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Examination of the presence of periodontitis and gingivitis in rats with induced diabetes mellitus

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SUMMARY

Introduction Diabetes mellitus (DM) is a state of chronic hyperglycemia that is a predisposing factor to caries, gingivitis, inflammation of periodontium, oral candidiasis, xerostomia and many other diseases of the oral cavity. Inflammation of the supporting tissue of the tooth is a chronic disease that destroys the supporting structure of the tooth, i.e. periodontal ligament and alveolar bone. The aim of this study was to examine using histological analysis the presence of periodontitis and gingivitis in rats with experimentally induced DM that were sacrificed after 14 and 30 days.

Material and methods The research was conducted on 42 Wistar rats. DM in experimental animals was induced by the use of Alloxan intraperitoneally. The first group (Exp_14) consisted of 16 rats in which DM was induced and sacrificed after 14 days, the second group (Exp_30) consisted of 16 rats in which DM was induced and they were sacrificed after 30 days, while the control consisted of 10 healthy rats.

Results Periodontitis and gingivitis in the first group of rats (Exp_14) were determined in 54.5% of cases, while in the second group (Exp_30) in 88% of cases. In the control group no case of periodontitis and gingivitis was recorded. A highly statistically significant difference was found between the examined groups (Chi-square = 14.685; $p < 0.001$).

Conclusion In the group of rats with experimentally induced DM that were sacrificed after 30 days, a significantly higher incidence of periodontitis and gingivitis was found compared to the group of rats that were sacrificed after 14 days.

Keywords: diabetes mellitus; periodontitis and gingivitis; histological analysis

INTRODUCTION

DM is a state of chronic hyperglycemia followed by disorder of metabolism of carbohydrates, fats and proteins that occurs as a result of an absolute or relative lack of insulin action [1]. Dentists are aware of the importance of DM in their patients because various oral conditions are associated with diabetes, including xerostomia, yeast infection as well as periodontitis and gingivitis [2]. An increase in catabolic and a decrease in anabolic processes lead to changes in cells in the body, which affects the occurrence of oral diseases such as periodontitis and gingivitis. Increased concentration of glucose in saliva and hyposalivation in patients with DM encourage conditions for an acidic environment and development of pathogenic bacteria. Periodontal inflammation of a tooth is chronic, multicausal, disease in the supporting tissue around the teeth. Patients with DM can have gingivitis, pathological changes in the supporting tissue of the teeth, resorption of alveolar bone and finally complete tooth loss [3, 4].

Periodontal inflammation is an irreversible inflammatory condition and represents a significant public health burden. It is present in more than 11% of adults and is one of the causes of teeth loss, which negatively affects speech, nutrition and quality of life. Periodontal disease is one of the most common diseases today, and the number of patients is increasing with age. Good preventive measures and adequate therapy reduce the percentage of tooth loss and improve the quality of life [5–8].

Numerous studies examined correlation between DM and periodontal inflammation. Păunică et al. examined the relationship between periodontal inflammation and DM and confirmed that DM affects the onset of periodontal disease, leading to its worsening, and that periodontitis negatively affects glycemic control and the course of diabetes [9].

Pathogenic processes that connect these two diseases are the focus of many studies. DM increases the risk of periodontitis by contributing to increased inflammation in the periodontal tissue. In diabetes, there is an increased deposition of advanced glycation end products (AGE) in the periodontal tissue, and the interactions between AGEs and their receptors (RAGE, the receptor for AGEs, which is found especially on macrophages) lead to activation of local immune system and inflammatory reactions first observed on gingiva [10–14].

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MATERIAL AND METHODS

The research was conducted after the approval of the Ethics Committee of the University Clinical Center in Banja Luka no. 01-9-192.2/15, Bosnia and Herzegovina. The sample consisted of 42 Wistar rats. The animals were two months old, with a body weight of 150-200 g. They were kept in group cages made of Plexiglas, with 12 hours of light (07:00-19:00) at an air temperature of 22°C (± 2) and a humidity of 60% ± 10%, with free access to food and water during of the experiment. At the beginning of the experiment, individuals were separated into appropriate test and control groups.

Rats were divided into the two experimental groups (Exp_14 and Exp_30) and one control group. The first (Exp_14) and second (Exp_30) groups each consisted of 16 rats with experimentally induced DM. Using Alloxan the first and second groups of rats (Exp_14 and Exp_30) were brought to experimentally induced DM. Alloxan solution was applied intraperitoneally in a dose of 100 mg per kilogram of body weight of rats. The protocol was repeated every other day until the measured value of glycemia did not exceed 200 mg/dcl (animal in hyperglycemia). Glycemia was measured with a blood glucose meter (ACCU ACEH, Roche) from tail vein blood. The achieved hyperglycemia was controlled by regular measurement (every other day) and maintained for 7 days. The control consisted of 10 healthy rats.

Rats from the first group were sacrificed after 14 days and from the second group after 30 days. Rats from the control group were sacrificed also after 30 days. For histological analysis the bones of the upper jaws together with the teeth after 48h of fixation (in 10% neutral buffered formalin) were decalcified in a nitric acid solution (no longer than 90 minutes). The decalcified samples were then washed with water and processed in an automated tissue processor Leica TP 1020 (Leica Byosistems) according to a standard protocol; dehydration in increasing concentrations of ethyl alcohol (70%, 96%, 100%); clarification in xylene, impregnation with liquid paraffin, after which selected tissue samples were molded into paraffin blocks. After cooling, the paraffin blocks were cut on a sliding microtome (Leica SM 2000R, Leica Byosistems) into sections 4-5 μm thick for histological analysis. For histological analysis, the cross-sections of the periodontal tissue were collected on appropriate glass slides and dried at 60°C. In an automatic staining processor (Leica ST4040 Linear stainer, Leica Byosistems), tissue sections were deparaffinized, rehydrated and rinsed in distilled water. After that, they were stained with the standard hematoxylin-eosin (HE) method. Definitive preparations were analyzed with a light microscope (Leica DM 2500, Leica Byosistems) and photographed with a camera connected to the microscope (Figure 1).

RESULTS

In the first experimental group (Exp_14) periodontitis and gingivitis were recorded in 54.5% of cases. In the second experimental group (Exp_30) periodontitis and gingivitis are recorded in 88.0% of cases. In the control group (healthy rats without hyperglycemia) not a single case of periodontitis and gingivitis was recorded (Table 1, Figure 1). There was highly statistically significant difference in the prevalence of periodontitis and gingivitis between the studied groups (Chi-square = 14.685; $p < 0.001$) (Table 1).

Table 1. Periodontitis and gingivitis in the studied groups
Tabela 1. Periodontitis i gingivitis kod analiziranih grupa

			Periodontitis and gingivitis Periodontitis i gingivitis		Total Ukupno
			Absent Odsutan	Present Prisutan	
Group Grupa	Exp_14 Eksp_14	N	5	6	11
		%	45.5%	54.5%	100%
	Exp_30 Eksp_30	N	3	22	25
		%	12%	88%	100%
	Control Kontrolna	N	12	0	12
		%	100%	0.0%	100%
Total Ukupno		N	20	28	48
		%	41.7%	58.3%	100%

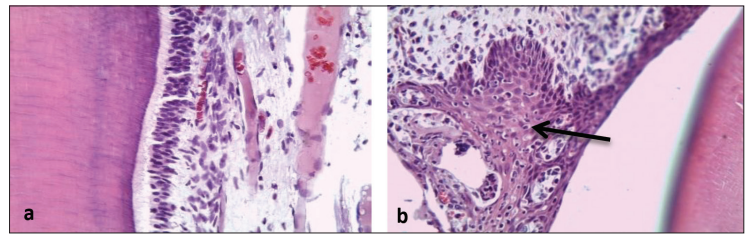

Figure 1. a) Cross-section of the periodontal ligament and jaw bone of a rat without morphological changes (HE × 400); b) View of the interdental part of the gingiva of a rat. Inflammation of the gingiva is observed (HE × 400).
Slika 1. a) Poprečni presek periodontalnog ligamenta i vilična kost pacova bez morfoloških promena (HE × 400); b) Prikaz interdentalnog dela gingive pacova. Uočava se zapaljenje gingive (HE × 400).

DISCUSSION

Periodontal inflammation is chronic inflammatory disease caused by the accumulation of dental plaque, consisted of bacteria that lead to a chronic and destructive inflammatory response resulting in tissue destruction, i.e. deterioration of the periodontal ligament, formation of periodontal pockets and resorption of alveolar bone. The risk of periodontitis is increased 2-3 times in people with DM compared to people without DM, and the level of glycemic control is crucial in determining the risk of the disease. Similar to other complications of diabetes, the risk of periodontitis increases with poorer glycemic control [15, 16].

Most of the research on inflammation of the tooth's supporting tissue and diabetes has focused on type 2 DM (probably because these diseases mainly occur in middle-aged adults), but type 1 DM is also associated with increased periodontal destruction in children and teenagers [17].

In our study, it was determined that periodontitis and gingivitis were present in groups of rats with experimentally induced DM, while no pathological changes in the periodontium were found in the group of healthy rats. In the first group of rats that were sacrificed after 14 days of introduction to DM, significantly smaller changes were

observed in the supporting tissues of teeth compared to the group of rats that were sacrificed after 30 days.

A large number of research studied the relationship between DM and inflammation of the supporting tissue of the tooth and found that patients with DM (including children and young adults) had an increased risk of periodontitis. Lalla et al. compared the periodontological status in children with DM and periodontological status in healthy children of the same age of 6-18 years. The results of their study indicated higher prevalence of periodontitis and gingivitis (20%) in children with DM than in healthy children (8%), which was in accordance with the results of this study [18].

Periodontitis is now known as a risk factor for worsening glycemic control and may increase the risk of diabetes complications. Choubaya et al. also investigated the association of periodontitis with the development and progression of diabetes in Wistar rats with induced DM. The study indicated a connection between glycemic levels and changes in the periodontium, i.e. the higher the level of glucose in the blood of rats, the greater the changes in periodontium [19].

Takai et al., using histological analysis, determined that the inflammatory reaction in the gingival tissue was higher and more intense in rats with DM compared to healthy rats. These results indicate that dental plaque is also an important factor for severe inflammatory processes of the periodontium and the importance of proper maintenance of oral hygiene in patients with DM [20].

Pepelassi et al., also using histological analysis, found that alveolar bone loss was significantly greater in rats with DM and periodontitis than in rats with only periodontitis or DM [21].

In patients with DM, it is necessary to raise the level of oral hygiene, in order to prevent accumulation of dental plaque, which is one of the factors in the development of gingivitis and other periodontal diseases. Also, the level of glycemia in the blood of DM patients depends primarily on diet, which is why it is important for patients to follow the instructions given by the endocrinologist. Wang et al. as well as Preshaw et al. pointed out important roles of dental team in patients with diabetes and periodontitis, in preventing the worsening of the clinical picture [22, 23].

CONCLUSION

In the group of rats with experimentally induced DM that were sacrificed after 30 days, significantly higher prevalence of periodontitis and gingivitis was found compared to the group of rats with experimentally induced DM that were sacrificed after 14 days, as well as healthy group of rats. The results of this study indicate a connection between DM and periodontal diseases, which is why it is necessary to propose a preventive program for patients with DM that includes dental procedures such as oral hygiene training and regular visits to dentist, detection of dental plaque, removal of soft and hard dental deposits and observation of the initial pathological changes of the periodontium and their treatment.

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Ispitivanje zastupljenosti parodontitisa i gingivitisa kod pacova sa indukovanim dijabetesom melitusom

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KRATAK SADRŽAJ

Uvod Dijabetes melitus (DM) stanje je hronične hiperglikemije koje predstavlja predisponirajući faktor karijesu, gingivitisu, inflamaciji parodoncijuma, oralnoj kandidijazi, kserostomiji i mnogim drugim oboljenjima usne šupljine. Zapaljenje potpornog tkiva zuba je hronično oboljenje koje razara potpornu strukturu zuba, odnosno parodontalni ligament i alveolarnu kost.
Cilj ove studije je bio da se histološkom analizom ispita zastupljenost parodontitisa i gingivitisa kod pacova sa ekperimentalno izazvanim DM-om koji su žrtvovani posle 14 i 30 dana.
Materijal i metode rada Istraživanje je sprovedeno na 42 pacova soja Vistar. DM kod eksperimentalnih životinja indukovan je upotrebom aloksana (Alloxan) intraperitonealno. Prvu grupu (Exp_14) činilo je 16 pacova, kod kojih je indukovan DM i koji su žrtvovani posle 14 dana; drugu grupu (Exp_30) činilo je 16 pacova kod kojih je DM indukovan posle 30 dana, dok je kontrolu činilo 10 zdravih pacova.
Rezultati Parodontitis i gingivitis u prvoj grupi pacova (Exp_14) uočeni su u 54,5% slučajeva, a u drugoj u grupi (Exp_30) u 88% slučajeva. U kontrolnoj grupi nije zabeležen nijedan slučaj parodontitisa i gingivitisa. Između ispitivanih grupa utvrđena je visoko statistički značajna razlika ($\chi^2 = 14{,}685$; $p < 0{,}001$).
Zaključak Kod grupe pacova sa eksperimentalno indukovanim DM-om koji su žrtvovani posle 30 dana utvrđena je značajno veća zastupljenost parodontitisa i gingivitisa u odnosu na grupu pacova koji su žrtvovani posle 14 dana.
Ključne reči: dijabetes melitus; parodontitis i gingivitis; histološka analiza

UVOD

DM je stanje hronične hiperglikemije praćeno poremećajem metabolizma ugljenih hidrata, masti i proteina koje nastaje kao posledica apsolutnog ili relativnog nedostatka dejstva insulina [1]. Stomatolozi su svesni važnosti dijagnoze DM-a kod svojih pacijenata jer su različita oralna stanja povezana sa dijabetesom, uključujući kserostomiju, kandidoznu infekciju, kao i parodontitis i gingivitis [2]. Povećanje kataboličkih a smanjenje anaboličkih procesa dovode do promena u ćelijama u organizmu, što utiče na nastanak oralnih oboljenja kao što su parodontitis i gingivitis. Povećana koncentracija glukoze u pljuvački i hiposalivacija kod pacijenata sa DM-om podstiče uslove za kiselu sredinu i razvoj patogenih bakterija. Zapaljenje potpornog tkiva zuba je hronično, multikauzalno oboljenje u potpornom tkivu oko zuba. Pacijenti sa DM-om mogu imati gingivitis, patološke promene potpornog aparata zuba, resorpciju alveolarne kosti i na kraju potpuni gubitak zuba [3, 4].

Inflamacija parodoncijuma je ireverzibilno inflamatorno stanje i predstavlja značajan teret za javno zdravlje. Zastupljen je kod više od 11% odraslih osoba i jedan je od uzroka gubitka zuba koji negativno utiče na govor, ishranu i kvalitet života. Parodontitis je jedna od najzastupljenijih bolesti današnjice, a broj obolelih raste s godinama. Dobrim preventivnim merama i adekvatnom terapijom smanjuje se postotak gubitka zuba i dolazi do poboljšanja kvaliteta života [5–8].

Brojne studije su se bavile ispitivanjem korelacije između DM-a i inflamacije parodoncijuma. Păunică i saradnici su se bavili ispitivanjem odnosa između inflamacije parodoncijuma i DM-a i na osnovu dobijenih rezultata potvrdili su da DM utiče na nastanak parodontalnog oboljenja, dovodeći do pogoršanja, a da parodontitis negativno utiče na kontrolu glikemije i tok dijabetesa [9].

Patogeni procesi koji povezuju ove dve bolesti su u fokusu mnogih istraživanja. DM povećava rizik od parodontitisa doprinoseći pojačanoj upali u parodontalnom tkivu. Kod dijabetesa dolazi do povećanog taloženja krajnjih produkata napredne glikacije (AGE) u parodontalnom tkivu, a interakcije između AGE i njihovih receptora (RAGE, receptor za AGE, koji se nalazi posebno na makrofagima) dovode do aktivacije lokalnog imunog sistema i upalnih reakcija koje se prvo uočavaju na gingivi [10–14].

MATERIJAL I METODE

Istraživanje je sprovedeno nakon odobrenja Etičkog odbora Univerzitetskog kliničkog centra u Banjoj Luci (Bosna i Hercegovina), br. 01-9-192.2/15. Uzorak se sastojao od 42 pacova soja Vistar. Životinje su bile stare dva meseca, s telesnom težinom od 150–200 g. Čuvane su u grupnim kavezima od pleksiglasa, na 12 sati svetlosti (07.00 – 19.00 časova), na temperaturi vazduha od 22°C (± 2) i vlažnosti od 60% ± 10%, pri čemu su imali slobodan pristup hrani i vodi tokom eksperimenta. Na početku eksperimenta, individue su razdvojene u odgovarajuće test i kontrolne grupe.

Pacovi su bili podeljeni u dve eksperimentalne grupe (Exp_14 i Exp_30) i jednu kontrolnu grupu. Prvu (Exp_14) i drugu (Exp_30) grupu je činilo po 16 pacova sa eksperimentalno indukovanim DM-om. Korišćenjem aloksana, prva i druga grupa pacova (Exp_14 i Exp_30) dovedene su u eksperimentalno indukovan DM. Rastvor aloksana je aplikovan intraperitonealno u dozi od 100 mg na kilogram telesne težine pacova. Protokol se ponavljao svakog drugog dana, sve dok izmerena vrednost glikemije nije prešla 200 mg/dcl (životinja u hiperglikemiji). Glikemija je merena aparatom za merenje glikemije (ACCU ACEH, Roche) iz krvi repne vene. Postignuta hiperglikemija je kontrolisana redovnim merenjem (svakog drugog dana) i održavana sedam dana. Kontrolna grupa se sastojala od 10 zdravih pacova.

Pacovi iz prve grupe su žrtvovani posle 14 dana, a iz druge grupe posle 30 dana. Pacovi iz kontrolne grupe su žrtvovani takođe posle 30 dana. Za histološku analizu su kosti gornjih vilica zajedno sa zubima posle 48 h fiksacije (u 10% neutralnom puferovanom formalinu) dekalcifikovane u rastvoru azotne kiseline (ne duže od 90 minuta). Dekalcifikovani uzorci su potom isprani tekućom vodom i obrađeni u automatizovanom tkivnom procesoru Leica TP 1020 (Leica Byosistems) po standardnom protokolu: dehidracija u rastućim koncentracijama etil-alkohola (70%, 96%, 100%), bistrenje u ksilolu, impregnacija tečnim parafinom, nakon čega su odabrani uzorci tkiva ukalupljeni u parafinske blokove. Za histološku analizu parafinski blokovi su nakon hlađenja sečeni na kliznom mikrotomu (Leica SM 2000R, Leica Byosistems) na preseke debljine 4–5 μm, a poprečni preseci potpornog aparata sakupljani su na odgovarajuća predmetna stakalca i sušeni na 60°C. U procesoru za automatsko bojenje (Leica ST4040 Linear stainer, Leica Byosistems) tkivni preseci su deparafinisani, rehidrirani i ispirani u destilovanoj vodi. Nakon toga su obojeni standardnom metodom hematoksilin-eozina (HE). Definitivni preparati su analizirani svetlosnim mikroskopom (Leica DM 2500, Leica Byosistems) i fotografisani kamerom povezanom sa mikroskopom (Slika 1).

REZULTATI

U prvoj eksperimentalnoj grupi (Exp_14) parodontitis i gingivitis su zabeleženi u 54,5% slučajeva. U drugoj eksperimentalnoj grupi (Exp_30) parodontitis i gingivitis su zabeleženi u 88% slučajeva. U kontrolnoj grupi kod zdravih štakora (bez hiperglikemije) nije zabeležen nijedan slučaj parodontitisa i gingivitisa (Tabela 1, Slika 1). Postoji visoko statistički značajna razlika zastupljenosti parodontitisa i gingivitisa između ispitivanih grupa ($\chi^2 = 14,685$; $p < 0,001$) (Tabela 1).

DISKUSIJA

Inflamacija parodoncijuma je hronično upalno oboljenje izazvano nakupljanjem zubnog plaka, čije bakterije dovode do hroničnog i destruktivnog upalnog odgovora koje za posledicu ima razaranje tkiva tj. propadanje periodontalnog ligamenta, nastanak parodontalnih džepova i resorpciju alveolarne kosti. Rizik od parodontitisa je povećan 2-3 puta kod osoba sa DM-om u poređenju sa osobama bez DM-a, a nivo kontrole glikemije je ključan u određivanju rizika oboljevanja. Slično drugim komplikacijama dijabetesa, rizik od parodontitisa se povećava sa lošijom kontrolom glikemije [15, 16].

Većina istraživanja o zapaljenju potpornog tkiva zuba i dijabetesu fokusirana je na DM tipa 2 (verovatno zato što se ove bolesti uglavnom javljaju kod odraslih osoba srednjih godina), ali DM tipa 1 je takođe povezan s povećanom destrukcijom parodonta kod dece i tinejdžera [17].

U našoj studiji utvrđeno je da su parodontitis i gingivitis zastupljeni u grupama pacova sa eksperimentalno indukovanim DM-om, dok u zdravoj grupi pacova nisu pronađene patološke promene u parodoncijumu. Kod pacova prve grupe koji su žrtvovani posle 14 dana od uvođenja u DM, uočene su značajno manje promene na potpornim tkivima zuba u odnosu na grupu pacova koji su žrtvovani posle 30 dana.

Veliki broj istraživanja bavi se proučavanjem veze između DM-a i inflamacije potpornog tkiva zuba, jer pored mnogih kliničkih manifestacija kod pacijenata sa DM-om (uključujući decu i mlade odrasle osobe) povećan je rizik od parodontitisa. Lalla i saradnici su poredili parodontološki status kod dece obolele od DM-a i parodontološki status kod zdrave dece, iste starosne dobi – od 6 do18 godina. Rezultati njihove studije su ukazali na veću zastupljenost parodontitisa i gingivitisa (20%) kod dece sa DM-om nego kod zdrave dece (8%), što je u skladu sa rezultatima ove studije [18].

Parodontitis je danas poznat kao faktor rizika za pogoršanje kontrole glikemije i može povećati rizik od komplikacija dijabetesa. Choubaya i saradnici su takođe ispitivali povezanost parodontitisa sa razvojem i napredovanjem dijabetesa kod pacova soja Vistar sa indukovanim DM-om. Studija je ukazala na povezanost nivoa glikemije sa promenama na parodoncijumu, odnosno što je veći nivo glukoze u krvi pacova utvrđene promene na parodoncijumu su bile veće [19].

Takai i saradnici su primenom histološke analize utvrdili da je upalna reakcija u gingivalnom tkivu bila viša i intenzivnija kod pacova sa DM-om u odnosu na zdrave pacove. Ovi rezultati ukazuju na to da je i dentalni plak važan faktor za teške upalne procese parodoncijuma i na značaj pravilnog održavanja oralne higijene kod pacijenata sa DM-om [20].

Pepelassi i saradnici su, takođe koristeći histološku analizu koja je korišćena i u ovom istaživanju, utvrdili da je gubitak alveolarne kosti značajno veći kod pacova sa DM-om i parodontitisom nego kod pacova koji su imali samo parodontitis ili DM [21].

Kod pacijenata sa DM-om neophodno je podići nivo održavanja oralne higijene, kako ne bi došlo do nakupljanja dentalnog plaka, koji predstavlja jedan od faktora nastanka gingivitisa i drugih parodontoloških oboljenja. Takođe, nivo glikemije u krvi kod pacijenata obolelih od DM-a zavisi prevashodno od ishrane, zbog čega je važno da se pacijenti pridržavaju uputstava dobijenih od endokrinologa. Wang i saradnici i Preshaw i saradnici ukazali su na značaj uloge stomatološkog tima kod pacijenata s dijabetesom i parodontitisom u sprečavanju pogoršanja kliničke slike navedenih oboljenja i njenom poboljšanju [22, 23].

ZAKLJUČAK

Kod grupe pacova sa eksperimentalno indukovanim DM-om koji su žrtvovani posle 30 dana utvrđena je značajno veća zastupljenost parodontitisa i gingivitisa u odnosu na grupu pacova sa ekperimentalno indukovanim DM-om koji su žrtvovani posle

14 dana, kao i u odnosu na zdravu grupu pacova. Rezultati ove studije ukazuju na povezanost DM-a i parodontalnih oboljenja, zbog čega je neophodno predložiti preventivni program za pacijente sa DM-om koji obuhvata stomatološke procedure od obuke održavanja oralne higijene i redovnih poseta stomatologu, detekciju zubnog plaka, uklanjanje mekih i tvrdih zubnih naslaga te uočavanje početnih patoloških promena parodoncijuma i njihovo lečenje.