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Vascular endothelial growth factor as a response of denture bearing tissues on mechanical stress in diabetes mellitus

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SUMMARY

Introduction Vascular endothelial growth factor (VEGF) is signal molecule enrolled in diabetes mellitus type 2 (DM type 2) oral complications, but there are no studies showing the relation between VEGF and pressure caused by denture wearing in diabetic conditions.

The aim of this study is to compare tissue VEGF levels in patients and animals with/without DM in conditions of chronic and acute pressure.

Methods Research was conducted on DM type 2 and healthy partial denture wearers for more than 5 years (78), candidates for teeth extractions and experimental animals of the Wistar rats (40). For chronic conditions, VEGF was measured in 2 mucosal samples covered and not covered by denture in all denture wearers. Demonstrating acute conditions, after 3 days of wearing experimental plate VEGF was measured in 2 gingival samples of palatal mucosa of DM (20) and control rats (20). The concentrations of VEGF (pg/ml) in human and animal tissues were measured by commercially available ELISA kit.

Results Tissue VEGF levels in control and diabetic partial denture wearers not covered by denture were without statistical difference. In comparison to noncovered tissue, VEGF decreased in samples covered by denture, being significantly lower in DM type 2, comparing to healthiest. VEGF levels in palatal mucosa without palatal base did not significantly differ in control and DM rats. VEGF levels under palatal base increased being significantly lower in DM rats comparing to controls.

Conclusion Both, chronic and acute mechanical stress caused by wearing palatal denture (plate) decreased the VEGF levels in diabetic conditions comparing the health's suggesting the altered homeostasis.

Keywords: compression; diabetes mellitus type 2; denture; mucosa; VEGF

INTRODUCTION

Diabetes mellitus type 2 (DM type 2) is associated with increased incidence of several oral conditions: periodontal disease, impaired healing of oral wounds and mucosa ulcerations-especially under the base of complete denture [1, 2]. Prosthetic rehabilitation of reduced masticatory function in DM patients is of great relevance having in mind the importance of good and well-balanced diet as a part of diabetes therapy. However, the presence of denture and its compression might compromise oral homeostasis [3]. Namely, subjected to denture wearing, oral mucosa has to endure mechanical loads of various levels and durations. In addition to the persistent load required for static support and retention, oral mucosa must be resistant to various levels and durations of acute and chronic load during functional and para functional behaviors [4]. Studies have shown morphological and histological changes in oral mucosa induced by chronic stress caused by wearing dentures in human subjects and rodents [5, 6, 7]. Examinations of mechanical compression on mucosa caused by denture base showed ischemia *in vivo* and disruption of multiple functions of endothelial basal cells [3, 8, 9]. Besides mentioned, mechanical forces and compression are also known to induce the secretion of soluble mediators, including cytokines and growth factors [10, 11, 12]. One of them, vascular endothelial growth factor (VEGF) is an angiogenetic signaling molecule that elicits cellular responses to hypoxia [13]. Tsuruoka et al. reported increased VEGF levels under experimental palatal plate in the connective tissues and osteoblasts in the periosteum of maxilla in healthy rats [12].

Studies have shown altered levels of VEGF in saliva, gingival tissues and crevicular fluids of diabetic subjects [14, 15], pointing this factor as one of the crucial mediators of oral complications in DM type 2. However, there is luck of data about changes in VEGF level as cellular

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response on mechanical loads cased by denture wearing in underlying tissues of diabetics.

The aim of this study was 1) to determine tissue VEGF levels produced during chronic pressure caused by wearing partial acrylic denture in patients with and without DM type 2, and 2) to determine tissue VEGF levels produced during acute pressure caused by experimental palatal plate in experimental animals with and without DM.

MATERIAL AND METHODS

Study population

Seventy-eight participants aged 45 to 64 years were included into the study. The study consisted of participants with diagnosed DM type 2 (42) recruited from Clinical department for endocrinology, diabetes and metabolic diseases, University Medical Center, Belgrade and participants without diabetes (36) from the Department of Prosthodontics, Faculty of Dental Medicine, University of Belgrade. Participants enrolled in the study were wearing maxillary partial removable acrylic dentures and mandibular complete dentures at least for 5 years and had teeth present with signs of severe periodontitis in the anterior and premolar maxillary regions [16, 17, 18]. The criteria for diabetic participants included the following: history of disease for approximately 5 years with a glycated hemoglobin A1c measurement less than 9 (HbA1c < 9) [16, 17]. Smokers, patients with systemic diseases or who were taking additional medication were excluded from the study. All study participants gave consent. The study was approved by University of Belgrade, Faculty of Dentistry Committee on the Ethics of Human Research (No. 36-32, April 12, 2010).

Sample collection

Each subject underwent periodontal examination and due to irreversible mobility and poor prognoses of the remaining teeth the treatment plan required extraction with fabrication of maxillary complete dentures and new mandibular complete dentures. During the extraction procedures approximately 1mm of tissue samples were obtained adjacent to the extracted teeth. For each patient, 2 samples were taken: the sample that was previously covered by partial denture (compressive sample) and the sample that was not covered by partial denture (non-compressive sample). The specimens were frozen in liquid-nitrogen and stored at -70°C until analysis.

Experimentally induced DM

Forty male Wistar rats weighing approximately 250 g each were used in this study. The rats were divided equally into 2 groups of 20 in each. The control group consisted of normal healthy rats. In another group, diabetes was induced using a single intraperitoneal injection of the pancreatic β-cell toxin monohydrate (alloxan, 140mg/kg; Sigma, St. Louis, MO, USA). Serum glucose level were measured (Accu-Chek; Roche Diagnostic, Indianapolis, IN, USA) and rats were considered to be diabetic when blood glucose levels exceeded 10 mmol/L. Experiments were started when animals were hyperglycemic for minimum 3 weeks. For each animal, 2 tissue samples were taken: the sample that was previously covered by experimental palatal plate (compressive sample) and the sample that was not covered by experimental palatal plate (non-compressive sample). The specimens were frozen in liquid-nitrogen and stored at -70°C until analysis.

All animal procedures were approved by the Ethical Committee of the Faculty of Dentistry, University of Belgrade (No. 36-32, April 12, 2010).

Preparation of experimental palatal plates and sample collection

For the animals in the group with experimental palatal plates (control group/DM group), following the procedure of Tsurioka et al [12], precision impression of the upper jaw was taken using vinyl-silicon impression material (Zhermack). A piece of wax approximately 0.6mm thickness was set on the occlusal surfaces of the molar teeth on the plaster cast. Methil-metacrylate plate (Biokril, Galenika, Serbia) extending from the first to the third molar teeth on both sides of palate was made and cured. After curing, the attached wax was removed. The palatal plate was set on the palatine mucosal surface of each animal with pressure and was fixed to the molar teeth on both sides using the same resin (Figures 1 and 2). In order to obtain animal tissue specimens, all rats were sacrificed 3 days after palatal plate wearing using an overdose of thiopental sodium. Maxillas were removed with palatal plates and tissue with/without palatal plate was prepared for VEGF quantification.

Tissue measurement of VEGF

The concentrations of VEGF in human and animal tissues (pg/mL) were measured by commercially available Enzyme-Linked Immunosorbent Assay (Human VEGFA ELISA Cell Culture Supernatant, Urine; RayBiotech and Rat VEGFA ELISA Kit for Cell Culture Supernatant, Urine; RayBiotech) according to the manufacturer's instructions [19]. Optical densities were measured at 450 nm with a microplate reader and the minimum detectable level of the test was 5.0 pg/mL.

Statistics

Results were presented as the mean ± standard deviation (SD). Comparisons between groups were done by using two-way ANOVA with repeated measures and Chi-squared test. One-way ANOVA was used to analyze group differences, and Bonferroni correction was performed for post-hoc comparisons. Independent samples Student's *t* test was performed to determine comparisons between appropriate group-time points. The statistical significance level was considered to be 5%. All data were analyzed using Stat for Windows 8, StatSoft, Inc, USA, 1984–2007.

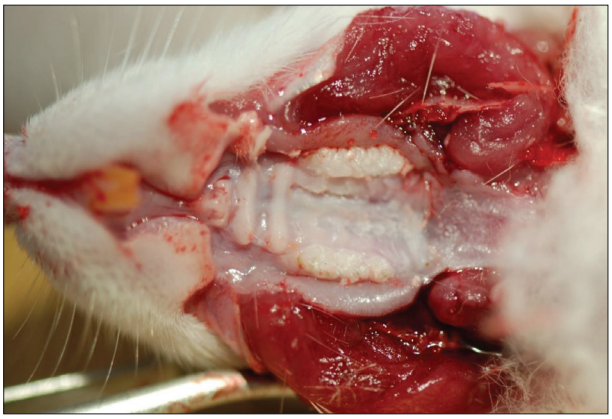

Figure 1. Palatal mucosa of rat before covering with experimental palatal plate
Slika 1. Palatinalna sluzokoža pacova pre pokrivanja eksperimentalnom palatinalnom pločom

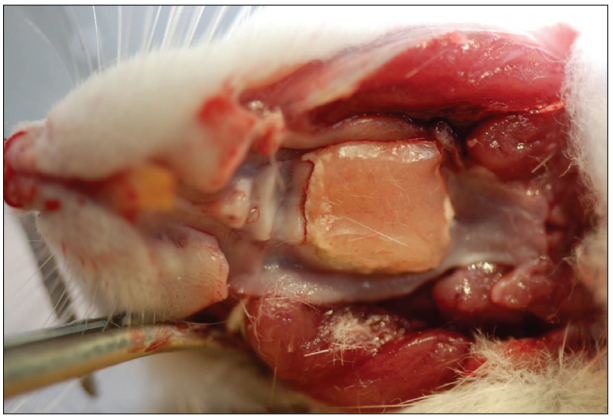

Figure 2. Experimental palatal plate on rat's maxilla
Slika 2. Eksperimentalna palatinalna ploča na maksili pacova

RESULTS

Age and gender were not considered in this study, because observed groups of participants consisted predominantly of male subjects (69%), aged between 45 and 65 years.

Table 1 shows tissue VEGF levels in non-compressive and compressive samples in healthy and DM type 2 partial denture wearers. VEGF levels in compressive samples were significantly lower in comparison to VEGF levels in non-compressive samples in both observed groups (DM type 2 and healthy). The VEGF decrease was 66% for diabetic compressive samples and 28% for compressive samples of healthy participants in comparison to non-compressive samples. DM type 2 denture wearers had significantly lower VEGF levels in compressive tissue samples comparing to healthy subjects, contrary to non-compressive samples where results showed no significant difference between healthy and DM type 2 partial denture wearers.

Table 1. Tissue VEGF in non-compressive and compressive samples in healthy and DM type 2 partial denture wearers
Tabela 1. Koncentracije VEGF u gingivi u uslovima akompresije i kompresije parcijalnom protezom kod zdravih ispitanika i ispitanika sa DM tipa 2

Participants Ispitanici	Tissue VEGF (pg/ml) Koncentracije VEGF u tkivu (pg/ml) X ± SD	
	Non-compressive samples Akompresivni deo gingive	Compressive samples Kompresivni deo gingive
Healthy (42) Zdravi (42)	37.64 ± 3.0	27.18 ± 1.0*
DM type 2 (36) DM tip 2 (36)	36.44 ± 4.9	12.58 ± 1.2***$

*p < 0.05, ***p < 0.001 – compressive vs non-compressive, $p < 0.05 – Healthy vs DM type 2 (ANOVA, Bonferroni post hoc test)
*p < 0,05, ***p < 0,001 – poređenje kompresije i akompresije, $p < 0,05 – poređenje zdravih i sa DM tipa 2 (ANOVA, Bonferonijev post hok test)

Characteristics of control rats and rats with DM referring blood glucose levels and body weight before experimental procedures, 21 days after DM induction and on the last day of experimental procedures -24th day (after 3

Table 2. Characteristics of control rats and rats with DM
Tabela 2. Karakteristike kontrolne grupe pacova i pacova sa eksperimentalno izazvanim DM

Parameters Parametri	Observation period Period posmatranja	Control rats (20) Kontrolna grupa (20)	DM rats (20) Indukovani DM (20)
	Before experimental procedures Pre eksperimentalnih postupaka	6.5 ± 1.8	6.4 ± 1.5
Blood glucose level (mmol/l) Nivo glukoze u krvi (mmol/l)			
	21st day 21. dan eksperimenta 24th day 24. dan eksperimenta	6.3 ± 1.3 6.4 ± 2.8	15.4 ± 3.2* 14.3 ± 0.2*
	Before experimental procedures Pre eksperimentalnih postupaka	245.0 ± 4.96	243.2 ± 5.0
Weight (g) Telesna masa (g)			
	21st day 21. dan eksperimenta 24th day 24. dan eksperimenta	300.0 ± 8.2 280 ± 5.6	200.0 ± 6.1* 180.0 ± 7.3*

*p < 0.01 – control vs DM (ANOVA, Bonferroni post hoc test)
*p < 0,01 – poređenje kontrolna grupe i DM (ANOVA, Bonferonijev post hok test)

Table 3. Tissue VEGF in non-compressive and compressive samples in control and DM rats
Tabela 3. Koncentracija VEGF tkiva u nekompresivnim i kompresivnim uzorcima kod kontrolnih i pacova sa eksperimentalno izazvanim DM

	Tissue VEGF (pg/ml) Koncentracije VEGF (pg/ml) X ± SD	
Animals Životinje	Non-compressive sample Nekompresivni uzorak	Compressive samples Kompresivni uzorak
Control (20) Kontrolne (20)	2.6 ± 0.1	9.56 ± 0.8*
DM (20) DM (20)	2.8 ± 0.2	6.34 ± 0.37*$

*p < 0.01 – compressive vs non-compressive, $p < 0.01 – control vs DM (ANOVA, Bonferroni post hoc test)
*p < 0,01 – poređenje kompresivnog uzorka i nekompresivnog uzorka;
$p < 0,01 – poređenje kontrolne grupe i DM (ANOVA, Bonferonijev post hok test)

days of wearing experimental palatal plate) are presented in Table 2. Results showed no significant difference in blood glucose levels and body weight before experimental procedures in observed groups. On 21st and 24th day, blood glucose level was increased and significantly higher in DM rats in comparison to healthy controls. At the 21st day, when experimental palatal plate was inserted, results showed decrease in body weight in DM animals contrary to controls and that resulted in significant difference in body weight in observed groups. On the last day of experimental procedures- after 3 days of wearing experimental palatal plate, both group of animals showed decrease of body weight, but DM rats had significantly lower body weight comparing to healthy controls.

Table 3 shows tissue VEGF levels in non-compressive and compressive samples in control and DM rats. Tissue VEGF levels are significantly increased in samples under experimental palatal plate (compressive samples), comparing to VEGF levels in tissue that is not covered by experimental palatal plate (non-compressive samples) in both observed groups. DM and control rats did not show statistical difference in tissue VEGF levels in non-compressive samples, contrary to compressive samples where VEGF levels are significantly lower in DM animals comparing to healthy controls.

DISCUSSION

Acute and chronic load during functional and para functional behaviors caused by dentures have the potential to cause deformation of underlying connective tissues [20, 21, 22]. Investigating the influence of the continuous compression of the removable partial denture on the blood flow in underlying mucosa of denture wearers, Akazawa and Sakurai [8] showed tissue ischaemia and delays in recovery of blood flow after release of compression. Tsouruka et al. reported that cells in the tissue, under experimental palatal plate, synthesize HSP70 and VEGF to maintain homeostasis [12]. However, the authors of this study identified lack of data concerning the influence of acute and chronic mechanical stress caused by denture wearing on VEGF tissue levels in diabetic conditions. To elucidate the effects of DM on acute changes of underlying oral mucosa, this study intended to stimulate the effects of denture wearing in an animal experimental model. Human model was used to show the influence of chronically mechanical stress on underlaying tissue at the cellular level in denture wearers with/without DM type 2. Following the study of Tsuruoka et al. [12], we did not consider the effects of used materials for acrylic experimental plate and partial denture on the underlying tissues. Namely, in accordance with Mori et al. [21] histopathological findings concerning heat-cured resin, Inoue et al. [23] showed minimal cytotoxic effect of 4-META/MMA-TBB, despite of monomer penetration in the tissue during polymerization.

Results of our study revealed increased tissue VEGF levels after 3 days wearing experimental palatal plate, in both observed group of animals: with and without DM. Our finding is in accordance with study of Tsuruoka et al., which found that VEGF staining was positive for vascular endothelial cells in connective tissues and for osteoblasts in periosteum of maxilla of healthy rats after 3 days wearing experimental palatal plate [12]. Having in mind studies that reported reduction of blood flow under experimental palatal plates resulting in ischaemia [8, 9], increased VEGF levels in rats tissue after 3 days wearing experimental palatal plate might be caused by hypoxic condition, a major stimuli for VEGF. Contrary to our results showing no statistically significance in tissue VEGF levels in non-compressive samples between DM and healthy animals (samples without experimental plate), there was significantly lower tissue VEGF increase under experimental palatal plate in rats with DM comparing the controls, suggesting that VEGF expression is altered under mechanical stress in diabetic conditions.

Analyzing model of chronic stress, our results showed significantly lower VEGF levels in tissue covered by partial denture (compressive samples) in comparison to VEGF levels in tissue that wasn't covered by partial denture (non-compressive samples). Mentioned decrease of tissue VEGF might suggest on oral mucosa adaptation and cellular response on mechanical stress in order to maintain oral homeostasis. Obtained result of significantly higher tissue VEGF decrease in compressive samples of DM type 2 partially denture wearers comparing to healthy subjects suggest on altered oral homeostasis in diabetic conditions. However, our results showed no statistical significance in tissue VEGF levels in non-compressive samples between DM type 2 and healthy denture wearers. Published data about tissue VEGF levels in diabetic periodontitis patients are conflicting. Contrary to Sakaliogllu et al. [23] who reported significantly higher VEGF levels in gingival supernatants of diabetic periodontitis patients comparing to controls, Keles et al. [24] found no significant difference in expression levels of VEGF mRNA in gingival tissues of periodontitis patients with and without DM type 2. However, many investigations showed tissue changes and alterations under denture base in diabetic patients and animals with experimentally induced DM [25–28] comparing to healthy controls, but our results for the first time introduced changes in tissue VEGF levels as cellular response on denture wearing in diabetic conditions.

CONCLUSION

Acute mechanical stress increases concentration of VEGF in palatal mucosa, while, chronically mechanical stress, conversely, decreases tissue VEGF levels. Lower VEGF levels were related in acute and chronic compression in diabetic conditions, suggesting altered homeostasis. Our research emphasizes the importance of prosthodontists education on denture maintenance in patients with DM type 2, as it contributes to a better understanding of the

cellular response and biological processes in the oral mucosa under mechanical stress.

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DISCLOSURE STATEMENT

The authors report no conflict of interest.

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Vaskularni endotelni faktor rasta kao odgovor mobilnim zubnim protezama kod pacijenata sa dijabetesom melitusom

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KRATAK SADRŽAJ

Uvod/Cilj Brojne studije ukazuju na vaskularni endotelni faktor rasta (VEGF) kao jedan od medijatora oralnih komplikacija dijabetesa melitusa tipa 2 (DM tip 2), ali nijedna nije pokazala efekte nošenja proteze na vrednosti VEGF kod dijabetičkih stanja.
Cilj ovog rada bio je da se odredi koncetracija VEGF u gingivi na mestima hroničnog i akutnog pritiska proteze kod pacijenata i eksperimentalnih životinja sa dijabetesom melitusom i bez njega.
Metode Istraživanje je sprovedeno na pacijentima i eksperimentalnim životinjama soja Vistar. U istraživanje su uključeni zdravi (42) i pacijenti sa DM tipa 2 (36), nosioci parcijalnih proteza duže od pet godina, sa indikacijom za vađenje zuba. Kod svakog pacijenta za merenje VEGF (model hroničnog pritiska) uzeta su po dva uzorka tkiva, kompresivni i akompresivni uzorak, tokom ekstrakcije zuba. Za merenje VEGF u palatinalnoj sluzokoži eksperimentalnih pacova posle tri dana nošenja eksperimentalne ploče (model akutnog pritiska) uzeta su dva uzorka gingive (pokrivene/nepokrivene eksperimentalnom pločom) kod kontrolne grupe (20) i pacova sa eksperimentalno indukovanim DM (20). Za kvantitativno merenje VEGF (pg/ml) korišćen je komercijalni ELISA kit.
Rezultati Koncentracije VEGF u sluzokoži koja nije pokrivena protezom bile su iste u kontrolnoj grupi i grupi pacijenata sa DM tipa 2. U kompresivnim uzorcima VEGF je smanjen u obe grupe, ali značajno niži kod dijabetičara u poređenju sa zdravim pacijentima. Koncentracije VEGF u sluzokoži bez palatinalne ploče bile su slične kod kontrolnih i pacova sa DM. U prisustvu palatinalne ploče koncentracije VEGF su bile povećane, međutim, značajno niže kod pacova sa DM u poređenju sa kontrolnom grupom.
Zaključak Hronični mehanički stres smanjuje VEGF za razliku od akutnog mehaničkog stresa koji povećava VEGF u palatinalnoj sluzokoži. Akutna i hronična kompresija povezane su sa nižim nivoima VEGF kod pacijenata sa DM, što ukazuje na izmenjenu homeostazu.
Ključne reči: kompresija; dijabetes melitus tip 2; proteza; sluzokoža; VEGF

UVOD

Dijabetes melitus tip 2 (DM tip 2) povezan je sa povećanom incidencom oralnih stanja kao što su: parodontopatija, usporeno zarastanje oralnih rana i ulceracija sluzokože – posebno ispod baze totalne proteze [1, 2]. Protetska rehabilitacija smanjene mastikatorne funkcije kod pacijenata sa DM je od velike važnosti, imajući u vidu značaj dobre i uravnotežene ishrane, kao deo terapije dijabetesa. Međutim, prisustvo proteze i njena kompresija mogu kompromitovati oralnu homeostazu [3]. Naime, nošenjem proteza, potporna tkiva moraju da izdrže mehanička opterećenja različitog nivoa i trajanja. Pored konstantnog opterećenja potrebnog za stabilizaciju i retenciju proteze, oralna sluzokoža mora biti otporna na različite nivoe i trajanje akutnog i hroničnog opterećenja tokom funkcionalnih i parafunkcionalnih navika [4]. Studije su pokazale morfološke i histološke promene u oralnoj sluzokoži izazvane hroničnim stresom zbog nošenja proteza kod ljudi i glodara [5, 6, 7]. Ispitivanja mehaničke kompresije na sluzokoži izazvane bazom proteze pokazala su ishemiju in vivo i višestruki poremećaj funkcija bazalnih ćelija endotela [3, 8, 9]. Pored navedenog, poznato je da mehaničke sile i kompresija indukuju lučenje rastvorljivih medijatora, uključujući citokine i faktore rasta [10, 11, 12]. Jedan od njih, vaskularni endotelni faktor rasta (VEGF), angiogenetski je signalni molekul koji izaziva ćelijske odgovore na hipoksiju [13]. Tsuruoka M. i saradnici [12] pokazali su povećanu ekspresiju VEGF ispod eksperimentalne ploče u palatinalnoj sluznici i osteoblastima u periostiumu maksile kod zdravih pacova.

Studije su pokazale izmenjene nivoe VEGF u pljuvački, gingivalnim tkivima i tečnosti gingivalnog sulkusa kod dijabetičara [14, 15], ukazujući na ovaj faktor kao jedan od ključnih medijatora oralnih komplikacija kod DM tipa 2. Međutim, malo je podataka o promenama u nivou VEGF kao ćelijskog odgovora na mehanička opterećenja izazvanog nošenjem proteze kod dijabetičara.

Na osnovu prikazanih podataka, cilj ove studije bio je 1) da se odredi nivo VEGF u tkivu na mestu hroničnog pritiska izazvanog nošenjem mobilne zubne proteze kod ispitanika sa DM tipa 2 i bez njega, i 2) da se odredi koncentracija VEGF u gingivi u uslovima akutnog pritiska ispod eksperimentalne palatinalne ploče u grupi životinja sa eksperimentalno izazvanim DM i kontrolnoj grupi životinja.

MATERIJAL I METODE

Ispitanici

Klinička studija je obuhvatala 78 ispitanika, oba pola, starosne dobi od 45 do 64 godine. U studiji su učestvovali ispitanici sa dijagnostikovanim DM tipa 2 (42) koji su lečeni na Klinici za endokrinologiju KBC „Zvezdara“ u Beogradu i ispitanici kod kojih nije dijagnostikovan DM (36) sa Klinike za stomatološku protetiku u Beogradu. Ispitanici su bili nosioci gornjih parcijalnih pločastih proteza (PPP) i donjih totalnih proteza (TP) u vremenskom periodu od pet godina, sa terminalnim stadijumom parodontopatije u prednjoj i premolarnoj maksilarnoj

regiji [16, 17, 18]. Kriterijum za uključivanje ispitanika sa DM tipa 2 u istraživanje bila je istorija bolesti pacijenata sa trajanjem oboljenja od najmanje pet godina i kontrolisana glikemija određena kroz vrednosti glikoziliranog hemoglobina (HbA1C < 9) [16, 17]. Ispitanici koji su bili pušači ili su imali prisustvo drugih sistemskih bolesti zbog kojih su uzimali određene medikamente su isključeni iz studije. Uključeni su svi ispitanici koji su dali saglasnost za učešće u ovoj studiji. Studiju je odobrio Etički odbor Stomatološkog fakulteta, Univerziteta u Beogradu (br. 36-32, 12. april 2010).

Uzimanje uzoraka

Svaki ispitanik je podvrgnut parodontološkom pregledu i zbog ireverzibilne mobilnosti i loše prognoze preostalih zuba plan lečenja zahtevao je ekstrakciju zuba sa izradom totalnih proteza u gornjoj i donjoj vilici. Tokom postupka ekstrakcije uzeti su uzorci tkiva veličine približno 1 mm pored izvađenih zuba. Za svakog pacijenta uzeta su dva uzorka: uzorak koji je prethodno bio pokriven parcijalnom protezom (kompresivni uzorak) i uzorak koji nije bio pokriven parcijalnom protezom (akompresivni uzorak). Uzorci su zamrznuti u tečnom azotu i čuvani na temperaturi -70° C do analize.

Eksperimentalno indukovan DM

Ispitivanje je izvedeno na 40 mužjaka pacova soja Vistar, telesne mase oko 250 g. Pacovi su podeljeni u dve grupe od po 20 u svakoj. Kontrolnu grupu su činili zdravi pacovi. U drugoj grupi dijabetes je indukovan upotrebom jedne intraperitonealne injekcije monohidrata toksina β-ćelija pankreasa (alloxan, 140 mg/kg; Sigma, St. Louis, MO, SAD). Izmeren je nivo glukoze u serumu (Accu-Chek; Roche Diagnostic, Indianapolis, IN, SAD) i DM je dijagnostikovan kada je nivo glukoze u krvi prelazio 10 mmol/L. Eksperimenti su započeti kada su životinje bile hiperglikemične najmanje tri nedelje. Za svaku životinju uzeta su dva uzorka tkiva: uzorak koji je prethodno bio pokriven eksperimentalnom palatinalnom pločom (kompresivni uzorak) i uzorak koji nije bio pokriven eksperimentalnom palatinalnom pločom (nekompresivni uzorak). Uzorci su zamrznuti u tečnom azotu i čuvani na temperaturi -70° C do analize.

Sva ispitivanja na životinjama odobrio je Etički odbor Stomatološkog fakulteta, Univerziteta u Beogradu (br. 36-32, 12. april 2010).

Priprema eksperimentalnih palatinalnih ploča i sakupljanje uzoraka

Za životinje u grupi sa eksperimentalnim palatinalnim pločama (kontrolna grupa / DM grupa), po proceduri Tsurioka i saradnika [12], uzet je precizni otisak gornje vilice adicionim silikonom (Zhermack). Parče voska debljine 0,6 mm je postavljeno na model preko okluzalnih površina molara. Preko voska je načinjena akrilatna ploča (Biokril, Galenika, Srbija) koja je bila ekstendirana duž palatuma od prvog do trećeg molara sa obe strane vilice. Nakon polimerizacije akrilata vosak je uklonjen. U daljem postupku, palatinalna ploča je bila postavljena na nepce pod kompresijom i fiksirana za molare sa obe strane korišćenjem istog akrilata (slike 1 i 2). Radi uzimanja biološkog materijala, životinje su bile žrtvovane anestetikom zoletil u letalnoj dozi tri dana posle postavljanja palatinalne ploče. Biološki materijal mekog tkiva pacova je uzet sa kompresivnih i akompresivnih mesta na kojima je ležala palatinalna ploča posle žrtvovanja životinja i odvajanja eksperimentalnih ploča od nepca, sa pripremom za kvantifikaciju VEGF.

Detekcija tkivnog VEGF

Za kvantitativno merenje VEGF u tkivu pacijenata i životinja (pg/mL) korišćen je komercijalni Enzyme-Linked Immunosorbent Assay kit (Human VEGFA ELISA Cell Culture Supernatant, Urine; RayBiotech and Rat VEGFA ELISA Kit for Cell Culture Supernatant, Urine; RayBiotech) u skladu sa uputstvima proizvođača [19]. Optičke gustine su izmerene na 450 nm čitačem mikroploča i minimalni nivo detekcije testa bio je 5,0 pg/mL.

Statistika

Rezultati su prikazani kao srednja vrednost ± standardna devijacija (SD). Poređenja između grupa su vršena korišćenjem testom ANOVA sa ponovljenim merenjima i testom Che-squared. Test ANOVA je korišćen za analizu grupnih razlika, a test Bonferroni za post-hoc poređenja. Da bi se odredila poređenja između odgovarajućih grupnih vremenskih tačaka, korišćen je Studentov t-test. Vrednosti $p < 0,05$ su se smatrale statistički značajnim. Svi podaci su analizirani pomoću Stat for Windovs 8, StatSoft, Inc, SAD, 1984–2007.

REZULTATI

U ovoj studiji starost i pol nisu uzeti u obzir, jer su posmatrane grupe učesnika pretežno bile muške osobe (69%), starosti između 45 i 65 godina.

U Tabeli broj 1 prikazane su koncentracije VEGF iz uzoraka prekrivenih protezom (akompresivni deo), kao i iz gingive koja je bila komprimovana protezom (kompresivni deo) kod zdravih ispitanika i ispitanika sa DM tipa 2. Koncentracije VEGF u kompresivnom delu gingive bile su značajno manje, kako u grupi zdravih ispitanika, tako i kod ispitanika sa DM tipa 2, u odnosu na koncentracije VEGF iz akompresivne gingive obe grupe. Naime, u grupi zdravih ispitanika koncentracija VEGF u kompresivnom delu gingive je bila za 28%, a kod ispitanika sa DM tipa 2 za 66% manja u odnosu na koncentracije ovog faktora rasta u akompresivnom delu gingive. Ispitanici sa DM tipa 2 imali su značajno manje koncetracije VEGF u kompresivnim uzorcima gingive u odnosu na zdrave ispitanike; suprotno tome uzorci gingive bez kompresije ne pokazaju razliku u koncetraciji VEGF između zdravih ispitanika i ispitanika sa DM tipa 2.

U Tabeli 2 prikazane su karakteristike kontrolnih pacova i pacova sa DM koje se odnose na nivo glukoze u krvi i telesnu težinu pre eksperimentalnih procedura, 21 dan posle indukcije DM i poslednjeg dana eksperimentalne procedure 24. dana (posle tri dana nošenja eksperimentalne palatinalne ploče). Rezultati nisu pokazali značajnu razliku u nivou glukoze u krvi i telesnoj težini pre eksperimentalnih procedura u posmatranim grupama. Nivo glukoze u krvi je povećan i značajno veći kod pacova sa DM u poređenju sa zdravim kontrolnim grupama 21. i 24. dana. Kada je postavljena eksperimentalna palatinalna ploča, 21. dana, rezultati su pokazali smanjenje telesne težine kod

životinja sa DM za razliku od kontrolne grupe, što je rezultiralo značajnom razlikom u telesnoj težini u posmatranim grupama. Poslednjeg dana eksperimenta, posle tri dana nošenja eksperimentalne ploče, obe grupe životinja su pokazale smanjenje telesne mase. Pacovi sa eksperimentalno izazvanim DM imali su značajno manju telesnu masu u odnosu na zdrave pacove.

Tabela 3 prikazuje nivoe VEGF u palatinalnoj sluznici, nekompresivnih i kompresivnih uzoraka, kod kontrolne grupe i pacova sa DM. Koncentracije VEGF u tkivu su značajno povećane u uzorcima ispod eksperimentalne palatinalne ploče (kompresivni uzorci), u poređenju sa koncentracijama VEGF u tkivu koje nije pokriveno eksperimentalnom palatinalnom pločom (nekompresivni uzorci) u obe posmatrane grupe. Zdravi pacovi, kao i pacovi sa eksperimentalno izazvanim DM, nisu pokazali statističku razliku u nivoima VEGF tkiva u nekompresivnim uzorcima, za razliku od kompresivnih uzoraka gde su nivoi VEGF značajno niži kod životinja sa eksperimentalno izazvanim DM u poređenju sa zdravim pacovima.

DISKUSIJA

Akutno i hronično opterećenje tokom funkcionalnih i parafunkcionalnih navika izazvanih protezama ima potencijal da izazove deformaciju potpornog tkiva [20, 21, 22]. Ispitujući uticaj kontinuirane kompresije mobilne parcijalne proteze na protok krvi u potpornoj sluzokoži nosilaca proteza, Akazava i Sakurai [8] pokazali su ishemiju tkiva i smanjenje protoka krvi u sluznici posle oslobađanja kompresije. Tsouruka i sar. su pokazali da ispod eksperimentalne palatinalne ploče ćelije u tkivu sintetišu HSP70 i VEGF za održavanje homeostaze [12]. Međutim, autori ove studije ne daju podatake o uticaju akutnog i hroničnog mehaničkog stresa izazvanog nošenjem proteze na koncentracije VEGF u tkivu ispitanika sa DM. Da bi se razjasnili efekti DM na akutne promene potpornog tkiva, ova studija je imala za cilj da stimuliše efekte nošenja proteza na eksperimentalnom modelu životinja. Ljudski model je korišćen da se pokaže uticaj hroničnog mehaničkog stresa na potporno tkivo, na ćelijskom nivou kod nosilaca proteza sa/bez DM tipa 2. Prateći studiju Tsuruoka i sar. [12], nismo razmatrali efekte korišćenih materijala za akrilatnu eksperimentalnu ploču i parcijalnu protezu na potporna tkiva. Naime, u skladu sa Mori i sar. [21], u studiji koju su sproveli Inoue i sar. [23], histopatološki nalazi koji se odnose na toplopolimerizujući akrilat pokazali su minimalni citotoksični efekat 4-META/MMA-TBB, uprkos penetraciji monomera u tkivo tokom polimerizacije.

Rezultati našeg istraživanja su otkrili povećanje nivoa VEGF u tkivima posle tri dana nošenja eksperimentalne palatinalne ploče, u obe posmatrane grupe životinja sa DM i bez njega. Naš nalaz je u skladu sa studijom Tsuruoka i sar. koja je otkrila da je povećana ekspresija VEGF kako u endotelnim ćelijama, tako i u vezivnom tkivu, osteoblastima i periostu gornje vilice posle tri dana nošenja palatinalne ploče kod zdravih pacova [12]. Imajući u vidu studije koje su pokazale smanjenje protoka krvi ispod eksperimentalnih palatinalnih ploča, što je praćeno ishemijom [8, 9], povećani nivoi VEGF u tkivu pacova posle tri dana nošenja eksperimentalne palatinalne ploče mogu biti uzrokovani hipoksičnim stanjem, glavnim faktorom za stimulaciju VEGF. Suprotno našim rezultatima koji ne pokazuju statističku značajnost za koncentraciju VEGF tkiva u nekompresivnim uzorcima između DM i zdravih životinja (uzorci bez eksperimentalne ploče), kod pacova sa DM došlo je do značajno manjeg povećanja koncentracije VEGF tkiva ispod eksperimentalne palatinalne ploče u poređenju sa kontrolnim grupama, što sugeriše da se ekspresija VEGF menja u uslovima mehaničkog stresa kod DM.

Analizirajući model hroničnog stresa, naši rezultati su pokazali značajno niže nivoe VEGF u tkivu ispod parcijalne proteze (kompresivni uzorci) u poređenju sa nivoima VEGF u tkivu koje nije bilo pokriveno parcijalnom protezom (nekompresivni uzorci). Pomenuto smanjenje tkivnog VEGF može ukazivati na adaptaciju oralne sluzokože i ćelijski odgovor na mehanički stres u cilju održavanja oralne homeostaze. Dobijeni rezultat značajno većeg smanjenja tkivnog VEGF u kompresivnim uzorcima kod nosilaca parcijalne proteze sa DM tipa 2 u poređenju sa zdravim ispitanicima ukazuje na izmenjenu oralnu homeostazu kod DM. Međutim, naši rezultati nisu pokazali statističku značajnost za nivoe VEGF tkiva u nekompresivnim uzorcima između DM tipa 2 i zdravih nosilaca proteza. Objavljeni podaci o nivoima VEGF u tkivima kod pacijenata sa DM i parodontopatijom su kontradiktorni. Za razliku od Sakaliogllua i sar. [23], koji su prijavili značajno više nivoe VEGF u uzorcima gingive kod pacijenata sa DM i klinički utvrđenom parodontopatijom u poređenju sa kontrolnom grupom, Keles i sar. [24] nisu našli značajnu razliku u ekspresiji VEGF mRNA u gingivalnim tkivima pacijenata sa parodontopatijom sa DM tipa 2 i bez njega. Međutim, mnoga istraživanja su pokazala tkivne promene ispod baze proteze kod pacijenata sa dijabetesom i životinja sa eksperimentalno izazvanim DM [25–28] u poređenju sa zdravim kontrolnim grupama. Međutim, naši rezultati su prvi put prikazali promene u nivoima VEGF u tkivima kao ćelijski odgovor na nošenje proteze kod ispitanika sa DM.

ZAKLJUČAK

Akutni mehanički stres povećava koncentraciju VEGF u palatinalnoj sluzokoži, dok hronični mehanički stres, obrnuto, smanjuje nivo VEGF u tkivu. Niže koncetracije VEGF su povezane sa akutnom i hroničnom kompresijom kod DM, što ukazuje na izmenjenu homeostazu. Naše istraživanje naglašava značaj edukacije stomatologa o održavanju proteza kod pacijenata sa DM tipa 2, jer doprinosi boljem razumevanju ćelijskog odgovora i bioloških procesa u oralnoj sluzokoži pod mehaničkim stresom.

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SUKOB INTERESA

Autori navode da nema sukoba interesa.