International Journal of Biomedicine 14(2) (2024) 324-328
http://dx.doi.org/10.21103/Article14(2)_OA15

ORIGINAL ARTICLE

Dentistry

INTERNATIONAL JOURNAL OF BIOMEDICINE

Cytological and Cytometric Analysis of Epithelial Cell Changes Under the Surface of Acrylate Prosthesis in Diabetic Patients

Erejeta Deva Kurshumliu[1], Gordana Kovacevska[2], Kujtim Shala[1], Blerta Musliu Krasniqi[3], Fisnik Kurshumiu[4*]

[1]*Department of Prosthodontics, University Clinical Dentistry Center of Kosovo, Prishtina, Kosovo*
[2]*Department of Prosthodontics, Dental School, University of Skopje "Ss. Cyril and Methodius," Republic of Macedonia*
[3]*Department of Pedodontics, Alma Mater Europaea, Campus College "Rezonanca," Prishtina, Kosovo*
[4]*Institute of Pathology, University Clinical Center of Kosovo; Medical Faculty, University of Prishtina, Prishtina, Kosovo*

Abstract

***Background*:** The aim of this study was to assess cytological alterations of the squamous epithelium of the alveolar ridge mucosal surface under the acrylate prosthesis in patients with type 2 diabetes (T2D).

Methods and Results: The subjects of interest were patients in whom the total acrylate prosthesis had been applied 3-5 years or more prior to the examination. The subjects were divided into two groups: 30 adult subjects with T2D (T2D group) and 30 adult subjects without T2D (control group). Both groups were over 49 years of age. Cytological smears were obtained by cytobrush from the mucosal surface of the gingival crest underlying the acrylate prosthesis. The following parameters were assessed: type of cells (basal, intermediate, superficial, superficial without nucleus, and parakeratotic), proportions of the types of cells, cytoplasmic diameter, nuclear diameter, and nucleus-to-cytoplasm ratio. An independent sample t-test was used to compare the two study groups. The percentage of superficial cells was significantly lower in the T2D group than in the control group (P=0.001). The T2D group tended to have a higher rate of parakeratotic cells (13.0±11.12 % vs. 8.07±7.92%, P=0.052). Superficial cells in the T2D group had a significantly lower mean cytoplasmic diameter than the control group (32.7947±8.61929µ vs. 36.6383±4.32228µ, P=0.03). Additionally, the nucleus-to-cytoplasm ratio of intermediate and superficial cells in the T2D group was significantly higher than in the control group (0.2407±0.07206 vs. 0.2000±0.03291, P=0.007 and 0.2573±0.06330 vs. 0.2280±0.03178, P=0.027).

Conclusion: The results of our study show that total acrylate prostheses in diabetic patients are responsible for the disrupted maturation of squamous epithelial cells. This is reflected in smaller superficial cells, increased parakeratosis, and the higher nucleus-to-cytoplasm ratio of intermediate and superficial cells.**(International Journal of Biomedicine. 2024;14(2):324-328.)**

Keywords: exfoliative cytology • liquid-based cytology • cytometric analysis • diabetes mellitus

For citation: Kurshumliu ED, Kovacevska G, Shala K, Krasniqi BM, Kurshumiu F. Cytological and Cytometric Analysis of Epithelial Cell Changes Under the Surface of Acrylate Prosthesis in Diabetic Patients. International Journal of Biomedicine. 2024;14(2):324-328. doi:10.21103/Article14(2)_OA15

Introduction

Type 2 diabetes (T2D) is an expanding global health problem. Contributing factors include genetics, obesity, physical inactivity, and advancing age.(1) Several oral diseases and disorders manifest themselves with greater frequency and severity in individuals with diabetes mellitus.(2) The situation is even worse in edentulous patients with diabetes mellitus who, on some occasions, happen to be wearers of total acrylic prostheses, given all the microvascular complications that may occur in relation to the disease.(3) Although the prevalence of complete tooth loss has declined over the last decade, edentulism remains a major disease worldwide, especially among older individuals.(4) The reasons for edentulism are

many. While it is primarily the result of microbial or genetic diseases that have strong individual and behavioral impacts, edentulism can be the result of iatrogenic, traumatic, or therapeutic causes, too.[5] Edentulism is manifested with masticatory difficulty, altered facial expression and difficulty in speech.[6,7] Most edentulous patients are rehabilitated by the application of total acrylic prostheses as a replacement for lost bone and teeth. Complete dentures restore the function of the jaw-tooth system and improve the aesthetic aspect of the face, which are very important to the life of an individual.[8] The effect of total prostheses on the oral mucosa has been the subject of a number of scientific studies with rather controversial results.[9-19] These studies analyzed the cytological features of oral mucosa. The major advantage of exfoliative cytology is the non-invasive character of the technique, which allows a simple and painless collection of mucosal cells from different layers of the epithelium for microscopic examination.[20] The cytological examination may be carried out by the conventional method and liquid-based cytology (LBC).[21]

In our study, we used LBC, which allows immediate fixation of cells while removing unwanted harvested material, e.g., blood cells, mucus, and debris. This technique provides for a thin cellular layer of evenly dispersed cells in a clear background.[22]

Although several studies have suggested a potential association between diabetes and oral mucosa,[23] the effect of total acrylic prosthesis on the underlying mucosa in diabetic patients is rarely reported.[24] The aim of this study was to assess cytological alterations of the squamous epithelium of the alveolar ridge mucosal surface under the acrylate prosthesis in T2D patients.

Materials and Methods

The subjects were selected from the patient records at the University Dentistry Clinical Center of Kosovo. The subjects of interest were patients in whom the total acrylate prosthesis had been applied 3-5 years or more prior to the examination. The subjects were divided into two groups: 30 adult subjects with T2D (T2D group) and 30 adult subjects without T2D (control group). Both groups were total acrylate prosthesis wearers and over 49 years of age. Exclusion criteria were patients with oral pathologies that may interfere with morphological features of the epithelial cells, such as stomatitis, candidiasis, and other infections, and patients with traumatic or tumor lesions.

Clinical protocol

Prior to cell collection, a questionnaire was filled out with relevant information, such as the patient's age, duration of T2D, type of treatment for T2D, duration of wearing a total prosthesis, causes of tooth loss, oral hygiene habits, smoking habits, alcohol consumption, daytime and/or nighttime wearing, and maintenance and hygiene of prostheses. Subjects underwent a clinical oral examination before sampling. The oral mucosa status was generally evaluated. The mucosa in the undersurface of the acrylate prosthesis was assessed for the following: color, surface, transparency, vascularity, edema, and evidence of recent or old traumatic lesions or tumors. Photographs were taken from the areas of interest and stored for analysis. Subsequently, disinfecting mouthwash was applied for a few seconds before sampling. A cytological sample was obtained using a cytobrush; the smear was taken from the mucosal surface of the gingival crest underlying the acrylate prosthesis. After the smear was scraped off from the surface, the cytobrush was placed in a cell collection vial for LBC.

Laboratory protocol

The cell suspension from the cell collection vial was placed in a Hettich cytospin centrifuge and subsequently applied on a glass slide. After additional fixation of the cell smear with 96% ethanol, the same was stained by the Papanicolaou method. This stain was applied because it provides the best morphological details and level of keratinization of the squamous epithelial cells. After staining, the slides were mounted with a mounting medium and coverslipped.

Cytological and cytometric analysis (Figures 1-4)

The microscope slides were scanned by a Microvisioneer slide scanner, using the Olympus CX41 microscope and the Microvisioneer Manual WSI 2020C-34 FL software. After scanning, the images were transferred and saved on the InstaSlide cloud platform. Cytometric analysis was performed using the Aperio Image Scope software (v12.4.3.5008). The following parameters were assessed: overall cellularity; ratio of basal-to-intermediate-to-superficial cells; presence and percentage of parakeratinized squamous epithelial cells; presence and percentage of anuclear surface epithelial cells (keratinization); the median diameter of basal, intermediate, and superficial cell cytoplasm; the median diameter of basal, intermediate, and superficial cell nuclei; the nucleus-to-cytoplasm ratio of basal, intermediate, and superficial cells.

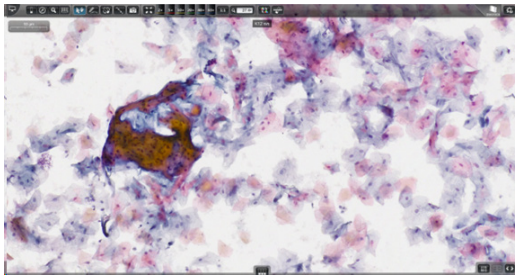

Fig 1. *Squamous epithelial cells in the cytological smear (Papanicolaou stain, 20x magnification; SlideViewer software).*

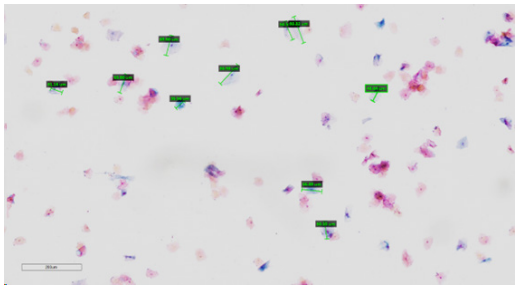

Fig. 2. *Measurement of cytoplasmic diameter (Papanicolaou stain, 10x magnification; Aperio Image Scope software).*

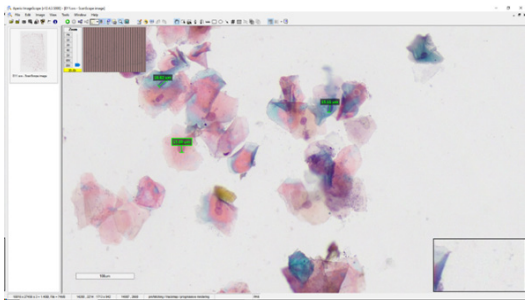

Fig. 3. *Measurement of nuclear diameter (Papanicolaou stain, 40x magnification; Aperio Image Scope software)*

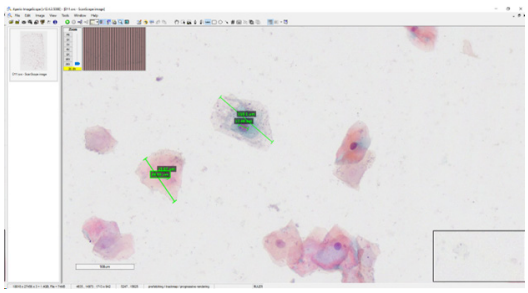

Fig. 4. *Measurement of nucleus-to-cytoplasm ratio (Papanicolaou stain, 40x magnification; Aperio Image Scope software)*

Statistical analysis was performed using the statistical software package SPSS version 23.0 (SPSS Inc, Armonk, NY: IBM Corp). For the descriptive analysis, results are presented as mean, standard deviation (SD), standard error of the mean (SEM). Means of 2 continuous normally distributed variables were compared by independent samples Student's t-test. A probability value of $P<0.05$ was considered statistically significant.

Results

Our results show no significant difference between the sample cellularity of the two study groups ($P=0.838$) (Table 1).

Table 1.

Cellularity of cytological smears in the study groups.

Cellularity	N	Mean	SD	SEM	*P*-value
T2D group	30	2.4667	0.68145	0.12441	0.838
Control group	30	2.5000	0.57235	0.10450	

In contrast, the percentage of superficial cells was significantly lower in the T2D group than in the control group ($P=0.001$) (Table 2). Also, the T2D group tended to have a higher rate of parakeratotic cells (13.0±11.12 vs. 8.07±7.92, $P=0.052$) (Table 2). Superficial cells in the T2D group had a significantly lower mean cytoplasmic diameter than the control group (32.7947±8.61929µ vs. 36.6383±4.32228µ, $P=0.03$) (Table 3). Additionally, the nucleus-to-cytoplasm ratio of intermediate and superficial cells in the T2D group was significantly higher than in the control group (0.2407±0.07206 vs. 0.2000±o.03291, $P=0.007$ and 0.2573±0.06330 vs. 0.2280±0.03178, $P=0.027$) (Table 4).

Table 2.

Percentage of types of squamous epithelial cells in the study groups.

Type of squamous epithelial cells (%)	N	Mean	SD	SEM	*P*-value
Intermediate (T2D)	30	27.2667	25.45304	4.64707	0.27
Intermediate (Control)	30	21.2333	15.48009	2.82627	
Superficial (T2D)	30	32.3333	25.20992	4.60268	0.001
Superficial (Control)	30	52.2667	19.22630	3.51023	
Parakeratotic (T2D)	30	13.0000	11.12003	2.03023	0.052
Parakeratotic (Control)	30	8.0667	7.91739	1.44551	
Anuclear (T2D)	30	27.7333	30.77161	5.61810	0.18
Anuclear (Control)	30	18.4333	21.69170	3.96034	

Table 3.

Cytoplasmic (CD) and nuclear (ND) diameter of intermediate and superficial cells in the study groups.

Type of squamous epithelial cells	N	Mean (µ)	SD	SEM	*P*-value
Intermediate CD (T2D)	30	38.4547	15.41475	2.81433	0.24
Intermediate CD (Control)	30	42.1333	7.62489	1.39211	
Superficial CD (T2D)	30	32.7947	8.61929	1.57366	0.03
Superficial CD (Control)	30	36.6383	4.32228	0.78914	
Intermediate ND (T2D)	30	8.6450	2.30501	0.42084	0.39
Intermediate ND (Control)	30	8.2587	0.82743	0.15107	
Superficial ND (T2D)	30	8.1860	2.11151	0.38551	0.87
Superficial ND (Control)	30	8.2523	0.70371	0.12848	

Table 4.

Nucleus-to-cytoplasm ratio (N:C) of intermediate and superficial cells in the study groups.

Type of squamous epithelial cells / N:C	N	Mean	SD	SEM	*P*-value
Intermediate / N:C (T2D)	30	0.2407	0.07206	0.01316	0.007
Intermediate / N:C (Control)	30	0.2000	0.03291	0.00601	
Superficial / N:C (T2D)	30	0.2573	0.06330	0.01156	0.027
Superficial / N:C (Control)	30	0.2280	0.03178	0.00580	

Discussion

Considering that changes in the oral mucosa of patients with diabetes mellitus are common, epithelial mucosal changes under the surface of acrylate prosthesis should be even more pronounced. The squamous epithelium undergoes constant replacement by cell migration and differentiation. Keratinization is a process of cytodifferentiation during which

the keratinocytes undergo maturation from their germinative state to finally differentiated stratum corneum.[9,10]

Cytological and cytometric changes of the oral mucosal epithelial cells in the immediate undersurface of the acrylate prostheses provide important morphological data about the level of irritation of the mucosa in diabetic patients. Various published studies have observed changes in the degree of keratinization of oral epithelium under prosthesis. Some of these studies show that oral epithelium under the prosthesis becomes more keratinized, while others show that the epithelium remains non-keratinized.[11] Many authors have concluded that total dentures affect keratinization, and most have reported increased keratinization in patients who wear total dentures. According to a study by Kumaresan and Jagannathan,[25] exfoliative cytology plays a major role in diagnosing clinically misinterpreted changes. Considering that the sensitivity and specificity of cytology are limited, the combination of computer-aided cytology and morphometry improves its accuracy. According to this study, this method should be used as a routine method for diagnosing mucosal lesions in the early stages. In a cytological study by Markov et al.,[12] if dentures were regularly removed at night, ortho keratinization (normal keratinization) occurred. They believed that rest at night made it possible for the oral mucosa to recover from the wear and tear caused by the dentures. According to a study by Watson and MacDonald,[13] the degree of keratinization was lower, and the stratum corneum was thinner in the epithelium under dentures. The complete dentures in these studies seemed to reduce the quantity and quality of the keratin layer. Our study has shown that the presence of a denture produced a more regular epithelium with few rete ridges and a thinner, less keratinized stratum corneum. In diabetic patients, due to the previously mentioned oral manifestations and increased susceptibility, these changes may have altered morphology and gravity. According to a study by Farhan and Yas,[26] diabetes is related to certain cytomorphometric changes in the oral mucosal cells. Our study shows that nuclear diameter increases while cytoplasmic diameter decreases in oral mucosal epithelial cells in T2D patients, compared to patients without diabetes. Parallel to these observations, our study shows that in the T2D group, superficial cells were smaller than in the control group. Similarly, there was also an increase in the nucleus-to-cytoplasm ratio in superficial and intermediary cells in T2D patients, compared to the control group. Additionally, our study shows an alteration of the differentiation process of the keratinocytes in diabetes mellitus with lesser numbers of superficial cells and an increased percentage of parakeratotic cells, compared to the control group. In our study, there was a higher percentage of superficial cells without a nucleus in the diabetes mellitus than in the control group; however, it was not statistically significant. We believe that with a larger study group, statistical significance could be established even regarding the percentage of superficial cells without a nucleus.

The alveolar ridge under the acrylic prosthesis seems to be a useful site for cell collection, considering that during chewing, the oral mucosa beneath the denture plays a critical role in distributing occlusal loads to the underlying bony ridge over a large denture-supporting tissue interface.[14,27,28] Acrylic-based resins are frequently used in daily dental practice for prostheses as they can provide essential properties and have the necessary characteristics for their use in diverse functions. During the polymerization of these materials, the residual monomer is released, which may be cytotoxic to oral mucosa. There is an assumption that residual monomers in the denture base, which is in direct contact with oral mucosa, might have a clinical impact on the tissue.[29-33] Some of the studies found that there is not only a quantitative reduction of keratinization but also acanthosis. Its frequency is considered even more significant in patients suffering from diabetes mellitus, considering the oral mucosa sensitivity in these patients. The results of our study support these observations.

In conclusion, the results of our study show that total acrylate prostheses in diabetic patients are responsible for the disrupted maturation of squamous epithelial cells. This is reflected in smaller superficial cells, increased parakeratosis, and the higher nucleus-to-cytoplasm ratio of intermediate and superficial cells. We believe that these results should be a driver for further research in exploring alternative materials or approaches for managing edentulism in diabetic patients.

Competing Interests

The authors declare that they have no competing interests.

References

1. Rathee M, Jain P, Singh S, Divakar S, Chahal S, Wakure P. Role of diabetes in the prosthodontic management of a completely edentulous patient. International Journal of Dental Research. Jun 2021; 3(1):50-5

2. Jivanescu A, Bratu EA, Goguta L, Borgnakke WS. Effect of Improvement of Complete Dentures on Quality of Life in Type 2 Diabetes. Diabetes, Stoffwechsel und Herz. 2013;22(4):207-211

3. Al-Maskari AY, Al-Maskari MY, Al-Sudairy S. Oral Manifestations and Complications of Diabetes Mellitus: A review. Sultan Qaboos Univ Med J. 2011 May;11(2):179-86. Epub 2011 May 15. PMID: 21969888; PMCID: PMC3121021.

4. Emami E, de Souza RF, Kabawat M, Feine JS. The impact of edentulism on oral and general health. Int J Dent. 2013;2013:498305. doi: 10.1155/2013/498305. Epub 2013 May 8. PMID: 23737789; PMCID: PMC3664508

5. Al-Rafee MA. The epidemiology of edentulism and the associated factors: A literature Review. J Family Med Prim Care. 2020 Apr 30;9(4):1841-1843. doi: 10.4103/jfmpc.jfmpc_1181_19. PMID: 32670928; PMCID: PMC7346915

6. Shinde J, Mowade T, Gupta P, Tekale R, Pande N, Deshmukh K, Lokhande T, Radke U. Satisfaction in conventional acrylic complete denture patient with and without denture liners - a systematic review. Pan Afr Med J. 2022 Aug 22;42:296. doi: 10.11604/pamj.2022.42.296.33035. PMID: 36415342; PMCID: PMC9643794

7. Olms C, Hix N, Neumann H, Yahiaoui-Doktor M, Remmerbach TW. Clinical comparison of liquid-based and conventional cytology of oral brush biopsies: a randomized

controlled trial. Head Face Med. 2018 May 29;14(1):9. doi: 10.1186/s13005-018-0166-4. PMID: 29843756; PMCID: PMC5975412.
8. Sun X, Meng L, Chen Y, Wang J, Wang Q. Efficacy and risk factors of traditional denture restoration versus biofunctional complete denture restoration system. Am J Transl Res. 2023 Jul 15;15(7):4755-4762. PMID: 37560251; PMCID: PMC10408514.
9. Menon RS, Dhakshaini MR, Gujjari AK, Hegde U. A clinical evaluation on the effect of astringent on keratinization of oral mucosa before and after the insertion of complete denture. Indian J Dent Res. 2019 May-Jun;30(3):347-351. doi: 10.4103/ijdr.IJDR_620_17. PMID: 31397406.
10. Shetty S; Gokul S. Keratinization and its disorders. Oman Med J. 2012 Sep;27(5):348-57. doi: 10.5001/omj.2012.90. PMID: 23074543; PMCID: PMC3472583.
11. Mneizel T.A study of the palatal keratinization levels beneath complete acrylic resin dentures. JRMS. June 2005; 12(1): 25-29
12. Markov NJ. Cytologic study of keratinization under complete dentures. J Prosthet Dent 1968;20:8-13.
13. Watson IB, MacDonald DG. Oral mucosa and complete dentures. J Prosthet Dent. 1982 Feb;47(2):133-40. doi: 10.1016/0022-3913(82)90176-7. PMID: 6173477
14. Mori S, Sato T, Hara T, Nakashima K, Minagi S. Effect of continuous pressure on histopathological changes in denture-supporting tissues. J Oral Rehabil. 1997 Jan;24(1):37-46. doi: 10.1046/j.1365-2842.1997.00443.x. PMID: 9049918.
15. Menon RS, Dhakshaini MR, Gujjari AK, Hegde U. A clinical evaluation on the effect of astringent on keratinization of oral mucosa before and after the insertion of complete denture. Indian J Dent Res. 2019 May-Jun;30(3):347-351. doi: 10.4103/ijdr.IJDR_620_17. PMID: 31397406.
16. Radke U, Kahar A, Zade P, Lambade D, Deshpande S, Radke P. Evaluation of Effect of Astringent on Oral Mucosa as a Non-surgical Preprosthetic Treatment Modality in Edentulous Patients: An In Vivo Study. J Indian Prosthodont Soc. 2014;14(Suppl 1):93-97. doi:10.1007/s13191-014-0372-0
17. Stig Gson Östlund. The Effect of Complete Dentures on the Gum Tissues, Acta Odontologica Scandinavica. 1958;16(1):1-41. doi: 10.3109/00016355809028181
18. Jani RM, Bhargava K. A histologic comparison of palatal mucosa before and after wearing complete dentures. J Prosthet Dent. 1976 Sep;36(3):254-60. doi: 10.1016/0022-3913(76)90179-7. PMID: 1066479.
19. Nedelman C, Gamer S, Bernick S. The alveolar ridge mucosa in denture and non-denture wearers. J Prosthet Dent. 1970 Mar;23(3):265-73. doi: 10.1016/0022-3913(70)90181-2. PMID: 5263308.
20. Verma R, Singh A, Badni M, Chandra A, Gupta S, Verma R. Evaluation of exfoliative cytology in the diagnosis of oral premalignant and malignant lesions: A cytomorphometric analysis. Dent Res J (Isfahan). 2015 Jan-Feb;12(1):83-8. doi: 10.4103/1735-3327.150339. PMID: 25709680; PMCID: PMC4336977.
21. Kokubun K, Nakajima K, Yamamoto K, Akashi Y, Matsuzaka K. Evaluation of oral brush liquid-based cytology for oral squamous cell carcinoma: a comparative study of cytological and histological diagnoses at a single center. BMC Oral Health. 2023 Mar 11;23(1):145. doi: 10.1186/s12903-023-02839-w. PMID: 36906554; PMCID: PMC10007825.
22. Olms C, Hix N, Neumann H, Yahiaoui-Doktor M, Remmerbach TW. Clinical comparison of liquid-based and conventional cytology of oral brush biopsies: a randomized controlled trial. Head Face Med. 2018 May 29;14(1):9. doi: 10.1186/s13005-018-0166-4. PMID: 29843756; PMCID: PMC5975412.
23. Nandita KP, Boaz K, Srikant N, Lewis AJ, Nidhi M. Oral epithelium in diabetics: A cytomorphometric correlation, Dental Hypotheses. Apr-Jun 2014;5(2):59-65. doi:10.4103/2155-8213.133428
24. Lee JH, Han JS, Han K, Lee SY. Association between Diabetes and the Use of Removable Dental Prostheses among the Korean Population. J Korean Med Sci. 2019 Oct 28;34(41):e262. doi: 10.3346/jkms.2019.34.e262. PMID: 31650717; PMCID: PMC6813424.
25. Kumaresan GD, Jagannathan N. Exfoliative cytology - A predictive diagnostic tool. International Journal of Pharmacy and Pharmaceutical Sciences,.2014;6(5): 1-3.
26. Farhan RS, Yas LS. Oral Finding and Cytomorphometric Analysis of Oral Mucosal Cells in Type 2 Diabetic Patients. International Journal of Medical Research & Health Sciences. 2018;7(6): 86-93
27. Kydd WL, Daly CH, Nansen D. Variation in the response to mechanical stress of human soft tissues as related to age. J Prosthet Dent. 1974 Nov;32(5):493-500. doi: 10.1016/0022-3913(74)90003-1. PMID: 4531483.
28. Jenny N. Beneath the Complete Denture Prosthesis: A Review. International Journal of Dental Sciences and Research. 2018;6(4):87-89. doi: 10.12691/ijdsr-6-4-2.
29. Gonçalves TS, Morganti MA, Campos LC, Rizzatto SM, Menezes LM. Allergy to auto-polymerized acrylic resin in an orthodontic patient. Am J Orthod Dentofacial Orthop. 2006 Mar;129(3):431-5. doi: 10.1016/j.ajodo.2005.10.017.
30. Lunder T, Rogl-Butina M. Chronic urticaria from an acrylic dental prosthesis. Contact Dermatitis. 2000 Oct;43(4):232-3. PMID: 11011931.
31. Martin N, Bell HK, Longman LP, King CM. Orofacial reaction to methacrylates in dental materials: a clinical report. J Prosthet Dent. 2003 Sep;90(3):225-7. doi: 10.1016/s0022-3913(03)00331-7. PMID: 12942054.
32. Koutis D, Freeman S. Allergic contact stomatitis caused by acrylic monomer in a denture. Australas J Dermatol. 2001 Aug;42(3):203-6. doi: 10.1046/j.1440-0960.2001.00517.x. PMID: 11488717.
33. Ruiz-Genao DP, Moreno de Vega MJ, Sánchez Pérez J, García-Díez A. Labial edema due to an acrylic dental prosthesis. Contact Dermatitis. 2003 May;48(5):273-4. doi: 10.1034/j.1600-0536.2003.00100.x. PMID: 12868970.

***Corresponding author:** *Fisnik Kurshumliu. Institute of Pathology, University Clinical Center of Kosovo; Medical Faculty, University of Prishtina, Prishtina, Kosovo. E-mail: fisnik.kurshumliu@uni-pr.edu*