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Biochemical markers in saliva in patients with oral cancer

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SUMMARY

Head and neck cancers account for 3% of all human cancers and are mainly localized in the oral cavity. Early detection of cancer is extremely important for reducing mortality and morbidity from this disease. In addition to clinical trials and histopathological findings, in the last two decades, with technological development, more specific and sensitive methods have been used in the analysis of salivary markers.

The aim of this study was to present a wide range of analyzed markers in saliva: different protein markers (total proteins, albumin, defensins, staterin, cystatins), epithelial and molecular markers (CA125, CA19-9, TPA, CEA, CYFRA 21-1, CD44), enzymes (LDH, ALP, MMP, SOD), cytokines (IL-6, IL-8, IL-1β, TNF-α), oxidative stress markers (8-OHdG, MDA), non-enzymatic antioxidants (glutathione, uric acid, albumin, vitamin C and E) etc. Collecting saliva is simple and painless for the patient, it does not require expensive equipment or specially trained staff, and it is possible to take saliva several times and in unlimited quantities. Extensive research that is increasingly being done with modern techniques indicates that saliva may be useful for early detection of the disease in the near future.

Keywords: saliva; oral cancer; biochemical markers

INTRODUCTION

Head and neck cancers account for 3% of all human cancers and are mainly localized in the oral cavity (48%). The most common (90%) are squamous cell carcinomas, with an incidence of over 300,000 cases per year worldwide. The tongue (over 40%) and floor of the mouth are the most common localizations of this tumor in the oral cavity, and it is less often localized in the area of the gingiva, buccal mucosa, labial mucosa and hard palate. The most important risk factors for the development of oral cancer are smoking, alcohol, tobacco smoke agents, human papilloma virus and others [1, 2, 3].

Early detection of oral cancer is extremely important for reducing mortality and morbidity from this disease. The diagnosis is made on the basis of clinical analyzes and histopathological findings after taking the biopsy. Recently, more work is being done on the potential use of non-invasive methods - "liquid biopsy" - for the detection of diagnostic and prognostic biomarkers in body fluids [4, 5]. Identification and characterization of markers in saliva would help in the diagnosis and monitoring of patients with pre-malignant and malignant lesions of the oral cavity, patients in the postoperative procedure or the application of adequate therapy [6-9]. In the monitoring of oral cancers, the term "salivaomics" has been introduced, for a wide range of technologies that investigate different types of molecules found in saliva [10].

The aim of this study is a detailed presentation of various markers that were analyzed in the saliva of patients with oral cancer.

PROTEIN MARKERS IN SALIVA

Protein markers in the saliva of patients with oral cancer are analyzed individually or in groups, for the purpose of early detection of the disease and timely application of appropriate therapy.

In the saliva of patients with oral cancer, the concentration of total proteins was increased compared to the control group [11, 12]. Other studies have shown that saliva of untreated patients has an increased concentration of total protein compared to treated patients with oral cancer and patients with precancerous lesions [13]. The authors believe that this is a consequence of locally increased protein synthesis. Sanjay et al. came to similar results in cancers with different degrees of differentiation, but the difference in total protein concentration was not statistically significant [14]. The opposite results were obtained, which indicate that the total proteins are reduced in the saliva of patients with oral cancer [15].

In the recent years, with technological developments, more specific and sensitive methods have been used to identify total salivary proteins or peptides, known as salivary proteomes. About a thousand proteins are present in saliva, with different roles in metabolic processes, immune regulation, cell adhesion, communication, etc. [16, 17].

Albumins are plasma proteins that are synthesized in the liver. The roles of albumin are maintaining oncotic pressure, regulation of blood pH, transport of various substances, but also antioxidant protection. Our studies indicate a significant decrease in the concentration of albumin in the saliva of patients with oral cancer compared to the control group [18]. This can be explained by

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the "consumption" of this antioxidant in neutralizing free radicals, which are excessively produced in these pathological conditions. In other studies, the opposite results were obtained [19].

The best known tumor suppressor gene is p53 (16-20 kb DNA) is localized on human chromosome 17. Mutation, inactivation and deletion of the p53 gene are also involved in the pathogenesis of oral tumors. Increased expression of the p53 gene has been found in saliva in oral squamous cell carcinoma. A high percentage (71 %) of tumor-specific mutations in the p53 gene has also been demonstrated in these patients [20].

Defensins are salivary peptides that exhibit antimicrobial activity and are extremely important in maintaining oral health [21]. They are proven in granular leukocytes - neutrophils, so they are called human neutrophil defensins. The concentration of defensin-1 in the saliva of patients with oral squamous cell carcinoma and other oral diseases (lichen planus, leukoplakia, inflammation) is significantly higher compared to saliva of the control group. This is in accordance with the fact that during many diseases in the oral cavity, a large number of neutrophils migrate from the blood through gingival sulcus to the oral environment [22].

Staterin is an acidic salivary protein that prevents the deposition of calcium phosphate in the excretory ducts of the salivary glands and regulates solubility of tooth enamel [21, 23]. In the saliva of patients with oral cancer, the concentration of staterin is reduced, and thus its function in oral cavity is reduced too [24].

Cystatins are proteins by chemical structure and inhibitors of the enzyme cysteine proteases by function. Cystatin SA-I, which has 14 kDa, has been detected in the saliva of patients with oral squamous cell carcinoma. This protein is more pronounced in the saliva of patients before treatment compared to the saliva of treated patients, so it may be a useful biomarker for patients with oral squamous cell carcinoma [25].

Epidermal growth factor (EGF) is a protein that plays a significant role in maintaining the homeostasis of the oral mucosa and mucosa of the upper gastrointestinal tract. It also promotes wound healing in the oral environment. Smoking and alcohol consumption have been shown to reduce the level of salivary EGF, which contributes to the development of oral carcinogenesis. In the saliva of patients with oral cancer, the concentration of EGF is reduced, and thus the possibility of renewal of the epithelium of the oral mucosa in these patients is reduced [26, 27].

SIALIC ACID, EPITHELIAL MARKERS, ENZYMES

Sialic acid is located at the terminal end of glycoproteins and glycolipids and plays a significant role in cell to cell interactions and development of cell adhesion important in malignant transformation [28]. Sialic acid concentration is increased in saliva of well-differentiated squamous cell carcinomas compared to poorly differentiated carcinomas [14]. Other authors have found its increased concentration relative to pre-malignant lesions in healthy individuals [11, 29]. After radiotherapy of patients with oral cancer, the level of sialic acid in the saliva is reduced, so it can be said that sialic acid is a sensitive tumor marker [30].

Epithelial markers (CA125, CA19-9, tissue polypeptide antigen, carcino-embryonic antigen, CYFRA 21-1) have an increased concentration in the saliva of patients with oral squamous cell carcinoma. In particular, the three analyzed markers (CYFRA-21, tissue polypeptide antigen, CA-125) were significantly increased (by 400 %), while for other markers no statistical significance was found [6]. Similar results were obtained by other authors for CYFRA 21-1 [31]. Analysis of these tumor markers in the saliva of patients with oral cancer may be suggested as an aid, rather than as a substitute, for other well-established diagnostic methods.

Molecular marker, the CD44 protein, can be converted to a soluble form by the action of proteases. It is increased in the saliva of patients with oral cancer compared to the control group. A perfect correlation between salivary CD44 molecules and grade and the degree of aggressiveness of the malignant lesion has been demonstrated. There is also a high statistically significant difference between patients with oral cancer and patients with pre-malignant lesions. This is indicated by the fact that the concentration of salivary CD44 between 19.2 and 20.4 ng/mL may indicate malignant transformation of lesions of the oral mucosa [32].

Enzymes, responsible for playing role in metabolic processes in cells, were also analyzed in saliva. The activity of lactate dehydrogenase and alkaline phosphatase is increased in the saliva of patients with oral squamous cell carcinoma [33, 34]. Merza et al. have demonstrated increased activity of these enzymes in the serum of patients with this disease [35]. The authors believe that the release of intracellular enzymes is increased from pathologically altered cells, rather than a consequence of increased biosynthesis. Matrix metalloproteinases (MMPs) are enzymes involved in the pathogenesis of oral cancer. Unregulated MMP activity in tumor tissues is one of the main factors of protein destruction (collagen, elastin, fibronectin). Increased activity of MMP-2 and MMP-9 has been demonstrated in the saliva of patients with oral squamous cell carcinoma (OSCC) [33, 36]. Peisker et al. demonstrated significantly increased MMP-9 activity in patients with OSCC compared to the control group (19.2 %), whether it was the first diagnosis or recurrence. The sensitivity of this marker was 100 % and the specificity 26.7 % [37].

CYTOKINES AND MARKERS OF OXIDATIVE STRESS

Cytokines represent a family of soluble, low molecular weight proteins or glycoproteins, which function as mediators and modulators of the immune response, inflammation, hematopoiesis, and development of malignant tumors. The concentration of interleukin-6 (IL-6), IL-8 and tumor necrosis factor (TNF-α), which

act as promoters in the process of carcinogenesis, was increased in the saliva of patients with oral cancer [38]. The most commonly determined cytokine in the saliva of patients with oral squamous cell carcinoma is IL-6 [39]. Brailo et al. have demonstrated increased concentrations of salivary IL-6 and IL-1β in patients with oral cancer compared to patients with leukoplakia. They leave the possibility to examine whether these cytokines are markers of malignant transformation of leukoplakia before oral cancer becomes clinically evident [40]. Other studies have indicated an increased content of IL-1, IL-6, TNF-α in the saliva of subjects with oral squamous cell carcinoma compared to patients with dysplastic oral lesions and control groups of subjects. Because of the above, salivary cytokines provide useful information on the behavior of epithelium in carcinogenesis and may be potential biochemical markers of oral cancer [41].

In the pathogenesis of oral cancer nowadays free radicals and oxidative stress are given increasing importance. Free radicals of oxygen and nitrogen lead to oxidative modification of proteins, lipids, DNA of oral tissue cells, which can result in their malignant alteration [42]. The most important biomarker of the degree of oxidative DNA damage is 8-hydroxy-deoxyguanosine (8-OHdG), the concentration of which is increased in the saliva of patients with oral squamous cell carcinoma [43]. The end product of lipid peroxidation is malondialdehyde (MDA). By analyzing the concentration of MDA, we showed that in the saliva of patients with periodontal disease there is an increase in its content compared to the group of healthy subjects [44]. Other authors have obtained similar results in the saliva of patients with oral squamous cell carcinoma [45].

In addition, toxic components from tobacco smoke affect the change in the antioxidant capacity of saliva [46]. Decreased antioxidant enzyme activity results in incomplete elimination of H_2O_2 from the oral environment, which reacts with other radicals and molecules to form much more reactive free radicals, which oxidatively damage biomolecules such as DNA, and that can lead to malignant transformation and oral cancer [47, 48]. The concentration of non-enzymatic antioxidants in saliva, such as glutathione, is also reduced. The authors explain this by the interaction of tobacco smoke aldehydes and SH groups of glutathione, when nonfunctional conjugates are formed [11, 46]. It is especially interesting to analyze the concentration of uric acid, as the main non-enzymatic antioxidant, which participates with about 70% in the total antioxidant capacity of saliva. Our and other studies showed that in the saliva of patients with oral cancer, who were smokers, the concentrations of uric acid and albumin were significantly reduced, compared to the group of healthy subjects. These results can be explained by the increased "consumption" of these antioxidants in neutralizing free radicals [18, 49]. Decreased concentrations of vitamins E and C in the saliva of patients with increase of histological grade of oral cancer have also been demonstrated [50]. From the above, it can be concluded that the analysis of the antioxidant capacity of saliva can be useful for improving preventive measures in the development of oral cancer, so, as in the case of periodontitis, the use of various antioxidants is recommended.

CONCLUSION

Based on this review, it was determined that biochemical composition of saliva changes in patients with oral cancer. Saliva analyzes in these patients have advantages and disadvantages. Saliva collection is simple and painless for the patient, it does not require expensive equipment or specially trained staff, which certainly goes in favor of cost-effectiveness. It is also possible to take saliva several times and in unlimited quantities. However, some of the present problems cannot be ignored. Individual biomarkers proven in saliva are not sensitive and specific enough to meet stringent diagnostic criteria. There is also the problem of extremely high saliva viscosity, due to the presence of mucopolysaccharides and mucoproteins, which can interfere with the analytical procedure. Despite these limiting circumstances, extensive research, increasingly done with modern techniques, indicates that saliva may be useful for early detection, diagnosis and monitoring of applied therapy for oral cancer in the near future.

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Biohemijski markeri u salivi obolelih od oralnog karcinoma

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KRATAK SADRŽAJ

Karcinomi glave i vrata predstavljaju 3% od svih karcinoma čoveka i uglavnom su lokalizovani u usnoj duplji. Rano otkrivanje karcinoma je izuzetno važno za smanjenje mortaliteta i morbiditeta od ove bolesti. Pored kliničkih ispitivanja i histopatološkog nalaza, u poslednje dve decenije, sa tehnološkim razvojem, koriste se specifičnije i osetljivije metode u analizi salivarnih markera.

Cilj rada bio je da se prikaže široka paleta analiziranih markera: različiti proteinski markeri (ukupni proteini, albumin, defenzini, staterin, cistatini), epitelni i molekularni markeri (CA125, CA19-9, TPA, CEA, CYFRA 21-1, CD44), enzimi (LDH, ALP, MMP, SOD), citokini (IL-6, IL-8, IL-1β, TNF-α), markeri oksidativnog stresa (8-OHdG, MDA), neenzimski antioksidansi (glutation, mokraćna kiselina, albumin, vitamin C i E) itd. Prednosti prikupljanja salive su jednostavnost i bezbolnost za pacijenta, to što ne zahteva skupu opremu ni posebno obučeno osoblje, a salivu je moguće uzeti više puta i u neograničenim količinama. Obimna istraživanja koja se sve više rade savremenim tehnikama ukazuju na to da saliva može biti od koristi za rano otkrivanje bolesti u bliskoj budućnosti.

Ključne reči: saliva; oralni karcinomi; biohemijski markeri

UVOD

Karcinomi glave i vrata predstavljaju 3% od svih karcinoma čoveka i uglavnom su lokalizovani u usnoj duplji (48%). Najčešće su to (90%) planocelularni karcinomi, čija je incidenca preko 300 000 slučajeva godišnje u svetu. Jezik (preko 40%) i pod usta su najučestalije lokalizacije ovog tumora u usnoj duplji, a ređe je lokalizovan u predelu gingive, bukalne mukoze, labijalne mukoze i tvrdog nepca. Najvažniji faktori rizika za nastanak karcinoma usne duplje su pušenje, alkohol, agensi duvanskog dima, humani papiloma virus i dr. [1, 2, 3].

Rano otkrivanje karcinoma usne duplje je izuzetno važno za smanjenje mortaliteta i morbiditeta od ove bolesti. Dijagnoza se vrši na osnovu kliničkih analiza i histopatoloških nalaza nakon uzimanja biopsije. U poslednje vreme sve se više radi na potencijalnoj upotrebi neinvazivnih metoda – „tečnoj biopsiji" – za detekciju dijagnostičkih i prognostičkih biomarkera u telesnim tečnostima [4, 5]. Identifikacija i karakterizacija markera u salivi bi pomogla u dijagnozi i praćenju pacijenata sa premalignim i malignim lezijama usne duplje, pacijenata u postoperativnom postupku, primeni adekvatne terapije [6–9]. U praćenju oralnih karcinoma uveden je termin "salivomi", za široku paletu tehnologija kojima se istražuju različite vrste molekula koji se nalaze u salivi [10].

Cilj rada je detaljan prikaz različitih markera koji su analizirani u salivi pacijenata sa oralnim karcinomima.

PROTEINSKI MARKERI U SALIVI

Proteinski markeri u pljuvački obolelih od oralnog karcinoma analiziraju se pojedinačno ili grupno, radi ranog otkrivanja oboljenja i pravovremene primene odgovarajuće terapije.

U pljuvački pacijenata obolelih od oralnog karcinoma povećana je koncentracija ukupnih proteina u odnosu na kontrolnu grupu [11, 12]. Druga istraživanja su pokazala da je u pljuvački netretiranih pacijenata povećana koncentracija ukupnih proteina u odnosu na tretirane pacijente sa oralnim karcinomom i pacijente sa prekanceroznom lezijom [13]. Autori smatraju da je to posledica lokalno povećane sinteze proteina. Sanjay i sar. su došli do sličnih rezultata kod karcinoma sa različitim stepenom diferentovanosti, ali razlika u koncentraciji ukupnih proteina nije bila statistički značajna [14]. Dobijeni su i suprotni rezultati, koji ukazuju da su ukupni proteini smanjeni u salivi obolelih od oralnog karcinoma [15].

U poslednjih nekoliko godina, sa tehnološkim razvojem, koriste se specifičnije i osetljivije metode u identifikaciji ukupnih proteina ili peptida salive, poznatih kao salivarni proteomi. U salivi je prisutno oko hiljadu proteina, sa različitim ulogama u metaboličkim procesima, imunoj regulaciji, ćelijskoj adheziji, komunikaciji itd. [16, 17].

Albumini su plazma proteini koji se sintetišu u jetri. Uloga albumina je u održavanju onkotskog pritiska, regulaciji pH krvi, transportu različitih supstancija, ali i u antioksidativnoj zaštiti. Naša istraživanja ukazuju na značajno smanjenje koncentracije albumina u pljuvački bolesnika sa oralnim karcinomom u odnosu na kontrolnu grupu [18]. To se može objasniti „potrošnjom" ovog antioksidansa u neutralisanju slobodnih radikala, koji se prekomerno stvaraju u ovim patološkim stanjima. U drugim istraživanjima su dobijeni suprotni rezultati [19].

Najpoznatiji tumor supresorski gen je p53 (16-20 kb DNA), koji je lokalizovan na humanom hromozomu 17. Mutacija, inaktivacija i delecija gena p53 uključena je i u patogenezu tumora oralne sredine. Povećana ekspresija p53 gena je ustanovljena u pljuvački kod oralnog skvamoznog karcinoma. Takođe je kod ovih pacijenata dokazan visok procent (71%) tumor-specifičnih mutacija na genu p53 [20].

Defenzini su peptidi pljuvačke koji ispoljavaju antimikrobno dejstvo i imaju izuzetan značaj u održavanju oralnog zdravlja [21]. Dokazani su u granuliranim leukocitima – neutrofilima, pa se nazivaju humani neutrofilni defenzini. Koncentracija defenzina-1 u pljuvački pacijenata sa oralnim skvamoznim celularnim karcinomom i drugim oralnim oboljenjima (lichen planus, leukoplakija, inflamacije) značajno je veća u odnosu na pljuvačku kontrolne grupe. To je u saglasnosti sa činjenicom da u toku mnogih oboljenja u usnoj duplji dolazi do migracije velikog broja neutrofila iz krvi kroz gingivalni sulkus u oralnu sredinu [22].

Staterin je kiseli protein pljuvačke koji sprečava taloženje kalcijum-fosfata u izvodnim kanalima pljuvačnih žlezda i reguliše rastvorljivost zubne gleđi [21, 23]. U pljuvački pacijenata obolelih od oralnog karcinoma smanjena je koncentracija staterina, a time je umanjenja i njegova funkcija u usnoj duplji [24].

Cistatini su po hemijskoj strukturi proteini, a po funkciji inhibitori enzima cistein proteaza. U pljuvački pacijenata sa

oralnim skvamoznim karcinomom dokazan je cistatin SA-I koji ima 14 kDa. Ovaj protein je više izražen u pljuvački pacijenata pre tretmana u odnosu na pljuvačku tretiranih pacijenata, tako da može biti koristan biomarker obolelih od oralnog skvamoznog karcinoma [25].

Epidermalni faktor rasta (EGF) jeste protein koji ima značajnu ulogu u održavanju homeostaze oralne mukoze i mukoze gornjih delova gastrointestinalnog trakta. Takođe, pospešuje zarastanje rana u oralnoj sredini. Dokazano je da pušenje i konzumiranje alkohola smanjuju nivo salivarnog EGF, što doprinosi razvoju oralne kancerogeneze. U pljuvački pacijenata obolelih od oralnog karcinoma smanjena je koncentracija EGF, a time je smanjena i mogućnost obnavljanja epitela oralne sluzokože kod ovih pacijenata [26, 27].

SIJALINSKA KISELINA, EPITELNI MARKERI, ENZIMI

Sijalinska kiselina se nalazi na terminalnom kraju glikoproteina i glikolipida i ima značajnu ulogu u ćeliji, ćelijskim interakcijama i u razvoju ćelijske adhezije značajne u malignoj transformaciji [28]. Koncentracija sijalinske kiseline je povećana u pljuvački dobro izdiferentovanih skvamoznih karcinoma u odnosu na slabo diferentovane karcinome [14]. Drugi autori su utvrdili njenu povećanu koncentraciju u odnosu na premaligne lezije i zdrave jedinke [11, 29]. Nakon radioterapije pacijenata sa oralnim karcinomom smanjen je nivo sijalinske kiseline u salivi, pa se može reći da je sijalinska kiselina senzitivni tumorski marker [30].

Epitelni markeri (CA125, CA19-9, tkivni polipeptidni antigen, karcino-embrionalni antigen, CYFRA 21-1) imaju povećanu koncentraciju u pljuvački obolelih od oralnog skvamoznog karcinoma. Posebno su tri analizirana markera (CYFRA-21, tkivni polipeptidni antigen, CA-125) značajno povećana (za 400%), dok za ostale markere nije utvrđena statistička značajnost [6]. Slične rezultate su dobili drugi autori za CYFRA 21-1 [31]. Analiza ovih tumorskih markera u pljuvački pacijenata sa oralnim karcinomom može se predložiti kao pomoć, a ne kao zamena, za druge afirmisane dijagnostičke metode.

Molekularni marker, protein CD44, može dejstvom proteaza da pređe u rastvorljivu formu. On je povećan u pljuvački pacijenata sa oralnim kancerom u odnosu na kontrolnu grupu. Dokazana je savršena korelacija između salivarnog CD44 molekula i gradusa i stepena agresivnosti maligne lezije. Takođe, postoji visoka statistički značajna razlika između pacijenata sa oralnim karcinomom i pacijenata sa premalignim lezijama. Na to ukazuje i podatak da koncentracija salivarnog CD44 između 19,2 i 20,4 ng/mL može ukazati na malignu transformaciju lezija oralne sluzokože [32].

U pljuvački su analizirani i enzimi, odgovorni za odigravanje metaboličkih procesa u ćelijama. U pljuvački pacijenata sa oralnim skvamoznim karcinomom povećana je aktivnost laktat-dehidrogenaze i alkalne fosfataze [33, 34]. Merza i sar. su dokazali povećanu aktivnost ovih enzima i u serumu pacijenata sa ovim oboljenjem [35]. Autori smatraju da je oslobađanje intraćelijskih enzima povećano iz patološki izmenjenih ćelija, pre nego što je posledica povećanja njihove biosinteze. Matriksne metaloproteinaze (MMP) jesu enzimi koji participiraju u patogenezi karcinoma oralne sredine. Neregulisana aktivnost MMP u tkivima tumora jedan je od glavnih faktora destrukcije proteina (kolagena, elastina, fibronektina). U pljuvački pacijenata sa oralnim skvamoznim karcinomom (OSCC) dokazana je povećana aktivnost MMP-2 i MMP-9 [33, 36]. Peisker i sar. dokazali su značajno povišenu aktivnost MMP-9 kod pacijenata sa OSCC u odnosu na kontrolnu grupu (19,2%), bilo da je u pitanju prvo dijagnostikovanje ili recidiv. Osetljivost ovog markera bila je 100%, a specifičnost 26,7% [37].

CITOKINI I MARKERI OKSIDATIVNOG STRESA

Citokini predstavljaju familiju solubilnih, niskomolekularnih proteina ili glikoproteina, koji funkcionišu kao medijatori i modulatori imunog odgovora, inflamacije, hematopoeze, razvoja malignih tumora. U salivi obolelih od oralnog karcinoma povećana je koncentracija interleukina-6 (IL-6), IL-8 i faktora nekroze tumora (TNF-α), koji deluju kao promotori u procesu kancerogeneze [38]. Najčešće određivani citokin u salivi pacijenata sa oralnim skvamoznim karcinomom je IL-6 [39]. Brailo i sar. su dokazali povećanu koncentraciju salivarnog IL-6 i IL-1β kod pacijenata sa oralnim karcinomom u odnosu na pacijente sa leukoplakijom. Oni ostavljaju mogućnost da se ispita da li su ovi citokini markeri maligne transformacije leukoplakija pre nego što oralni karcinom postane klinički evidentan [40]. Druge studije su ukazale na povećan sadržaj IL-1, IL-6, TNF-α u pljuvački ispitanika sa oralnim skvamoznim karcinomom u odnosu na pacijente sa displastičnim oralnim lezijama i kontrolnim grupama ispitanika. Zbog navedenog, citokini salive pružaju korisne informacije o ponašanju epitela u karcinogenezi i mogu biti potencijalni biohemijski markeri karcinoma oralne sredine [41].

U patogenezi oralnog karcinoma danas se sve veći značaj poklanja slobodnim radikalima i oksidativnom stresu. Slobodni radikali, kiseonički i azotni, dovode do oksidativne modifikacije proteina, lipida, DNK ćelija oralnih tkiva, što može imati za posledicu njihovu malignu alteraciju [42]. Najznačajniji biomarker stepena oksidativnog oštećenja DNK jeste 8-hidroksidezoksiguanozin (8-OHdG), čija je koncentracija povećana u salivi pacijenata obolelih od oralnog skvamoznog karcinoma [43]. Krajnji produkt lipidne peroksidacije jeste malondialdehid (MDA). Analizom koncentracije MDA, mi smo pokazali da u pljuvački obolelih od periodontalnih bolesti dolazi do porasta njegovog sadržaja u odnosu na grupu zdravih ispitanika [44]. Drugi autori su dobili slične rezultate u salivi pacijenata sa oralnim skvamoznim karcinomom [45].

Pored toga, toksične komponente iz duvanskog dima utiču na promenu antioksidativnog kapaciteta salive [46]. Smanjenje aktivnosti antioksidativnih enzima ima za posledicu nepotpunu eliminaciju H_2O_2 iz oralne sredine, čijom se reakcijom sa ostalim radikalima i molekulima stvaraju mnogo reaktivniji slobodni radikali, koji vrše oksidativno oštećenje biomolekula kao što je DNK, a što može dovesti do maligne transformacije i nastanka oralnog karcinoma [47, 48]. Takođe je smanjena i koncentracija neenzimskih antioksidanata u salivi, kao što je slučaj sa glutationom. Autori ovo objašnjavaju interakcijom aldehida duvanskog dima i SH grupa glutationa, kada se formiraju nefunkcionalni konjugati [11, 46]. Posebno je interesantno analiziranje koncentracije mokraćne kiseline, kao glavnog neenzimskog antioksidanta, koji učestvuje sa oko 70% u ukupnom antioksidativnom kapacitetu salive. Naša i druga istraživanja pokazuju da je u salivi pacijenata sa oralnim karcinomom, koji su bili pušači, značajno smanjena koncentracija mokraćne kiseline i albumina, u odnosu

na grupu zdravih ispitanika. Ovi rezultati mogu se objasniti pojačanom „potrošnjom“ ovih antioksidanata u neutralisanju slobodnih radikala [18, 49]. Takođe je dokazana smanjena koncentracija vitamina E i C u salivi pacijenata sa rastom histološkog gradusa oralnog karcinoma [50]. Iz navedenog, može se zaključiti da analiza anitoksidativne sposobnosti pljuvačke može biti od koristi za unapređenje preventivnih mera u nastanku oralnog karcinoma, pa se, kao i u slučaju parodontopatije, preporučuje upotreba raznih antioksidanasa.

ZAKLJUČAK

Na osnovu ovog preglednog rada utvrđeno je da se kod pacijenata sa oralnim karcinomom menja biohemijski sastav salive. Analize salive kod ovih pacijenata imaju prednosti i nedostatke. Prikupljanje salive je jednostavno i bezbolno za pacijenta, ne zahteva skupu opremu ni posebno obučeno osoblje, što svakako ide u prilog ekonomičnosti rada. Takođe je moguće salivu uzeti više puta i u neograničenim količinama. Međutim, neki prisutni problemi se ne mogu ignorisati. Dokazani pojedinačni biomarkeri u pljuvački nisu osetljivi i dovoljno specifični da zadovolje stroge dijagnostičke kriterijume. Postoji i problem izuzetno velike viskoznosti salive, zbog prisutnih mukopolisaharida i mukoproteina, što može ometati analitičku proceduru. Uprkos ovim ograničavajućim okolnostima, obimna istraživanja, koja se sve više rade savremenim tehnikama, ukazuju da saliva može biti od koristi za rano otkrivanje, dijagnozu i praćenje primenjene terapije kod oralnih karcinoma u bliskoj budućnosti.