Ovo je bio i izazov za naša istraživanja.

Pretpostavlja se da limfociti mogu imati dvojaku ulogu:

1. protektivnu, protiv infektivnog agensa i
2. mogu doprineti nastanku tkivne destrukcije.

Te dve aktivnosti limfocita jedna drugu isključuju. Postoje podaci da oba ova faktora (protektivni i destruktivni) mogu egzistirati istovremeno.[5,6]

Postoje osobe koje su „visoko suspektne" na pojavu i razvoj parodontalnih oboljenja. Ovaj termin govori o aspektima životnih navika, faktorima sredine i urođenim osobenostima. U proučavanju faktora rizika, bitno je identifikovati jedan ili nekoliko individualnih faktora koji mogu biti u vezi sa ispitivanom bolešću. U slučaju multipnih faktora kao model može poslužiti kombinacija faktora koji čine da se napravi razlika između osoba sa visokim i niskim rizikom.[7]

Istrazivanja su pokazala da se početne promene na gingivi u vidu inflamacije javljaju 4 dana nakon plak akomulacije. Posle 7 dana

tency and surface structure. Gingivitis appears under the chemical and toxic impact of the dental plaque micro-flora, as well as under the effects of the host's local immunologic reaction, and represents the consequence of homeostatic mechanisms which unavoidably cause irreversible cell damages.

Numerous systemic diseases, like infections by the virus of herpes, blood dyscrasia (leukemia), autoimmune diseases (pemphigus) and disorders of metabolism, are manifested in the gingiva with the changes characteristic of gingivitis. Certain medications, such as anticholinergics, antihistamines or antidepressants, cause lesions in the gingiva.[1]

Untreated gingivitis leads to further penetration of pathologic agents into other periodontal tissues. The inflammation spreads from the tooth attachment and causes separation of the gums from the teeth and formation of periodontal pockets. The changes in the alveolar bone are reflected in the resorption. It finally results in loosening, migration and falling out of the teeth.[2]

It has not been cleared out so far to which extent the destruction of periodontal tissues is directly caused by bacterial agents or by the indirect effects of the host's reaction to pathogens.[3]

The observations that T and B lymphocytes are present in juvenile periodontopathy and that they do not exist in the healthy gingiva provoked the investigation of their role in periodontal diseases.[4]

It is assumed that lymphocytes have a twofold role:

1. they are protective (for the host) and act against the infectious agents; and
2. they may contribute to the tissue destruction.

The mentioned lymphocyte activities exclude one another. There is evidence that both factors (protective and destructive) can simultaneously exist.[5,6]

There are persons who are „highly suspected" for the appearance and development of periodontal diseases. This term refers to the lifestyle aspects, environment factors and innate characteristics. In the research of risk factors, it is important to identify one or several individual factors that may be related to the studied disease. In case of multiple factors, a

akomulacije plaka u inflamiranom infiltratu otkrivena je dominantnost mononuklearnih leukocita. Plazma ćelije su bile lokalizovane na periferiji oštećenja. Kolagena destrukcija je bila prisutna u 15% gingivalnih promena.[8]

Posle dve do tri nedelje plak akomulacije dolazilo je do povećanja plazma ćelija u afektivnoj zoni kao i B limfocita na periferiji oštećenja. Makrofagalne ćelije su otkrivene u lamini propriji u gingivalnom džepu. U sulkusnom epitelu otkrivena je dominacija neutrofilnog infiltrata.[9]

Brecx i sar. (1987. god.) dokazali su da posle šest meseci neodržavanja oralne higijene, dominiraju granulociti i limfociti. Od svih ćelija 10% pripadalo je plazma ćelijama, što ide u prilog hronicitetu bolesti što su i naša istraživanja pokazala.

Postoje dokazi da posle šest meseci adekvatne oralne higijene nisu otkrivene plazma ćelije[10].

Cilj

Cilj ovog rada je bio da se:

1. kliničkim indeksima proceni stanje gingive (interdentalne papile, marginalne gingive i alveolarne gingive),

2. utvrdi priroda inflamatornog infiltrata gingive kod dece,

3. identifikuje imunofenotipski profil celularnog infiltrata pomoću monoklonskih antitela izmenjene gingive,

4. izvrši subtipizacija T i B limfocita kod izmenjene gingive i

5. izvrši komparativna analiza nađenog stanja gingive i patohistološkog i imunocitohemijskog nalaza.

Materijal i metode

Istraživanje je obuhvatilo 86 ispitanika, uzrasne strukture od 12 do 18 god., podjednake polne zastupljenosti: 44 ispitanika iz Osnovne škole „Ivan Goran Kovačić" u Niškoj Banji i 42 ispitanika iz Srednje Ekonomske škole u Nišu. Po starosnoj zastupljenosti bilo je 22 ispitanika uzrasta od 12 god. (10 devojčica i 12 dečaka), 22 ispitanika uzrasta od 14 god. (10 devojčica i 12 dečaka). Broj ispitanika od 16 godina je bio 21 (12 devojčica i 9 dečaka), a u uzrasnoj grupi od 18 godina bio je 21 ispitanik (12 devojčica

combination of factors may be used as a model to make the distinction between high-risk and low-risk persons[7].

The research has proved that the initial changes in the gingiva in the form of inflammation appear 4 days after the plaque accumulation. After 7 days from the plaque accumulation, the dominance of mononuclear leukocytes was observed in the inflammatory infiltrate. Plasma-cells were localized in the periphery of the damage. Collagen destruction appeared in 15% of the gingival changes.[8]

After 2-3 weeks of the accumulation of plaque, the increase in plasma-cells in the affected zone and B-lymphocytes in the damage periphery was observed. Macrophages and lymphocytes were discovered in the lamina propria of the gingival pocket. The sulcate epithelium was dominated by neutrophil infiltrate.[9]

Brecx et al.(1987) proved that after 6 months without practicing oral hygiene granulocytes and lymphocytes were predominant, while 10% of all the cells belonged to the group of plasma-cells.

There are proofs that plasma-cells disappear after 6 months of perfect oral hygiene practice.[10]

Objective

The objective of this paper was:

1. To evaluate the status of the gums (interdental papilla, marginal gingiva and alveolar gingiva) by clinical indices - PMA index,

2. To determine the nature of gingival inflammatory infiltrate in children,

3. To identify the immuno-phenotypical profile of cellular infiltrate by means of monoclonal antibodies of the affected gingiva,

4. To sub-typify T and B lymphocytes in the affected gingiva,

5. To do the comparative analysis of the found condition of the gingiva to the pathohistologic and immunocytochemical findings.

Material and methods

The research included 86 subjects of 12-18 years of age, with both sexes equally represented: 44 subjects from the elementary school "Ivan Goran Kovačić" in Niška Banja Spa and 42 subjects from the secondary school of Economics in Niš. According to the age representation there were 22 subjects of 12 years of age (10 girls and 12 boys), 22 subjects of 14 years

i 9 dečaka). Kontrolnu grupu su činili ispitanici koji su pripadali izabranom tj. ispitivanom uzorku, a kod kojih nisu nadjeni elementi izmenjene gingive (zdrava) (n=10). Rad je planiran prema principima „Dobre kliničke prakse" (DKP).

PMA gingivalnim indeksom po Schouru i Massleru evidentirano je stanje interdentalne papile (P), marginalne gingive (M) i pripojne gingive (A). Stanje gingive je numerički prezentovano.

Biopsijski uzorci gingive uzeti su od 52 ispitanika radi patohistološke i imunocitohemijske analize, kod dece koja su imala jasnu ili izraženu inflamaciju gingive a kod koje je bila indikovana ekstrakcija zuba. Biopsijski uzorci gingive obrađeni su na Institutu za patologiju u Nišu. Deo materijala je obrađivan klasičnim histološkim ispitivanjem. Gingivalni uzorci fiksirani su u 5% formalinu, a zatim ukalupljeni u parafinu. Potom su pravljeni mikrotomski isečci debljine 5 mikrometara i bojeni Gimsom i hematoxylin-eosinom. Drugi deo materijala obrađivan je za imunocitohemijska istraživanja tako što su gingivalne biopsije zamrzavane u tečnom azotu, a zatim su na kriostatu pravljeni isečci debljine 10 mm. Imunofenotipizacija vršena je pomoću imunoalkalne fosfataze (APAAP).

Rezultati

Od ukupnog broja pregledanih ispitanika 28 (32,6%) je bilo sa zdravom gingivom. U grupi ispitanika sa izmenjenom gingivom 50 (58,1%) ispitanika je imalo lakši oblik promena

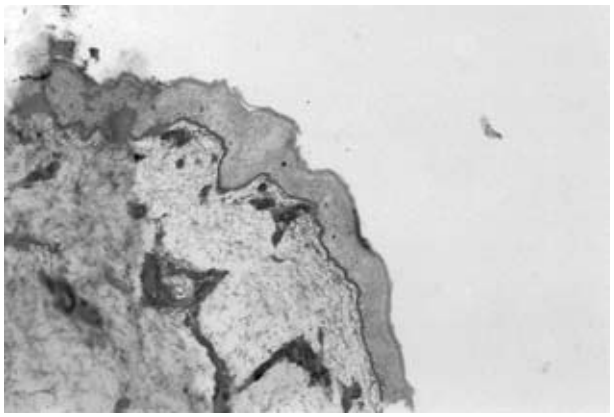
of age (10 girls and 12 boys). The number of persons studied of 16 years old was 21 (12 girls and 9 boys). The control group contained the gingival samples of 10 subjects in which gingivae did not have any inflammation elements (healthy gingivae). The work was planned according to the principles of "A Good Clinical Practice".

The conditions of inter-dental papilla (P), marginal gingiva (M), and tooth attachment (A) were registered with the PMA index according to the method of Schour and Massler. The condition of gingiva was numerically presented.

Gingival biopsy samples were taken from 52 subjects because of the pathohistologic and immunocytochemical analysis from the children who had clear or expressive inflammation of gingiva in which the extraction of teeth was indicated. The gingival biopsy samples were taken for immunocytochemical tests at the institute of Pathology in Niš. One part of the material was processed by the classical histologica testing. Gingival samples were first fixed in 5% formaldehyde and subsequently molded in paraffin. Microtomic sections of 5 μm in thickness were then cut and stained with Gims and Hematoxylin-eosin. The other part of the material was processed for immunocytochemical study. Namely, gingival biopsies were frozen in liquid nitrogen and then cut in the cryostat into 10 μm thick sections. The immune-phenotypic categorization was made by the application of immune-alkaline phosphates (APAAP).

Results

Out of the total number of examined subjects, 28 (32.6%) had healthy gingivae. In the group of subjects with changed gingivae, 50



Slika 1. Histološki nalaz zdrave gingive ukazuje na jasno uočljivu subepitelnu bazalnu membranu
Figure 1. Histologic findings of healthy gingiva showed a clearly distinctive sub-epithelial basal membrane

Slika 2. Kod inflamirane gingive skvamozni epitel pokazuje upadljivu hiperplaziju
Figure 2. In inflamed gingivae squamous epithelium develops noticeable hyperplasia

na gingivi, tj. GI (gingivalni indeks) se kretao u opsegu od 1 do 2 i 8 (9,3%) ispitanika je imalo teži oblik obolele gingive tj. GI je bio veći od 2. Nijedan ispitanik nije imao GI od 0 do 1. Obolela gingiva je klinički registrovana kod 67,4% ispitanika.

Za komparaciju nađenih promena u inflamiranoj gingivi koristila nam je zdrava gingiva koju smo izdvojili kod 10 ispitanika iz ukupnog uzorka od 28 ispitanika kod kojih je bila zdrava gingiva, a kod kojih je bila indikovana ekstrakcija zuba.

Histološki nalaz zdrave gingive ukazuje na jasno uočljivu subepitelnu bazalnu membranu (slika 1) i normalnu gingivalnu vaskularizaciju (slika 3).

Kod inflamirane gingive skvamozni epitel pokazuje upadljivu hiperplaziju, što se dobro uočava sa monoklonalnim antitelom za cytokeratin (APAAP, CYTOKERATIN). Ovakav nalaz smo registrovali kod 52 ispitanika (slika 2).

Uočene su i promene u dijametru krvnih sudova (vazodilatacija) kao i promene u njihovoj strukturi (povećana permeabilnost) (slika 4).

U procesima inflamacije eksudativnog tipa, registrovane su promene na gingivi, koje su klinički imale vidljivo promenjenu boju (crvena – slabijeg ili jačeg intenziteta). U blažim formama, tj. kod ispitanika gde je GI bio u opsegu od 1 do 2, u početnim fazama bolesti, crvenilo je bilo prisutno samo na vrhovima interdentalnih papila i na marginalnoj gingivi. Kod uznapredovalih formi zapaljenja, što smo našli kod ispitanika sa GI većim od 2, promene su bile prisutne i na pripojnoj gingivi. Zbog istanjen-

subjects (58.1%) had mild forms of gingival changes, i.e. GI (gingival index) varied in the range from 1 to 2, while 8 subjects (9.3%) had more severe forms of diseased gingivae, i.e. GI>2. None of the studied subjects expressed GI ranging from zero to 1. The status of gingival disease was registered in 67.4% of the studied subjects.

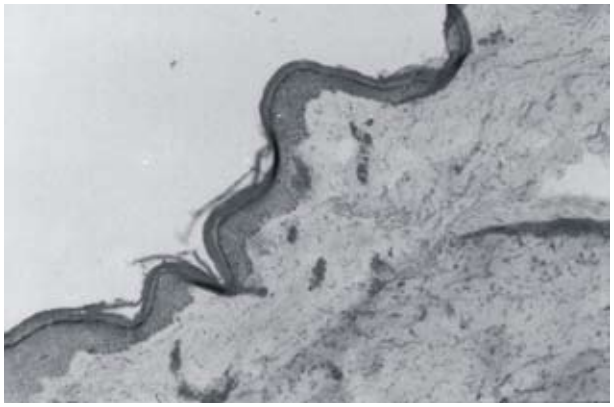
For the comparison of the changes found in inflamed gingiva we used healthy gingiva which we found in 10 studied subjects from the total sample of 28 subjects in whom there was healthy gingiva and in whom there was the extraction of teeth indicated.

Histologic findings of a biopsy sample taken from healthy gingiva showed a clearly distinctive sub-epithelial basal membrane (figure 1) and normal gingival vascularization (figure 3).

In inflamed gingivae squamous epithelium develops noticeable hyperplasia, which can be well observed by means of monoclonal antibody for cytokeratin (APAAP, CYTOKERATIN) (figure 2).

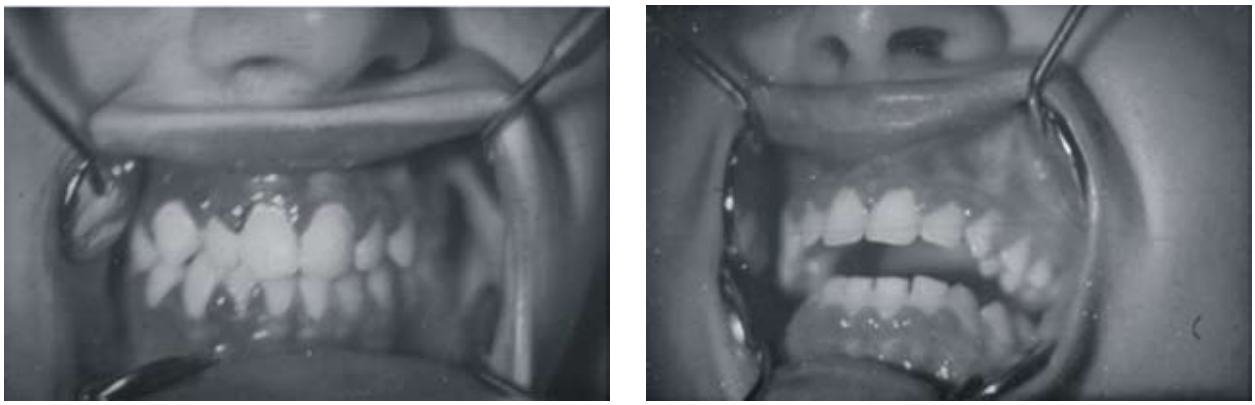
Alterations of blood vessel diameter (vasodilatation) were noticed, as well as changes in their structure (increased permeability) (figure 4).

In the inflammatory processes of the exuding type, gingiva changed in color. It became reddish of higher or lower intensity. At the beginning of the disease, only free, marginal gingiva and inter-dental papillary peaks were red. With the progression of the disease, the redness spreads to other parts of gingival tooth attachment. Due to its thinning, epithelium has become more transparent so the blood vessels of gingiva could easily be observed (figure 5).



Slika 3. Biopsijski uzorak zdrave gingive sa normalnom gingivalnom vaskularizacijom
Figure 3. Biopsy sample of a healthy gingiva with normal gingival vascularization

Slika 4. Uočene su promene u dijametru krvnih sudova (vazodilatacija), kao i promene u njihovoj strukturi (povećana permeabilnost)
Figure 4. Alterations of blood vessel diameter (vasodilatation) were noticed, as well as changes in their structure (increased permeability)



Slika 5. Gingiva je bila bogata vezivno tkivnim elementima i njena boja je bila stoga bleda
Figure 5. Gingiva was richer with connective tissue and its color was therefore paler

osti epitela, veće njene transparentnosti, bilo je moguće uočiti krvne sudove krzna (slika 5).

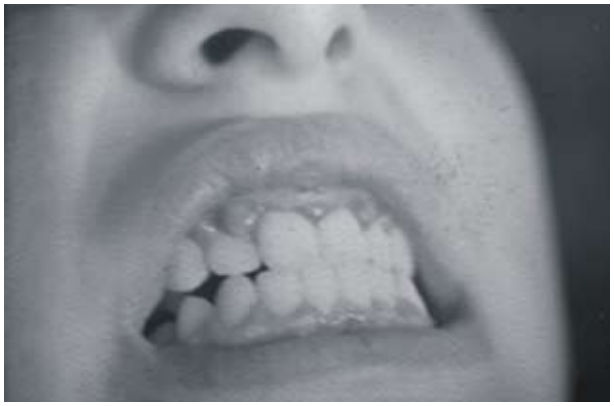
Samo je 6 ispitanika imalo zapaljenje gingive proliferativnog tipa. Zbog bogatstva vezivno tkivnih elemenata, boja gingive kod ovih ispitanika je bila bleda sa prominentnim interdentalnim papilama (slika 6). Gingiva je kod ovih ispitanika bila čvršće konzistencije.

Različita veličina i oblik gingive su bili u zavisnosti od stepena oštećenja krvnih sudova. Naime, veće oštećenje je dovelo do izraženijih promena (povećana veličina i oblik gingive) što nalazimo kod 9,3% ispitanika kao posledica povećane propustljivosti krvnih sudova za ekstravazalnu tečnost i ćelijski infiltrat (neutrofilni granulociti, makrofagalne ćelije, limfociti), najizraženije u slobodnoj gingivi. Zbog prisutne eksudacije i ćelijskog infiltrata, gingiva je kod ovih ispitanika, kao i kod ispitanika kod kojih je GI indeks bio od 1 do 2 (blaža forma), bila izrazito mekana, sunđerasta sa znacima krvarenja spontano ili u početnim fazama bolesti na provokaciju.

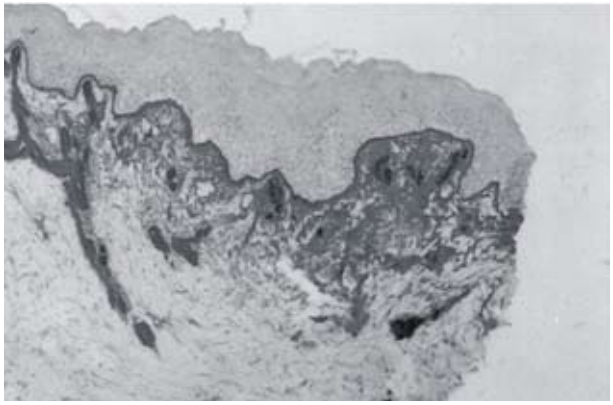
Only 6 studied persons had inflammation of the proliferating type. In the inflammation processes dominated by proliferation, gingiva was richer with connective tissue and its color was therefore paler (figure 6).

The gingiva size and shape are directly related to the level of damage in blood vessels, leading to their increased permeability for extravascular fluids and cell infiltrates (neutrophil granulocytes, macrophages and lymphocytes), which is particularly manifested in free gingiva. In such cases, gingiva becomes edematous, enlarged, with prominent inter-dental papillae that are exposed to subsequent damaging due to mastication. Gingival consistency depends on the inflammation process type. Thus, in the inflammatory processes of the exuding type, due to the disintegration of connective tissue elements, gingiva becomes markedly soft, spongy, liable to provoked or spontaneous bleeding.

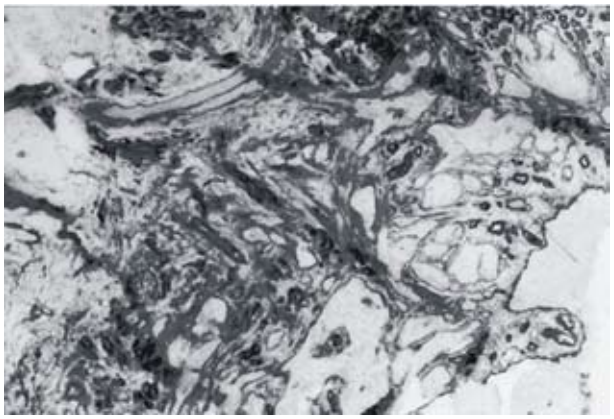
The proliferating type of inflammation is characterized by the gingiva of harder consistency. The destruction of connective tissue is clinically manifested by the loss of firmness



Slika 6. Veličina i oblik gingive direktno su povezani sa stepenom oštećenja krvnih sudova
Figure 6. The gingiva size and shape are directly related to the level of damage



Slika 7. Kolagena vlakna gube kontinuitet, postaju disharmonična i kidaju se
Figure 7. Collagen fibers lose continuity, become disharmonic and tear off



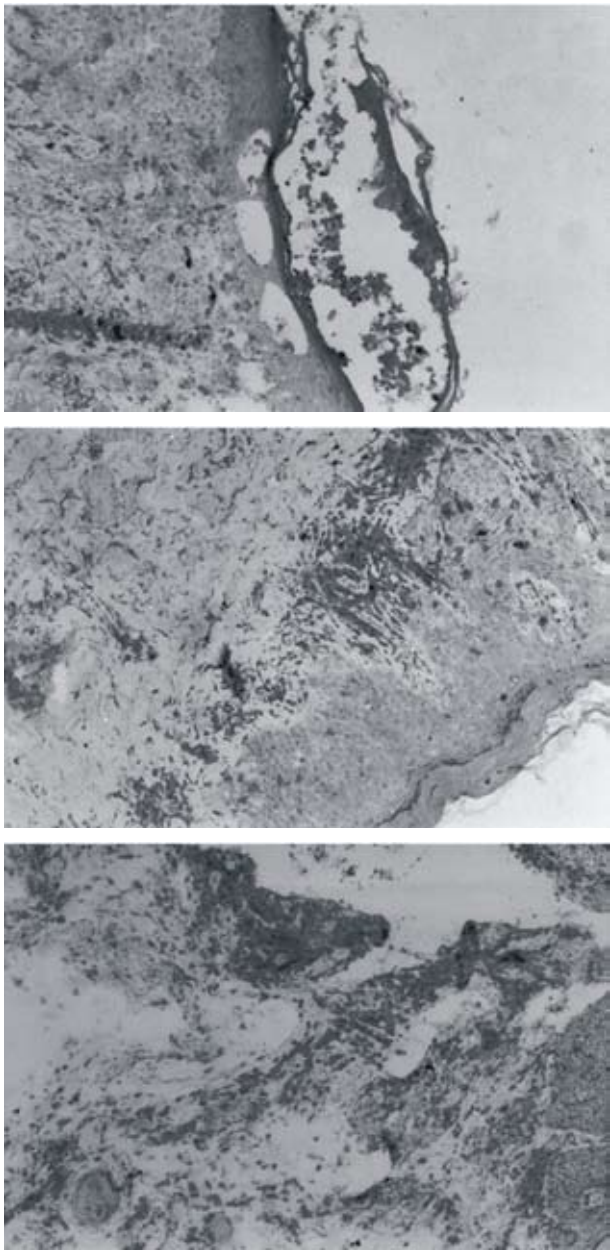
Slika 8. Normalna arhitektura je potpuno uništena
Figure 8. The normal architecture is completely ruined

Inflamatorni infiltrat i imunofenotipski profil celularnog infiltrata izmenjene gingive kod klinički potvrđenog gingivitisa, odgovarao je ispitanicima kod kojih je GI bio od 1 do 2 (58,1%). Koncentracija limfocitnih i monocitnih produkata je niska ili na nivou seruma. Struktura ćelijskih elemenata je odgovarala površinskom zapaljenju. U nekim uzorcima pronađeni su limfociti infiltrati, koji su se pretežno sastojali iz T-ćelija (slika 9) sa malo B-ćelija i plazma ćelija (slika 10).

Manji broj ispitanika tj. njih 8 (9,3%) imao je teži oblik promena na gingivi – GI je bio veći od 2. Destrukcija vezivnog tkiva na gingivi kod ovih ispitanika se manifestovala gubitkom čvrstine i elastičnosti. Zbog gubitka potpore registrovana je blaža pokretljivost zuba kod ovih ispitanika, što je vodilo bolest u pravcu parodontitisa. Zbog gubitka kontinuiteta kolagenih vlakana, registrovan je gubitak arhitekture normalne strukture (slika 7, slika 8). Kod ovih ispitanika je nađena i promena T-ćelijske infiltracije sa dominacijom B-ćelijske i plazma ćelijske infiltracije. Ovaj nalaz nam je bio dokaz da gingiva koja je imala klinički izražene promene, izraženu inflamaciju sa početnim znacima parodontitsa, takođe je imala i promene celularnog i imunonološkog statusa. Zapravo, radilo se o hroničnim procesima i pomeranju gingivitisa ka parodontitisu. Prisustvo Russellovih telašaca kao dokaz pojačane i izražene plazmocitne aktivnosti dopunjuje nalaz kod ove grupe ispitanika (GI veći od 2) (slika 11).

Diskusija

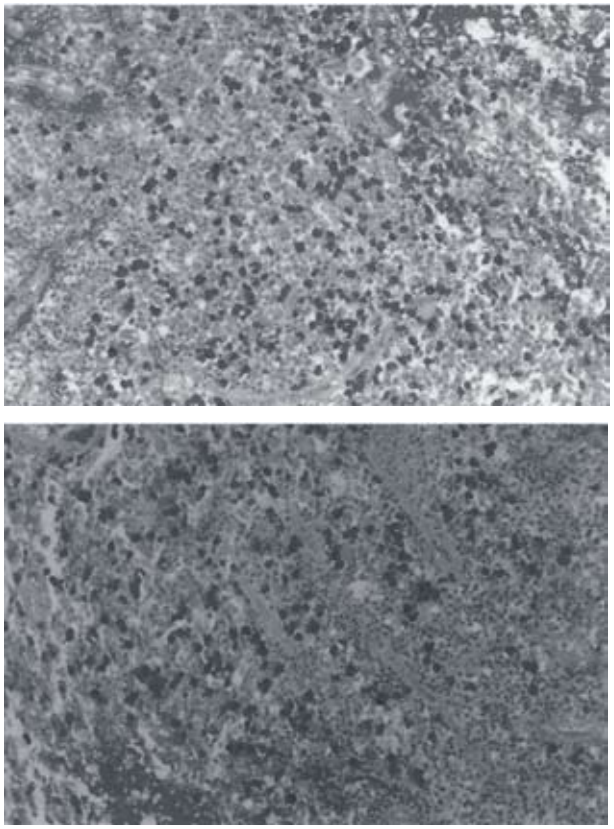
Obolela gingiva sa karakterističnim znacima i simptomima, koji prate gingivitis registrovana



Slika 9. Infiltracija sa dominacijom T ćelija interepitelno, subepitelno i duboko u tkivu
Figure 9. Infiltration with the domination of T cells inter-epithelial, sub-epithelial and deeper into the tissue

and elasticity. With the progression of these processes, the teeth lose their support in the jaw and drift from their original position, which already represents periodontitis. Collagen fibers lose continuity, become disharmonic and tear off (figure 7), so the normal architecture is completely ruined (figure 8).

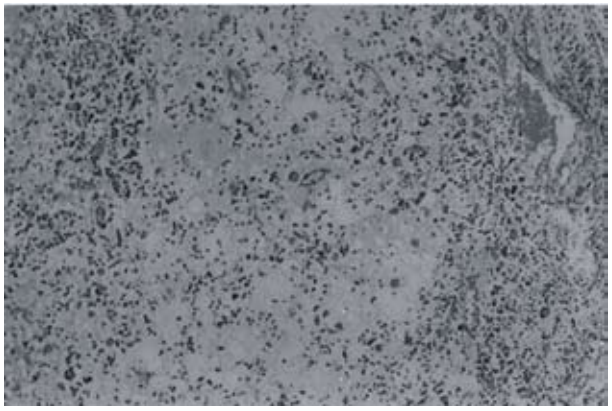
The concentration of lymhocyte and monocyte products is also changed in gingivitis. Namely, it is low or on the serum level. Such structure of cellular elements corresponds to a surface inflammation of gingiva. This blockade may be supplemented with reduced lympho-



Slika 10. Infiltracija sa dominacijom B ćelija i plazma ćelija
Figure 10. Infiltration with the domination of B cells and plasma cells

je kod 67,4% ispitanika. Đajić i sar. su (1980. god.) evidentirali da u ekonomski razvijenim zemljama sveta čak 90% dece ima obolelu gingivu.[11]

Biopsijski uzorci inflamirane gingive, uzeti od ispitanika sa klinički vidljivim promenama na gingivi (67,4%), pokazuju promene u dijametru krvnih sudova i njihovoj strukturi. Uočena je angiogeneza koja se dovodi u vezu sa povećanjem propustljivosti krvnih sudova. Istovremeno zbog proteolitičke destrukcije bazalne membrane izvornog suda došlo je do migracije ćelija prema središtu zapaljenja, što se klinički manifestovalo edemom gingive. Proliferisane ćelije endotela vremenom sazrevaju, formiraju kapilarne cevi, koje se u daljem procesu rasta susreću, spajaju i tako formiraju vaskularne petlje. Vremenom dolazi do diferentovanja vaskularne mreže i njenog formiranja pored kapilara, arteriola i venula. Otkrivena angiogeneza kod naših ispitanika potvrđuje nastale promene u gingivi. Angiogeneza nastala u zapaljenjskim procesima gingive je pokušaj reparacije vezivnog tkiva. Nađeni nalaz se poklapa i sa nalazima brojnih istraživaca.[12–14]



Slika 11. Ruselova telašca kod subjekata sa hroničnom gingivalnom inflamacijom
Figure 11. Russell's corpuscles in the subjects with a chronic gingival inflammation

cytic infiltrate, which is registered by a minimal existence of T-cell population (figure 9), B cells and plasma cells (figure 10). However, the change of T-cell infiltration with the domination of B cells and plasma cells implies that the process is chronic and that gingivitis has progressed towards periodontitis.

We also notice a high level of antibodies Ig of the A group, as a secretory Ig, which represents the first defense line and the local immunological reaction. The pathohistologic findings registered Russell's corpuscles in the subjects with a chronic gingival inflammation (figure 11).

Discussion

Affected gingivae with the characteristic signs and symptoms of gingivitis were registered in 67.4% of the examined subjects. Recorded that almost 90% of the children in economically developed countries in the world suffer from gingival diseases.[11]

Biopsy samples of the inflamed gingiva (67,4%), showed changes in the diameter and structure of blood vessels. The angiogenesis was also registered, which was related to the increase of blood vessel permeability. Namely, due to the proteolytic destruction of the basal membrane of the original vessel, cells simultaneously migrated toward the inflammation center. Proliferated endothelium cells matured and formed capillary tubes, which contacted, merged and formed vascular lumps in the process of further growth. In time, a vascular network was differentiated and formed alongside capillaries, arterioles and venules. The observed

Vaskularne reakcije sa manifestnim promenama u dijametru i strukturi krvnih sudova neminovno dovode do eksudacije, tj. izlaska tečnosti, krvnih ćelija i proteina, što zavisi od vrste i intenziteta dejstva patogena. Nađeni patohistološki nalaz kod naših ispitanika je bio u korelaciji sa klinički manifestnim promenama u smislu prisutnog edema, veličine, oblika i konzistencije gingive u 58,1% ispitanika.[15,16] Kod ispitanika kod kojih je dijagnostikovan proliferativni tip zapaljenja pronađen je veći broj makrofagalnih ćelija, fibrocita i kolagenih vlakana u vezivnom tkivu.[17]

Aktivnost lizozomskih enzima se dešava u svakoj ćeliji onog trenutka kada je ona napadnuta patogenom, dok će se aktivnost neutrofilnih lizozomskih enzima ispoljiti i kada nisu izložene direktnom dejstvu istog. Enzimska aktivnost lizozoma neutrofila je u inflamaciji najizraženija. Oni poseduju veliki broj enzima: kisele proteaze koje razgrađuju peptide, neutralne proteaze koje razgrađuju protein, kolagenaze koje razgrađuju kolagen, peroksidaze, dekstranaze i dr. Mnogobrojne bakterije kao što su: actinomycetem comitans, bacteroides intermedia, campilobacter rectus i dr. produkuju toksine: leukotoksin, endotoksin, fibroblast inhibitorni faktor, fibrinolizin, fosfolipazu i dr. koji omogućavaju leukocitnu funkciju i određuju stepen parodontalnog zdravlja tj. bolesti. Vaskularne promene se dešavaju i pod dejstvom medijatora inflamacije koje produkuju medijatorske ćelije (mastociti, bazofili, fagociti i trombociti). Medijatorske ćelije u sadejstvu sa mikroorganizmima dentalnog plaka, preko svojih produkata dovode do promena u tkivu gingive. Akutno zapaljenje je reakcija tkiva kao prvi odgovor na dejstvo patogena. Nađeni nalaz izmenjene gingive sa znacima akutnog zapaljenja nalazimo u našem istrazivanju kod ispitanika sa GI od 1 do 2. Iste nalaze dobili su i drugi istraživači.[15,16]

Inflamacija gingive je praćena promenama elektrohemijske reakcije, uz pad pH sredine i aktiviranja brojnih enzima. Pri tom dolazi do narušavanja integriteta vezivno tkivnih elemenata. Prisutan ćelijski infiltrat potiskuje kolagena vlakna te ona postaju rastresitija. Tečnost, koja je prisutna u zapaljenjskom tkivu, dovodi do labavljenja snopova kolagenih vlakana. Tako razdvojeni fibrili omogućuju lakši prodor ćelijskih elemenata i kolagenaze. Zbog gubitka intermolekularne veze, molekuli kolagena po-

angiogenesis, i.e. proliferation of small blood vessels, represents an attempt of the connective tissue reparation. This finding coincides with the findings of numerous researchers.[12–14]

Vascular reactions with manifested changes in the diameter and structure of blood vessels unavoidably lead to exudation, i.e. passing out of fluid, blood cells and proteins, in dependence of the pathogen type and intensity of action.[15,16] As distinguished from the inflammation of the exuding type, proliferating inflammation processes result in a multiplication of macrophages, fibrocytes and collagen fibers in the connective tissue.[17]

The activity of lysosomal enzymes begins in each cell the moment it gets attacked by a pathogen, while the activity of neutrophil lysosomal enzymes is expressed even if the cells are not exposed to direct effects. The enzymic activity of neutrophil lysosomes is the most expressed in inflammation. They contain a great number of enzymes: acid proteases that dissolve peptides, neutral proteases that digest protein, collagenases that dissolve collagen, peroxidases, dextranases, acid phosphatases, amino-peptidases, etc. Numerous bacteria, such as: Actinobacillus actinomycetemcomitans, Bacteroideus intermedia, Campilobacter rectus and other, produce the toxins: leukotoxin, collagenase, endotoxin, fibroblast, inhibitory factor, fibrinolysin, phospholipase, etc, which enable leukocytic function and determine the level of periodontal health or disease. Vascular changes also appear under the impact of the inflammation mediators produced by mediatory cells (mastocytes, basophiles, phagocytes and thrombocytes). It has been proved that mediatory cells, in cooperation with the dental plaque microorganisms, cause changes in the gingival tissue by their products. Acute inflammation is a reaction of the tissue as the first answer to the impact of pathogens.[15,16]

The inflamed gingiva, accompanied with altered electro-chemical reaction, decreased pH value in the environment and activation of numerous enzymes, characterizes by a disturbed integrity of connective tissue elements. The present cell infiltrate pressed collagen fibers, so they became looser. The fluid, registered in the inflamed tissue, caused slackening of collagen fiber bundles. Thus separated, fibrils facilitated the penetration of cell elements, collagenases and other numerous enzymes and toxins. Due to the loss of inter-molecular connection,

staju nezaštićeni te lakše podležu dejstvu kolagenaze, što nalazimo u našem istraživanju kod ispitanika sa GI većim od 2, gde su postojali i elementi promena ne samo na gingivi već i na ostalim strukturama parodoncijuma (9,3%).

Fibroblasti su oštećenji, veze između ćelija nestaju te se tako smanjuje i njihova adhezivnost. Intercelularni prostori postaju širi, što omogućuje dalji prodor patogenu. U početku inflamacije, ćelijski infiltrat samo potiskuje kolagena vlakna, ali kako inflamacija progredira tako se ćelijski infiltrat povećava u krznu gingive, što uzrokuje kidanje i rastresitost do potpunog gubitka kolagena. Ovako dobijen patološki nalaz sa oštećenjem fibroblasta, prisustvom ćelijskog infiltrata, izmenjenom arhitekturom kolagenih vlakana poklapa se sa nalazima drugih autora.[18–20]

U kom je stanju parodont zavisi od stanja kolagenih vlakana, njihovog oblika i broja. Stepen oštećenja kolagenih vlakana je u pozitivnoj korelaciji sa inflamacijom, što se poklapa sa našim rezultatima.

Model patogeneze koji definiše kritičku stazu za ispoljavanje bolesti uključuje bakterijsku etiologiju i reakciju domaćina, koja je uglavnom određena genima. Ako osoba ima lošu oralnu higijenu, može se razviti gingivitis, što možda neće biti dovoljno da izazove prelaz ka parodontitisu. Međutim, moguće je da vremenom stekne nove visoko virulentne mikroorganizme koji su sposobni da izbegnu klirens domaćina i da do oboljenja ipak dođe.[21]

Kod gingivitisa je u cervikalnoj tečnosti koncentracija limfocitnih i monocitnih produkata niska ili na nivou seruma. Ova struktura ćelijskih elemenata odgovara površinskom zapaljenju koje ne prodire duboko u tkivo, verovatno zato što su neutrofili u mogućnosti da kontrolišu infekciju. Proređeni limfocitni infiltrat se pretežno sastoji iz T-ćelija sa malo B-ćelija ili plazma ćelija. Nađeni nalaz kod naših ispitanika poklapa se sa nalazima drugih istraživača.[22] Međutim, kada patogen izbegne neutrofilno sito, bakterije i/ili njihovi produkti prodiru dublje u tkivo i započinje prelaz od gingivitisa ka parodontitisu. Istovremeno dolazi i do promene limfocitne populacije, pri čemu se T-ćelije zamenjuju B-ćelijama i plazma ćelijama. U ovoj fazi, ako domaćin može brzo organizovati reakciju antitelima, koja predstavlja zadovoljavajuću zaštitu, onda su bakterije u privremenom mirovanju. Bolest je blaga ili

unprotected collagen molecules succumbed easily to the impact of enzymes, primarily to collagenase (9,3%). Fibroblasts are damaged, connections among cells disappear and their adhesive capacity decreases. Intra-cellular space expands, enabling further penetration of harmful agents. At the inflammation start, the cell infiltrate only presses collagen fibers, but as the inflammation progresses, the cell infiltrate increases in the gingiva, which causes tearing apart and loosening of collagen until it is completely destroyed. The obtained pathologic finding with a visible fibroblast damage, presence of cell infiltrate and changed architecture of collagen fibers corresponds to the findings of other authors.[18–20]

The status of periodont depends on the status of collagen fibers, their number and shape. The level of collagen fiber damage stands in a positive correlation with the inflammation.

The model of pathogenesis defining the critical path of the disease demonstration includes bacterial etiology, as well as the reaction of the host that is mainly determined by genes. If a person performs poor oral hygiene, gingivitis may develop, but it may not grow into periodontitis. However, in time this person may get new highly virulent microorganisms, which could evade the clearance of the host and provoke the disease.[21]

In case of gingivitis, the concentration of lymphocytic and monocytic products in the cervical fluid was low or on the serum level. Such structure of cell elements corresponded to a surface inflammation that does not penetrate deep into the tissue, probably because neutrophils were capable of controlling the infection. Reduced lymphocytic infiltrate predominantly consisted of T-cells, with a few B-cells or plasma cells. The obtained finding correlates with the results reached by other researchers.[22] However, when the pathogen evades the neutrophil sieve, bacteria and/or their products penetrate deeper into the tissue and the transition from gingivitis into periodontitis starts. Findings differed in such cases, namely, there was a change in lymphocyte population, where T-cells were substituted by B-cells and plasma cells. In this phase, if the host is able to organize a quick antibody reaction as a sufficient protection, bacteria remain temporarily still and the disease is mild and „limited“. The obtained finding, related to the predominance of B-cellular and

„ograničena“. Prisutan nalaz sa dominacijom B-ćelijske infiltracije i plazma ćelija govori u prilog da se radi o hroničnom oboljenju, što se i klinički poklapa sa nađenim stanjem na gingivi. Kod ispitanika sa GI većim od 2, tj. kod 9,3% ispitanika imunološka reakcija domacina je u velikoj meri određena genima, što bitno utiče na prirodu zaštitne reakcije antitelima kao i na jačinu zapaljenjske reakcije. Naš nalaz se poklapa sa rezultatima koje srećemo u istraživanjima brojnih autora.[23]

Ali ako antitela ne obezbede dovoljan klirens, dolazi do umnožavanja bakterija, aktivacije monocitnog sistema i progresije bolesti. Smanjena odbrambena sposobnost domaćina omogućava izraženo dejstvo virulentnih patogena. Kada je domaćin ugrožen, klirens neutrofila ili reakcija monocita i T-ćelija pomeraju se više ka zapaljenjskom nego ka zaštitnom bilansu.[24]

Prisustvo Russellovih telašaca u patohistološkom nalazu kod težih oblika gingivitisa sa prisutnim znacima parodontitisa, što nalazimo kod 9,3% ispitanika, govori da se radi o izraženoj sekretornoj aktivnosti plazmocita u pokušaju da se odbrana dovede na nivo neutrofila ili bolest dovede u stanje mirovanja. Do sličnih podataka su došli i drugi istraživači.[25,26]

Zaključak

Procenom stanja gingive kod ispitanika kliničkim indeksima (PMA indeks) došli smo do rezultata da 67,4% ispitanika ima izmenjenu gingivu, tj. neki oblik gingivitisa.

Patohistološkim i imunološkim analizama izmenjene i zdrave gingive došlo se do zaključka da se imunofenotipski profil celularnog infiltrata razlikuje i da je u direktnoj zavisnosti od stanja gingive kod ispitanika, od zdrave, blago inflamirane, jasnije inflamacije i promenjene gingive sa već prisutnim početnim znacima parodontitisa.

Izvršena subtipizacija T i B limfocita kod izmenjene gingive ukazuje da je prisustvo ćelijske populacije u zavisnosti od biopsijskog uzorka uzetog sa gingive sa različitim kliničkim nalazom, a da je direktno u korelaciji sa stanjem gingive sa koje je uzorak uzet.

plasmocytic infiltration, bears witness that it is a chronic disease, which coincided with the clinically found status. In the studied persons with GI higher than 2 (9,3%) the immunological reaction of the host is largely determined by genes, substantially influencing the nature of the protective antibody reaction, as well as the severity of the inflammatory reaction. Similar results have been observed in the findings of Zafiropoulos et al.[23]

Nevertheless, if the antibodies cannot provide sufficient clearance, bacteria multiply, monocyte system is activated and the disease progresses. The decreased defensive capability of the host enables a pronounced impact of virulent pathogens. In case the host is endangered, the clearance of neutrophils or the reaction of monocytes and T-cells shift more toward the inflammatory as compared to the protective balance.[24]

Russell's corpuscles registered in the pathohistologic findings confirm that this is the case of a chronic gingival inflammation – 9,3% subjects. Namely, these corpuscles reflect a marked secretory activity of plasmocytes in the attempt to elevate the defense to the level of neutrophils or to bring the disease in the state of rest. The attained finding matches with the results achieved by other researchers as well.[25,26]

Conclusion

By the research of the condition of gingiva in the persons studied by PMA index, we concluded that 67,4% persons have affected gingiva, that is, a certain kind of gingivitis.

By means of pathohistologic and immune analysis of the affected gingival, it is concluded that the immuno-phenotypical profile of cellular infiltrate is different in the case of healthy, slightly inflamed, clearly inflamed gingiva and in gingiva with the present signs of parodontitis.

The sub-typifying of T and B lymph glands in the affected gingiva shows that the presence of the cell population depends on biopsy sample taken from the gingiva with the different clinical findings, and that it is in direct correlation with the condition of the gingiva from which the sample was taken.

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