

HISTOPATHOLOGICAL ASPECTS OF UNDER-DENTURE ORAL MUCOSA AREA IN ACRYLIC REMOVABLE DENTURES WEARERS

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ABSTRACT

Aim of the study: The aim of this study was to highlight the histopathological lesions of the oral mucosa adjacent to acrylic removable denture. **Material and methods:** The histopathological study was performed on 21 fragments of human oral mucosa, collected from subjects wearing removable acrylic dentures, who presented at the Clinic of Dental Prosthetics and Oral Rehabilitation of the Faculty of Dentistry in Craiova, between January 2018 and May 2020. **Results:** The most common lesions in the epithelium were acanthosis, parakeratosis, associated with the presence of papillomatosis and hypertrophy of connective papillae. The lesions of the lamina propria were mostly of the chronic granulomatous inflammatory type or of the lymphoplasmocytic type, with macrophages in some places. **Conclusions:** The possibilities of the oral mucosa to react in the contact area with a removable partial acrylic denture are multiple, ranging from simple adaptive histopathological changes to the appearance of acute or chronic histopathological lesions.

Key words: denture stomatitis, acrylic removable denture, histopathological lesion

INTRODUCTION

Denture stomatitis is an inflammatory condition of the oral tissues that come in contact with a removable denture (1) and has a multifactorial aetiology (2). It is estimated that denture stomatitis affects up to 70% of patients wearing removable dentures (3).

The physiology of the oral cavity changes with the application of dentures in the

prosthetic field (4). An acrylic removable denture placed adjacent to a large area of the oral mucosa causes changes in the oral mucosa and oral epithelium (5), having an adverse effect on epithelial cells and causing various disorders at cellular level (6).

Regarding the intensity of the reactions of the oral mucosa, it has been shown that the oral mucosa responds less intensely to irritation than skin, due to its anatomical and functional

features (7).

Different studies have shown that the labial mucosa reacts more frequently than mucosa from other regions of the oral cavity (8).

An ideal material for the denture base should have adequate mechanical and physical properties, in addition to biocompatibility and aesthetics (9). Polymethyl-methacrylate (PMMA) has multiple advantages, such as: excellent aesthetics, adequate strength, low water absorption, low solubility and easy technological processing. However, this material also has some disadvantages, such as: polymerization shrinkage, low impact resistance, low fatigue strength and poor bending (10). However, acrylic resins are the most widely used material for manufacturing removable dentures (11).

The aim of this study was to highlight the histopathological lesions of the oral mucosa adjacent to an acrylic removable denture. Although the materials from which the dentures were made belonged to the same category, acrylic resins, the clinical changes of the under-denture oral mucosa varied. It started from the null hypothesis that the histopathological changes of the oral mucosa adjacent to an acrylic removable denture are similar, in all situations, because the materials from which the dentures were made were part of the same class, acrylic resins.

MATERIAL AND METHODS

The histopathological study was performed on a total of 21 fragments of human oral mucosa, collected from subjects wearing removable acrylic dentures, who presented at the Clinic of Dental Prosthetics and Oral Rehabilitation of the Faculty of Dentistry in Craiova, between January 2018 and May 2020. The study was approved by the Ethics Commission of the University of Medicine and Pharmacy of Craiova, in accordance with the

Helsinki Declaration of 1975, as revised in 2008. Subject selection was made based on the inclusion and exclusion criteria.

The criteria for including subjects in the study were: a. adult subjects, over 18 years of age; b. adult subjects with partial or complete edentulism, wearing complete or partial removable acrylic dentures.

The criteria for excluding subjects from the study were: a. subjects under 18 years of age; b. subjects with intact dental arches; c. subjects with complete or partial edentulism who did not wear a removable acrylic denture.

Each study participant was informed about the study objectives and gave their written consent. Clinical parameters recorded for the participants included in the study were: gender, age, place of residence, the presence or absence of clinical lesions of the under-denture oral mucosa, duration of use of removable acrylic dentures, topography of mucosal fragments collected.

Oral mucosal fragments were collected only from participants who, following clinical examination and complementary investigations, required the extraction of an unrecoverable tooth or surgical excision of marginal mucosal hyperplasia, caused by an unstable removable denture, and were approximately 3-4 mm in size.

In the first stage of processing, the collected oral mucosa fragments were washed with saline to remove blood traces, and subsequently fixed in 10% solution of formic aldehyde (formalin) at neutral pH for 48-72 hours. The samples were processed by the classical histological paraffin embedding technique at the Laboratory of Pathology, Emergency County Hospital of Craiova. The staining method used for the study of under-denture human oral mucosa fragments was Hematoxylin-eosin (HE) for diagnostic evaluation.

The study was performed compared to the

normal histological structure of oral mucosa. Sampling of human oral mucosa from an area other than the under-denture oral mucosa was not possible because the subjects did not give their consent.

The statistical research method used the Chi-square test, with $\alpha = 5\%$, the p value $<0.05\%$ being considered statistically significant.

RESULTS

Mucosal fragments were taken from 7 subjects without clinical changes in the under-denture oral mucosa and from 14 subjects who showed clinical changes, wearing upper and/or lower removable acrylic dentures (Table 1), female and male, aged between 46 and 75 years, who came from urban and rural areas (Figure 1). The period of use of removable acrylic dentures by study participants ranged from 3 years to 7 years.

Table 1. Topographic distribution of fragments of oral mucosa collected

Topography of mucosal fragments collected	Maxillary arch	Mandible arch	Total
Number of fragments taken	11	102	21

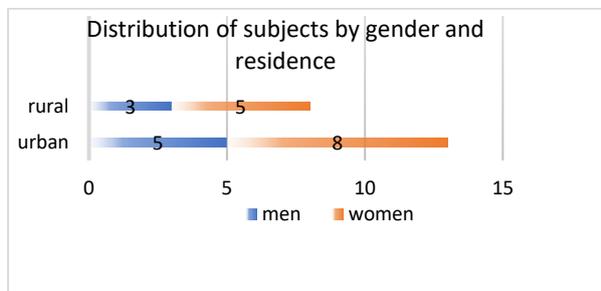


Figure 1. Distribution of subjects by gender and residence

The histopathological lesions of the sampled mucosal fragments were varied and intricate.

Changes were observed in the epithelium, the basal membrane and the lamina propria (Table 2).

Table 2. Types of lesions in relation to their location

Location	Epithelium	Lamina propria
Lesion type	-acanthosis -parakeratosis -hyperkeratosis with parakeratosis -hypertrophy of the cells of the spinous layer	-intense granulomatous reactions -hemorrhagic micro-foci -ulcerative lesions

Statistical analysis by chi-square test did not show a statistical association between the intensity of the inflammatory reactions of the studied mucosal fragments and the clinical variables ($p > 0.05$).

The most common lesions in the epithelium were acanthosis, parakeratosis, associated with the presence of papillomatosis and papillary crest hypertrophy. The papillomatosis lesion was characterized by bipotential differentiation or alternation of hypertrophic connective papillae with atrophic, thin, elongated papillae. In some cases, intraepithelial inflammatory infiltrate could be observed (Figure 2, Figure 3).

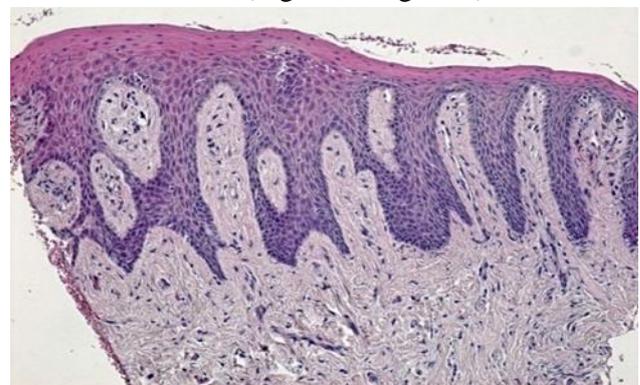


Figure 2. Oral mucosal fragment with alternating atropho-hypertrophic papillomatosis, hyperkeratosis with parakeratosis, spinous hypertrophy cells HE x100

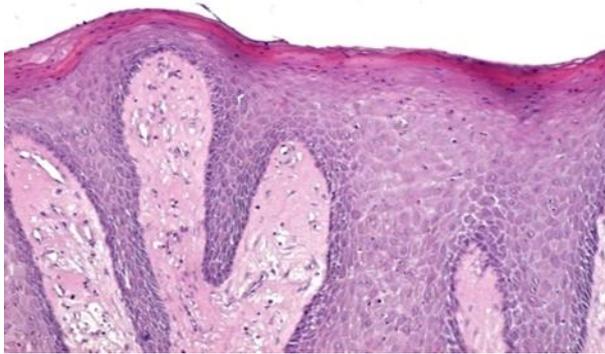


Figure 3. Oral mucosal fragment with bipotentially differentiated papillomatosis, hyperkeratosis with parakeratosis, intraepithelial inflammatory infiltrate
HE x200

Some mucosal fragments showed old ulcerations with superficial, pseudonodular fibroplastic reaction, chronic perilesional inflammatory reaction with diffuse lymphocytes arranged in a sclerocollagen stroma, focal blood infiltrates, superficial micro-hemorrhages (Figure 4).

In other samples, a granulomatous reaction was shown in the lamina propria, with numerous numerous pseudoangiomas vessels with hyperemia, fibrocytic stroma with blood infiltrates and lymphocyte type (Figure 5).

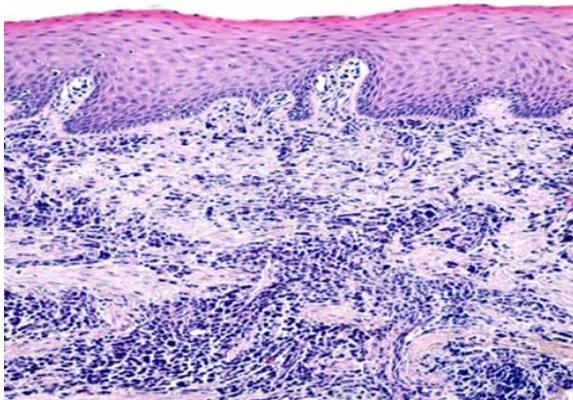


Figure 4. Oral mucosal fragment with hypertrophic connective papillae, rare intrapapillary inflammatory elements, parakeratosis; chronic inflammatory infiltrate
HE x100

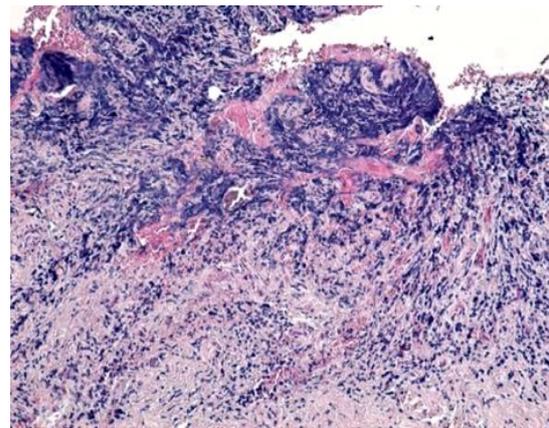


Figure 5. Extended ulceration fragment with superficial fibroplastic reaction, perilesional inflammatory reaction with pseudonodular disposition, HE x100

The lesions of the lamina propria were mostly of the chronic granulomatous inflammatory type or of the lymphoplasmocytic type, with macrophages in some places. Sometimes, the moderate pseudonodular inflammatory reaction predominated, delimited by scleroconjunctival tracts and micro-hemorrhages. (Figure 6, Figure 7).

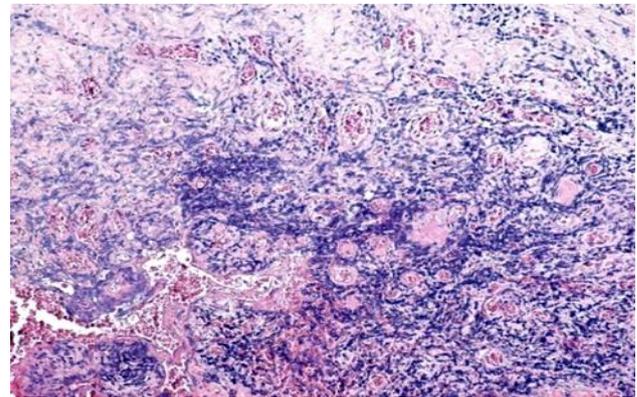


Figure 6. Lamina propria fragment with intense granulomatous reaction, inflammatory and hematic infiltrate
HE x100

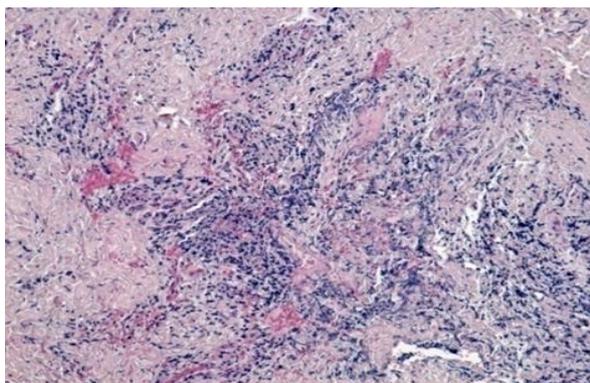


Figure 7. Fragment of the deep lamina propria with moderate pseudonodular inflammatory reaction, delimited by sclero-conjunctival bays and micro-hemorrhages HE x100

DISCUSSION

The histopathological lesions of the oral mucosa, highlighted in its contact areas with an acrylic removable denture, are still a topic of debate for many authors. The oral mucosa-prosthesis interface is a dynamic structure in which bidirectional exchanges take place, so that the surface of the removable denture causes changes in the mucosa, but also the oral environment causes changes in the surface of the denture. The complexity of these biological processes is all the greater, as the oral environment varies from individual to individual and even in the same individual during a day.

The histological sections analyzed in the present study microscopically, revealed a variety of alterations of the components of the under-denture oral mucosa, which evolved progressively. From a topographical point of view, the mucosal lesions in the wearers of acrylic removable dentures started at the level of the covering epithelium, subsequently, they affected the basal membrane, with the development of a defence reaction at the level of lamina propria. In terms of severity, mucosal changes ranged from adaptive alterations to chronic or acute inflammatory reactions.

Adaptive histopathological changes of the covering epithelium were highlighted by exaggerated reactions of normal cell differentiation in the attempt to adapt the mucosa to the acrylic denture. Thus, the most common lesions consisted of an increase in the number of layers of the covering epithelium, an aspect that characterizes the phenomenon of acanthosis, associated with the differentiation of papillomatosis, with the hypertrophy of the connective papillae.

At the same time, the cellular turn-over had an accentuated rhythm, fact indicated by the presence of hyperkeratosis, associated with parakeratosis, which varied from a discrete character to an accelerated one.

A study by *Le Bars et al.* on fragments of oral mucosa with stomatitis induced by wearing removable acrylic dentures highlighted the presence of mucosal inflammation associated with areas of hyperplasia and atrophy. At epithelial level, the authors noted the presence of incomplete parakeratosis or the complete absence of keratin and a reduced thickness of epithelial tissue. The authors also stated that at epithelial level, different types of epithelial differentiation are conditioned by the regional characteristics of basal cells, characteristics that may be affected by changes in the oral environment (12).

Other oral mucosal fragments examined showed an atrophic covering epithelium, but with hyperkeratosis with parakeratosis, the cells of the intermediate layer being ballooned, with vacuolar cytoplasm or weakly eosinophilic.

A histological study by Reichart et al. on nineteen fragments of oral mucosa taken from patients diagnosed with denture stomatitis revealed a partially atrophic squamous epithelium that covered the wide and long papillae of the connective tissue. The basal epithelial cells were arranged in 1-2

rows, highlighting different degrees of keratinization, from orthokeratosis in 17 cases to mild parakeratosis in 5 cases (13). In our study, some fragments showed high intraepithelial connective papillae, and on other fragments an atrophic-hypertrophic alternation of them was noticed. Le Bars et al. claim that in the under-denture oral mucosa, atrophic-hypertrophic changes are due to the pressure to which the mucosa is subjected during mastication, and inflammatory changes are due to prolonged wear of the denture or the action of pathogens in the microbial plaque (12).

On some of the histological sections diagnosed with bipotential papillomatosis with hyperkeratosis with parakeratosis, at the epithelial level, inflammatory infiltrate was observed. Similar results were mentioned in a histopathological study performed by Janosi et al. on fragments of hyperplastic mucosa, collected from a patient wearing a maxillary acrylic denture. The authors stated that at the epithelial level a moderate thickening was observed with the presence of a strong inflammatory lymphoplasmocytic infiltrate, in which lymphocytes dominated, with the presence of rare plasma cells and polymorphonucleate cells (14).

At the lamina propria level, the most common lesions found consisted in the presence of chronic and acute inflammatory infiltrate, which was accompanied by old ulceration lesions or hemorrhagic lesions. The cellular elements present were most often arranged in a fibroparous and fibrocytic stroma, suggesting the tendency to limit the lesion. Thus, the main lesions in the lamina propria observed in the present study were: extensive ulceration with fibropar reaction accompanied by perilesional inflammatory reaction, intense granulomatous lesion with inflammatory and haematic infiltrate and pseudonodular inflammatory lesion delimited

by sclero-conjunctival tracts and along with microhemorrhages.

A study by Bergendal et al. highlighted the presence of lymphocytes and plasma cells and the presence of an intense inflammatory reaction, and the fact that zonal connective tissue was replaced with granulation tissue in the uner-epithelial connective tissue of removable acrylic dentures wearers. The authors also observed a low number of histiocytes present, and eosinophils were observed only occasionally (15).

Similar results were obtained by Morimoto et al., highlighting the presence of an intense inflammatory reaction, with numerous lymphocytes and plasma cells in the lamina propria (16).

In addition to the papillomatosis lesion present in most of the analyzed fragments, but with various shapes, in 5 cases we also encountered hemorrhagic and ulcerative lesions accompanied by differentiated organized inflammatory infiltrate in different types of fragments.

In rare cases, the presence of eosinophils, vasodilation and leukocyte exudate has been noted, which involves, in addition to triggering inflammatory mechanisms, the activation of immune mechanisms in response to local aggressions induced by the denture (16, 17).

CONCLUSIONS

The null hypothesis that all analyzed oral mucosa fragments show the same type of lesion has not been confirmed. Although most fragments were diagnosed histopathologically with papillomatosis, there were also fragments of, ulcerative lesions and hemorrhagic lesions. 1. Of the 21 fragments of under-mucosa oral mucosa examined, 13 were taken from female subjects, and from a topographical point of view 11 fragments were taken from the

maxillary arch.

2. The possibilities of the oral mucosa to react in the contact area with a removable acrylic denture are multiple, ranging from simple adaptive histopathological changes to the appearance of acute or chronic histopathological lesions.

3. The histological picture of the epithelial lesions had a polymorphic character, with alterations such as: acanthosis, parakeratosis, hyperkeratosis with parakeratosis, spinous cell hypertrophy, ulcerative and hemorrhagic lesions.

4. At the level of the lamina propria, multiple and varied histopathological lesions were highlighted: ulceration with superficial fibroplastic reaction, intense granulomatous reaction, pseudonodular inflammatory reaction delimited by sclero-conjunctival tracts and micro-hemorrhages.

5. The analysis of the correlations between the studied clinical variables and the intensity of the inflammatory reactions at the level of the lamina propria of the studied under-denture oral mucosa fragments did not show a statistically significant association.

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