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EVALUACIJA SALIVARNOG PROTOKA I SALIVARNOG LAKTOFERINA NAKON RADIOTERAPIJE KOD MALIGNIH NEOPLAZMI GLAVE I VRATA

EVALUATION OF SALIVARY FLOW RATE AND SALIVARY LACTOFERRIN AFTER RADIOTHERAPY OF HEAD AND NECK MALIGNANT NEOPLASMS

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Sažetak

Uvod: Kserostomija kao rezultat smanjenog protoka pljuvačke najčešća je komplikacija nakon zračenja kod pacijenata sa malignim neoplazmama glave i vrata, koja značajno smanjuje kvalitet života. Upotreba volumetrijski modulirane radijacione terapije (VMAT) preciznija je u primeni doza, budući da okolna zdrava tkiva tako mogu ostati pošteđena od negativnog uticaja radijacije.

Cilj istraživanja bio je da se proceni protok pljuvačke i laktoferin pljuvačke kod pacijenata sa malignim neoplazmama glave i vrata nakon sprovedene radioterapije.

Materijali i metode: Istraživački uzorak čine 24 pacijenta tretirana različitim tehnikama radijacije; kod svih je određen protok pljuvačke u minutu i bihemijskim analizama je kvantitativno određena koncentracija laktoferina.

Rezultati: Veće prosečne vrednosti protoka pljuvačke dobijene su kod pacijenata tretiranih manjom dozom radijacije, do 60 Gy, i to kod onih tretiranih tehnikom volumetrijske modulirane radijacione terapije. Na osnovu rezultata linearne korelacije između ispitivanih varijabli laktoferina i količine pljuvačke u ml/min kod ispitanika, može se zaključiti da je korelacija između ovih dveju varijabli statistički značajna, dok je korelacija negativna, a koeficijent korelacije je sa vrednošću $r(24) = -0,903$; $p < 0,01$.

Zaključak: Protok pljuvačke je veći kod pacijenata tretiranih volumetrijski modulisanom radioterapijom; pritom, koliko je doza zračenja manja, toliko su veće vrednosti izlučene pljuvačke u minutu. Može se zaključiti da povišene vrednosti laktoferina imaju antiinflamatorne odlike i da su dobar pokazatelj upale, što može biti i korisna komponenta za zaštitu pljuvačnih žlezda od negativnih efekata terapije radijacijom.

Cljučne reči: hiposalivacija, maligne plazme, volumetrijska modulirana radioterapija, salivarni proteini, laktoferin

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Abstract

Introduction: The most common postradiation complication in patients with head and neck neoplasms resulting from decreased salivary flow rate is xerostomia which affects the quality of life of patients in a negative way. On account of that, the application of volumetric modulated radiation therapy (VMAT) is found to be more precise in dose application, thus the surrounding healthy tissues can be spared from the negative influence of radiation.

The aim of this study was to evaluate the salivary flow rate and salivary lactoferrin levels in patients with head and neck malign neoplasms following radiation therapy.

Material and Methods: The research sample consisted of 24 patients treated with different radiation techniques, in which the salivary flow rate was measured for one minute. Additionally, the concentration of lactoferrin was quantitatively evaluated using biochemical analyses.

Results: Higher average values were obtained in patients treated with a lower dose of radiation with up to 60 Gy, especially in those treated with a volumetric modulated radiotherapy technique. Therefore, from the results of linear correlation between the evaluated variables of lactoferrin and salivary flow volume in ml/min, it can be concluded that the correlation between these two variables is statistically significant whereby the correlation is negative with the coefficient of correlation $r(24) = -0.903$; ($p < 0.01$).

Conclusion: The salivary flow rate is higher in patients treated with volumetric modulated radiotherapy, whereby the lower the dose of radiation, the higher the values of excreted saliva per minute. Therefore it can also be concluded that the higher values of lactoferrin are considered to have anti-inflammatory characteristics and are indicators of inflammation, which can be used for prevention of the salivary glands from negative effects of the radiation therapy.

Key words: hyposalivation, malignant neoplasms, volumetric modulated radiotherapy, salivary proteins, lactoferrin

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Uvod

Tokom tretmana radioterapijom pacijenti se suočavaju sa teškim posledicama, poput ogromnog poremećaja funkcije žlezda i izraženog stepena hiposalivacije, što dovodi do subjektivnog osećaja kserostomije. Radijacija prouzrokuje oštećenja žlezda, smanjenje njihove funkcije, kao i promenu u sastavu pljuvačke¹. Protok pljuvačke u minutu najčešće je smanjen ispod 0,1 ml/min do 0,2 ml/min^{2,3}. Protok se smanjuje za 50% – 60% nakon prve nedelje terapije; takođe, uočavaju se promene njegovog viskoziteta, nivoa elektrolita, kapaciteta pufera i pH vrednosti³. Posledice se uglavnom odnose na ukupnu dozu zračenja, dozu zračenja po frakciji, zapreminu i distribuciju doze, kombinaciju sa hemoterapijom i trajanje lečenja⁴. Mehanizam nastanka uticaja terapije radijacijom na pljuvačne žlezde nije u celosti razjašnjen. Smatra se da se matične i progenitorne ćelije parotidne žlezde nalaze u regionu koji sadrži glavne izvodne kanale. Zračenje ovih mesta rezultuje izmenjenom količinom proizvedene pljuvačke^{6,7}. Osim toga što laktoferin svojom antibakterijskom i antiinflamatornom ulogom ozbiljno učestvuje u lokalnom oralnom imunitetu, smatra se i da poseduje radioaktivnu funkciju pljuvačnih žlezda, mada mehanizmi kojima se to odvija još nisu u potpunosti definisani^{8,9}. Jedan od mehanizama kojim se postiže radioprotektivni efekat jeste uticaj laktoferina na proliferaciju ćelija i ciklin D1 – posredovano napredovanje ćelijskog ciklusa. Iz ovih razloga, smatra se da laktoferin može biti korisna komponenta za zaštitu pljuvačnih žlezda od negativnih efekata terapije radijacijom¹⁰. Terapija zračenjem utiče na sastav pljuvačke i donosi značajne promene; pritom, nivo laktoferina smatra se dobrim pokazateljem upale nakon radioterapije.

Cilj istraživanja bio je da se proceni protok pljuvačke i laktoferin pljuvačke kod pacijenata sa malignim neoplazmama glave i vrata nakon sprovedene radioterapije.

Materijali i metode

U istraživanje su bila uključena 24 pacijenta tretirana dvema tehnikama radioterapije; kod svih je bio izmeren protok pljuvačke, a biohemijskim analizama određena je vrednost laktoferina kao posebnog parametra u postradijacionom periodu.

Na Univerzitetnoj klinici za radioterapiju i onkologiju u Skoplju izvršena je selekcija pacijenata prema unapred definisanim kriterijumima uključivanja i isključenja i podacima o vrsti radioterapije.

Introduction

During the radiation treatment, patients face severe consequences such as large obstruction of the salivary glands function and a high degree of hyposalivation, which leads to the subjective feeling of xerostomia. The radiation causes the obstruction of the salivary glands, a decrease in their function and changes in the saliva compounds¹. The salivary flow rate is usually reduced under 0.1–0.2 ml/min^{2,3}. It is reduced by 50–60% after the first week of therapy, changes are also registered in the viscosity of the saliva, the level of electrolytes, puffer capacity and the pH value³. The after-treatment consequences are mainly related to the total dose of radiation, the dose of radiation per fraction, the volume and distribution of the doses, the combination with chemotherapy and the duration of the treatment⁴. The mechanism by which radiation therapy influences the salivary glands is not completely clarified. It is considered that the stem cells and the progenitor cells of parotid glands are located in the region of the main salivary ducts. The effect of radiation on these structures leads to the modification of the produced saliva volume^{6,7}. Lactoferrin, with its antibacterial and anti-inflammatory role, significantly contributes to local oral immunity and is considered to have radioprotective function in the salivary glands as well. However, the mechanisms that contribute to this are still not quite precisely defined^{8,9}. One of the mechanisms that provide the radio protective effect of lactoferrin is its influence on the cell proliferation and cyclin-D1-mediated progression of the cell cycle. For these reasons, lactoferrin is considered to be a useful component in the prevention of salivary glands from the negative influence of radiation therapy¹⁰. Radiation therapy affects the saliva composition and generates significant changes, whereby the lactoferrin level is considered to be a valuable indicator of inflammation after radiation therapy.

The aim of this study was to evaluate the salivary flow rate and salivary lactoferrin levels in patients with head and neck malign neoplasms following radiation therapy.

Materials and Methods

In this study, 24 patients treated with two different techniques of radiation therapy were evaluated for the salivary flow rate measurement, and by using biochemical analysis, lactoferrin values were defined as a separate parameter in the post-radiation period.

Svi ispitanici koji su deo istraživanja dobrovoljno su potpisali formular o informisanosti i saglasnost da njihovi podaci budu korišćeni u ovoj studiji. Pregled pacijenata i uzimanje uzoraka izvršeno je na Klinici za maksilofacijalnu hirurgiju u Skoplju. Sakupljanje uzoraka i merenje protoka pljuvačke vršeno je tako što su ispitanici pljuvali u posebne kolektore za pljuvačku (SimplyOFy). Ovi uređaji su fabrički izrađeni i imaju kapacitet akumulacije 2 ml pljuvačke. Ispitanicima su date instrukcije da ne unose hranu, ne puše i ne upotrebljavaju proizvode oralne higijene najmanje 30 minuta pre korišćenja uređaja i da usta ispiraju vodom. Kako bi se smanjio uticaj dnevnih varijacija u proizvodnji pljuvačke, sakupljanje je vršeno u isto doba dana kod svih ispitanika, i to od devet do dvanaest sati. Količina pljuvačke očitavana je na graduisanoj traci koja se nalazi na samom uređaju, a vrednosti su izražene u ml/min; naime, sakupljanje je trajalo pet minuta, a dobijena vrednost delila se sa pet da bi se dobila vrednost po minutu (Slika 1).

Epruvete sadrže sredstvo za stabilizovanje pljuvačke, koje se nalazi na poklopcu i koje se aktivira u roku od jednog minuta nakon što se promućka. To omogućuje da pljuvačka ostane stabilna i do deset meseci nakon sakupljanja. Kako bi se obezbedila stabilnost uzoraka i omogućio biološki integritet uzoraka za dalja biohemijska ispitivanja, uzorci su zamrzavani na temperaturi od -80 stepeni. Za stimulaciju lučenja pljuvačke koristili smo elektro-stimulativne uređaje SaliPen, inovativne medicinske uređaje dizajnirane da stimulišu proizvodnju pljuvačke. Stimulacija ovim uređajima trajala je po pet minuta kod svakog pacijenta.

Biohemijska analiza sprovedena je u Institutu za eksperimentalnu i kliničku biohemiju u Skoplju; tu se laktoferin kao poseban parametar kvantitativno određivao ELISA metodom.

The selection of patients was done by preliminary defined inclusive and exclusive criteria and data for the radiation therapy type, which was conducted in the University Clinic of Radiation Therapy and Oncology in Skopje. The patients were treated with three-dimensional conformal radiation therapy and volumetric modulated radiation therapy. All of the participants in the study have voluntarily signed an informed consent form for their data to be used in this study. Patients' examinations and sample collections were performed in the Clinic of Maxillofacial Surgery in Skopje. The sample collections for salivary flow rate measurement were done by spitting out the saliva in special saliva collectors—SimplyOFy. These devices are factory produced with capacity of 2 mL for saliva collecting. The participants were instructed not to consume food or smoke cigarettes to restrain using products for oral hygiene at least 30 minutes before using the device, and to rinse the mouth with water only. To minimize the daily variations of saliva production, the collecting process was performed at the same time of the day in every participant, from 9 to 12 o'clock. The volume of saliva was measured using a graduated ruler on the device, and the values were expressed in ml/min, with the saliva being collected for a duration of 5 minutes. To get the value for one minute, the obtained value was divided by 5 (Figure 1).

The test tubes contain an ingredient that provides stabilization of the saliva, placed under the cap and activated by manually shaking the tube for one minute. This process enables the stability of the saliva for even 10 months after its collection. In order for the samples to remain stable and biologically integrated for further biochemical analyses, the samples were frozen at a temperature of -80 degrees Celsius.



Slika 1. SimplyOFy graduisane epruvete za sabiranje i evidentiranje količine pljuvačke

Figure 1. SimplOFy graduated test tubes for saliva collection and volume measurement

Za određivanje koncentracije laktoferina u pljuvački primenjen je komercijalni ELISA Kit – ab 200015-Human Lactoferrin Simple Step firme Abcam, i to prema uputstvima datim od proizvođača. Za analizu je korišćeno razblaživanje laktoferina 1 : 10.000. Svi uzorci su analizirani u duplikatu i upoređeni su sa osam standarda kita. Apsorbacije su dobijene na talasnoj dužini od 450 nm, a ploča kita je analizirana korišćenjem Awareness Technology, Inc. ChemWell 2910 Chemistry Analyzer.

Dobijene vrednosti iz definisanih parametara obrađene su i prikazane sa deskriptivnom statistikom distribucije i Pearsonovim koeficijentom korelacije pomoću računarskog softvera SPSS Statistics.

Rezultati

U nastavku se prikazuju dobijeni rezultati vrednovanja 24 ispitanika, koji su podeljeni u dve grupe prema primenjenoj tehnici i klasifikovani na osnovu ispitivanih parametara.

1. Rezultati srednje dobijene vrednosti stimulisanog protoka pljuvačke izražene u ml/min prema dozi zračenja, mesec dana nakon radioterapije

U prvoj grupi – 3D CRT: kod ispitanika koji su bili tretirani dozom radijacije do 60 Gy, prosečna količina produkovane pljuvačke iznosila je 0,1 ml/min; kod ispitanika tretiranih dozom do 66 Gy, prosečna dobijena vrednost bila je 0,08 ml/min; kod ispitanika koji su bili tretirani dozom do 70 Gy, količina sakupljene pljuvačke bila je prosečne vrednosti 0,05 ml/min. U drugoj grupi – VMAT: kod ispitanika koji su bili tretirani dozom radijacije do 60 Gy, prosečna količina produkovane pljuvačke bila je 0,3 ml/min; kod ispitanika tretiranih dozom do 66 Gy, prosek je iznosio 0,26 ml/min; kod ispitanika koji su bili tretirani dozom do 70 Gy, prosečna količina sakupljene pljuvačke bila je 0,1 ml/min. Rezultati su prikazani u Tabeli 1.

2. Rezultati prosečne linearne korelacije između ispitivanih varijabli laktoferina i količine protoka pljuvačke u ml/min

Na osnovu dobijenih rezultata prosečne linearne korelacije između ispitivanih varijabli laktoferina i količine protoka pljuvačke u ml/min kod ispitanika, može se zaključiti da je korelacija između ovih dveju varijabli statistički značajna, pri čemu je korelacija negativna, a koeficijent korelacije je sa vrednošću $r(24) = -0,903$; $p < 0,01$. Drugim rečima, što je protok pljuvačke u minutu manji, to je količina laktoferina u pljuvački veća. Rezultati su prikazani u Tabeli 2 i Grafikonu 1.

For stimulation of the salivary secretion, we used electro-stimulating devices SaliPen, innovative medical devices created for saliva production stimulation. The use of these devices for stimulation was 5 minutes of duration in each patient.

The biochemical analysis was done in the Institute for Experimental and Clinical Biochemistry in Skopje, where lactoferrin as a special parameter was quantitatively evaluated using the ELISA method. The commercial Human Lactoferrin ELISA Kit (ab 200015) Simple Step produced by Abcam Company was used to evaluate the concentration of lactoferrin in saliva samples according to the manuals provided by the manufacturer. To analyze lactoferrin, a 1:10,000 dilution was used. All of the samples were analyzed in duplicate and compared with eight standards from the kit. Adsorptions were obtained with wave length of 450 nm, and the kit plate was analyzed by using Awareness Technology, Inc. ChemWell 2910 Chemistry Analyzer.

The values obtained from the defined parameters were processed and presented with descriptive statistics for distribution and Pierson's coefficient of correlation using the computer software SPSS Statistics.

Results

The results obtained from the evaluation of 24 participants divided into two groups based on the used technique are displayed in the following paragraphs and are classified in the order of parameter evaluation.

1. Results of the average value of stimulated salivary flow rate expressed in ml/min according to the radiation dose, one month after radiotherapy

In the first group (3DCRT), in the participants treated with 60 Gy dose of radiation, the average volume of produced saliva was 0.1 ml/min; in the participants treated with a dose of 66 Gy, the average value was 0.08 ml/min; in the participants treated with 70 Gy dose of radiation, the average volume of collected saliva was 0.05 ml/min. In the second group (VMAT), in the participants treated with 60 Gy dose of radiation, the average volume of produced saliva was 0.3 ml/min; in the participants treated with a dose of 66 Gy, the average value was 0.26 ml/min; in the participants treated with 70 Gy dose of radiation, the average volume of collected saliva was 0.1 ml/min. The obtained results are displayed in Table 1 below.

Tabela 1. Prosečna dobijena vrednost protoka pljuvačke izražena u ml/min prema dozi zračenja kod ispitanika obeju grupa

Table 1. Average value of salivary flow rate expressed in ml per minute according to the dose of radiation in the two groups of participants

Doza radijacije Dose of radiation	up to 60 Gy (n = 8)	up to 66 Gy (n = 10)	up to 70 Gy (n = 6)
3D CRT	0.1 ml/min	0.08 ml/min	0.05 ml/min
VMAT	0.3 ml/min	0.26 ml/min	0.1 ml/min

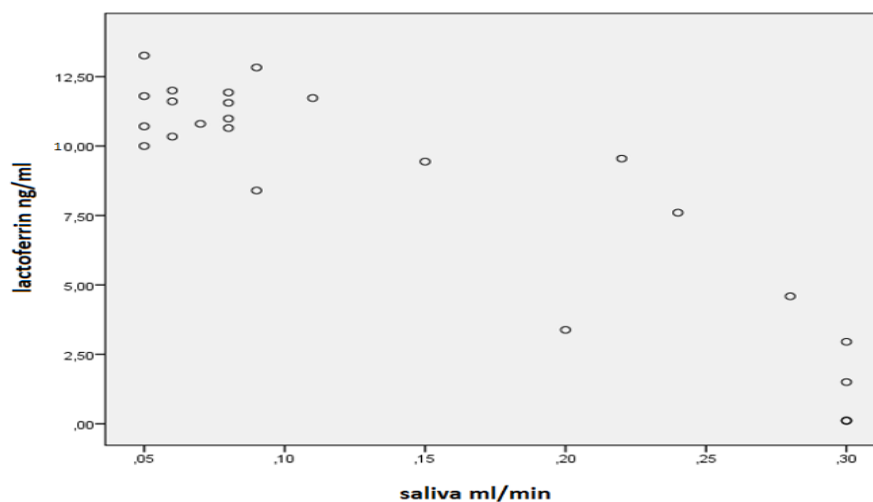
Tabela 2. Rezultati prosečne linearne korelacije između ispitivanih varijabli laktoferina i količine protoka pljuvačke u ml/min, sa Pearsonovim koeficijentom korelacije

Table 2. Results from the linear correlation between the evaluated variables of lactoferrin and volume of salivary flow in ml/min presented with Pierson's coefficient of correlation

Correlations

lactoferrin (ng/ml)	Pearson Correlation	1	-.903**
	Sig. (2-tailed)		.000
	N	24	24
saliva flow rate ml/min	Pearson Correlation	-.903**	1
	Sig. (2-tailed)	.000	
	N	24	24

** Correlation is significant at the 0.01 level (2-tailed).



Grafikon 1. Grafički prikaz rezultata linearne korelacije između ispitivanih varijabli laktoferina i količine protoka pljuvačke u ml/min

Figure 2. Results from the linear correlation between the evaluated variables of lactoferrin and volume of salivary flow in ml/min

Diskusija

Pored prosečne doze zračenja tokom radioterapije, u novijim istraživanjima postoje dokazi da regioni u pljuvačnim žlezdama koji sadrže matične ćelije imaju veći uticaj na pojavu hiposalivacije. Smatra se da su matične i progenitorne ćelije parotidne žlezde locirane u regionu koji sadrži glavne izlučne kanale. Zračenje ovih mesta rezultuje izmenjenom količinom proizvedene pljuvačke^{6,7}. Kserostomija nije uvek povezana sa smanjenjem protoka pljuvačke, već se može manifestovati i nezavisno. Kada kserotomiju uzrokuje terapija radijacijom, u najvećem broju slučajeva je ireverzibilna i trajna^{11,12}. Primena volumetrijski modulirane terapije zračenjem je, zahvaljujući naprednoj tehnologiji, preciznija od konvencionalne metode; ona se zasniva na upotrebi kompjuterski kontrolisanih linearnih akceleratora koji precizno primenjuju doze zračenja na tačno određeno mesto na kome je tkivo zahvaćeno tumorom. Uvođenjem nove metode – volumetrijski modulirane terapije zračenjem (VMAT) – kserostomija koja prati zračenje kod pacijenata svedena je na značajan nivo^{13,14}. IMRT tehnika i VMAT, moduliranjem intenziteta snopova zračenja u više manjih delova, omogućuju da doza radijacije bude prikazana preciznije u trodimenzionalnom obliku tretiranog tumora. Ovaj metod takođe pomaže da veće doze radijacije budu fokusirane striktno na tumor i da okolna zdrava tkiva budu što je moguće manje izložena štetnom dejstvu zračenja^{20,21}.

U istraživanju Christophera Hoynea i sar.¹⁴, u svrhu očuvanja funkcije submandibularne žlezde, bili su primenjeni metodi IMRT i VMAT. Istraživanje je obuhvatilo deset pacijenata. Glavna doza radijacije bila je značajno smanjena sa 34,5 Gy ± 4,8 Gy na 31,5 Gy ± 5,5 Gy upotrebom tehnike radijacijske terapije sa modularnim intenzitetom (IMRT). Na osnovu dobijenih rezultata nakon terapije zaključeno je da je moguće očuvati funkciju pljuvačne žlezde primenom novih IMRT i VMAT metoda ove terapije¹⁴.

U radu čiji su autori William M. i sar.¹⁵ utvrđeno je da je IMRT korišćen za smanjenje doze zračenja i za smanjenje posledica kserostomije. Naime, svaka doza radijacije koja je prevazilazila 39 Gy uzrokovala je trajnu ablaciju protoka pljuvačke i sa stimulacijom i bez stimulacije.

2. Results from linear correlation between the evaluated variables lactoferrin and volume of salivary flow in ml/min

From the obtained results of linear correlation between the evaluated variables of lactoferrin and volume of salivary flow in ml/min in participants, it could be concluded that the correlation between these 2 variables was negative and was statistically significant with the coefficient of correlation $r(24) = -0.903$; ($p < 0.01$). In other words, the smaller the salivary flow rate in a minute, the higher the amount of lactoferrin in the saliva. The results are displayed in Table 2 and Figure 1.

Discussion

In addition to the average dose of radiation received during radiotherapy, recent research has shown that the main cause of hypo-salivation is due to the areas in the salivary glands that contain stem cells. These stem cells and the progenitor cells are located in the region of the parotid salivary gland that contains the main salivary ducts. As a result, radiation to these regions can often lead to a change in the volume of produced saliva^{6,7}. Xerostomia is not always related to a decrease in the salivary flow, but it can also be manifested independently. When xerostomia is caused by radiation therapy, it is frequently irreversible and permanent in most cases^{11,12}.

The application of volumetric modulated radiation therapy due to improved technology is significantly more precise than the conventional method because it is based on the use of computer-controlled linear accelerators that apply radiation doses precisely to a specified place of the tumor-affected tissue^{16,17}. By introducing a new volumetric-modulated radiation therapy (VMAT), xerostomia following the radiation treatment is significantly reduced in affected patients^{13,14}.

The IMRT technique and VMAT enable a more precise application of the radiation dose to the tumor being treated. By modulating the intensity of the beams of radiation in multiple smaller parts, the radiation can be delivered in a three-dimensional form, resulting in higher doses of radiation being focused on the tumor itself while minimizing the exposure of surrounding tissues to the harmful effects of radiation^{20,21}.

In a study by Christopher Hoyne et al.¹⁴ the use of IMRT and VMAT was recommended to spare the function of the submandibular salivary gland.

Na osnovu rezultata je takođe zaključeno da primena IMRT-a zaobilaženjem submandibularnih žlezda dugoročno rezultuje smanjenom kserostomijom, bez povećanja rizika od lokalno-regionalnih recidiva¹⁵.

Nutting C. M. i sar.¹⁸ dokazali su da je 40% ispitanika imalo posledice kserostomije nakon tretmana VMAT-a. Nakon dvanaestomesečnog praćenja tretiranih pacijenata, kserostomija je registrovana kod 73 od ukupno 82 ispitanika. Pokazalo se da je uznapredovali stepen kserostomije značajno ređi u grupi ispitanika lečenih terapijom zračenjem modulisanog intenziteta (38%) nego u grupi pacijenata lečenih konvencionalnom terapijom zračenjem (74%)¹⁹.

Istraživanjem Zhong-He Wang i Chao Yana bila su obuhvaćena ukupno 52 pacijenta koja su primila VMAT, uz očuvanje barem jedne od parotidnih žlezda. Od toga, kod 26 pacijenata sa niskim rizikom recidiva očuvane su obe submandibularne žlezde; kod preostalih 26 pacijenata sa visokim rizikom recidiva submandibularne žlezde nisu očuvane. Kserostomija i protok pljuvačke bili su praćeni u pet intervala (pre VMAT-a i posle dva meseca, šest meseci, 12 meseci i 18 meseci). U prvoj grupi pacijenata bilo je upotrebljeno zračenje intenziteta 30 Gy, dok je u drugoj grupi intenzitet zračenja bio 57,4 Gy. Rezultati su pokazali da je kserostomija bila na značajno nižem nivou nakon dva meseca i nakon šest meseci od VMAT-a u grupi sa očuvanim submandibularnim žlezdama; međutim, nije bilo značajne razlike u rezultatima za kserostomiju ni u jednoj grupi ispitanika nakon 12 i 18 meseci terapije^{21,22}. Nakon lečenja zračenjem u trajanju od dve godine, kserostomija je registrovana kod samo 29% pacijenata koji su lečeni VMAT-om; kod pacijenata koji su lečeni konvencionalnom terapijom pak procenat pojave kserostomije kao posledice bio je značajno veći – 83%⁵. Stoga, prema Chrisu Nuttingu, modifikovana VMAT terapija zračenjem treba da se koristi kao zlatni standard kod pacijenata sa visokim rizikom od kserostomije izazvane zračenjem¹⁸. U našem istraživanju, kod pacijenata tretiranih volumetrijskom modulisanom terapijom sa dozom radijacije do 60 Gy, prosečna količina proizvedene pljuvačke iznosila je 0,3 ml/min, odnosno 0,26 ml/min kod pacijenata tretiranih dozom radijacije do 66 Gy.

The study evaluated 10 patients. The main dose of radiation was significantly reduced from 34.5 ± 4.8 Gy to 31.5 ± 5.5 Gy using the IMRT technique. According to the obtained results, it can be concluded that it is possible to spare the function of the salivary gland by using the new IMRT and VMAT methods in this therapy¹⁴.

In the study by William M. et al.¹⁵ and the use of IMRT technique was recommended to reduce the consequences such as xerostomia after the radiation. The obtained results showed that any radiation dose above 39 Gy caused permanent ablation of the salivary flow, with or without stimulation. The study also concluded that using IMRT to bypass the submandibular glands resulted in reduced xerostomia in the long term without increasing the risk of local-regional recurrences¹⁵.

Nutting C. M., et al.¹⁸ found that 40% of patients treated with VMAT experienced xerostomia. After a 12-month follow-up of treated patients, xerostomia was registered in 73 of the total number of 82 participants. The study also found that advanced-stage xerostomia was significantly less common in the group of participants treated with intensity-modulated radiation therapy (38%), compared to the group of participants treated with conventional radiation therapy (74%)¹⁹.

Zhong-He Wang and Chao Yan analyzed 52 patients treated with VMAT and with at least one of the parotid glands spared. Twenty-six patients with a small risk for recurrence were treated with sparing both of the submandibular glands, on the other hand, in the 26 patients with a high risk for recurrence, the submandibular glands were not spared. Xerostomia and salivary flow rate were measured before VMAT and at 2, 6, 12 and 18 months after treatment. The radiation with the intensity of 30 Gy was used in the first group of patients and 57.4 Gy in the second group. The obtained results showed significantly lower levels of xerostomia at 2 and 6 months after VMAT therapy in the group of participants with spared submandibular glands, however, no significant difference was found in the results for xerostomia in the two groups of participants at 12 and 18 months after the therapy^{21,22}.

After 2 years of radiation treatment, xerostomia was registered only in 29% of the patients treated with VMAT, compared to 83% of the patients treated with conventional

U istraživanju koje je sproveo Keskin analiziran je nivo laktoferina pre, za vreme i posle radioterapije – ispostavilo se da su vrednosti laktoferina u pljuvački značajno povećane za vreme tretmana, a da nakon radioterapije značajno opadaju. Međutim, istaknuto je i da u literaturi nisu dostupna druga istraživanja na ovu temu, potrebna da se napravi poređenje²⁴. Ipak, pominje se radioprotektivno dejstvo laktoferina, koji, osim što je antiinflamatorni protein, može biti koristan u prevenciji postradijacionih efekata na pljuvačne žlezde. Eliasson i sar. ukazali su na povećane vrednosti laktoferina kod svih ispitivanih uzoraka pljuvačke²⁵. Takođe, u istraživanju Marangoni-Lopesa i sar. predstavljeni su rezultati koji ukazuju na nizak protok pljuvačke i na povećane vrednosti proteina i laktoferina kod svih ispitanika posle radioterapije²⁶. Postoje i drugi rezultati slični onima dobijenim u ovom istraživanju, tj. oni koji potvrđuju statistički značajnu korelaciju između smanjenih vrednosti protoka pljuvačke i povećane koncentracije laktoferina.

Zaključak

Na osnovu navedenih rezultata može se zaključiti da tretman VMAT-om pruža mogućnost očuvanja funkcije pljuvačke, što dokazuje poboljšan stepen pljuvačke, a smanjen stepen kserostomije. Ujedno, može se zaključiti o povećanim vrednostima laktoferina kao antiinflamatornog proteina i dobrog indikatora informisanosti nakon radioterapije, a može biti i korisna komponenta za zaštitu pljuvačnih žlezda od negativnih efekata terapije zračenjem. Ipak, ova tema ostaje otvoreno polje za dalja, opsežnija istraživanja.

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radiation therapy where xerostomia occurred as a complication following therapy with a significantly higher rate⁵. Due to the finding of Chris Nutting, modified radiation therapy VMAT is now considered and used as the gold standard in patients with a high risk of radiation-induced xerostomia¹⁸. In our study, in the patients treated with volumetric modulated radiotherapy in a dose of up to 60 Gy of radiation, the average quantity of produced saliva was 0.3 ml/min, while in the patients treated with a dose of up to 66 Gy of radiation, the average quantity of saliva was 0.26 ml/min.

In the study conducted by Keskin, lactoferrin levels in saliva samples were evaluated before, during and after the radiation therapy. The results showed that the levels of lactoferrin significantly increased during the treatment, and then significantly decreased after the treatment. However, they noted that there were no studies on this subject available for comparison²⁴. The anti-inflammatory effect of lactoferrin can also be used to prevent post-radiation effects on the salivary glands. Eliasson et al. found increased lactoferrin levels in all evaluated saliva samples²⁵. Further, Marangoni-Lopes et al. found decreased salivary flow, and increased levels of proteins and lactoferrin in all study participants after the radiotherapy²⁶. These findings are similar to the results of our study, which also found a statistically significant correlation between decreased levels of salivary flow and increased concentration of lactoferrin.

Conclusion

Based on the obtained results, it can be concluded that VMAT treatment offers a possibility to spare the salivary function, which is proved by the improved level of salivary flow, and the decreased level of xerostomia. Further, it can be concluded that the higher values of lactoferrin are considered to have anti-inflammatory characteristics and are indicators of inflammation, which can be used in the prevention of the salivary glands from negative effects of the therapy with radiation. All in all, the subject of this study is still open for further and wider investigations.

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