PHD THESIS
ABSTRACT

IMPLICATIONS OF GROWTH FACTORS IN PSEUDEPITHELIOMATOUS HYPERPLASIA OF THE ORAL CAVITY

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ABSTRACT

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Key-words: pseudoepitheliomatous hyperplasia, growth factors, immunohistochemical markers
INTRODUCTION

Pseudoepitheliomatous hyperplasia (PEH) or Heck’s disease is an epithelial proliferation, less inconstant and conjunctive, as a response to a chronic irritative stimulus, affecting the mucous surfaces and the skin. Thus, it is considered that pseudoepitheliomatous hyperplasia is a benign reactive lesion, characterized by the epithelium hyperplasia, in the form of epithelial "tongue-like" projections in the dermis or chorion, often presenting a pseudo invasive aspect (Grunwald M.H., Lee J.Y., Ackerman A.B., 1988; Ju D.M., 1967).

Being a condition that develops secondarily to another condition, its real incidence is difficult to estimate. It affects both sexes almost equally, and the patients’ age varies a lot, the literature highlighting cases between 11 and 80 years old.

We were motivated to choose this theme especially by its multi factorial etiopathogenicity, the association with other pathologies, namely infectious, neoplastic, dermatoses with chronic irritations and inflammations and other pathological processes, but mainly from the practitioner’s point of view the interest was brought by the problems that this pathology gives in relation to the certainty diagnosis, which most often leads to confusions (Zayour M., Lazova R., 2011).

By the performed study, we aimed at completing and contributing to the clarification of certain aspects regarding the etiopathogenesis and certainty diagnosis of this condition.

The histopathological and immunohistochemical study lasted for 3 years, including 47 cases of oral pseudoepitheliomatous hyperplasias, the histological material, selected bewteen 2012 and 2014, coming from the cases of the Laboratory of Pathological Anatomy within the Emergency Clinical County Hospital of Craiova.

KNOWLEDGE STAGE
CHAPTER 1
PSEUDOEPITHELIOMATOUS HYPERPLASIA – EPIDEMIOLOGICAL, ETIOPATHOGENIC AND CLINICAL ASPECTS

The first case of pseudoepitheliomatous hyperplasia was mentioned in 1896 as an epidermal proliferation in a case of lupus vulgaris (El-Khoury J., Kibbi A.G., Abbas O., 2012). This hyperplasia is also called pseudocarcinomatous hyperplasia, invasive acanthosis, invasive epidermal hyperplasia, carcinomatous hyperplasia (Sarangarajan R, Vaishnavi Vedem VK, Sivadas G et. al, 2015; Kao G.F., Farmer E.R., 2000).
The etiopathogeny of pseudoepitheliomatous hyperplasia is not completely known, as this reactive lesion of the mucous surfaces and skin seems to develop as a response to a variety of infectious, neoplastic, inflammatory or traumatic stimuli (Zarovnaya E, Black C, 2005). Pseudoepitheliomatous hyperplasia is a lesion associated to another pathology, being found in a series of pathogenic conditions (Sapp J.P., Eversole L.R., Wysocki G.P., 2004; Scully C., 1999; Speight P.M., 2007). Thus, in the etiology of pseudoepitheliomatous hyperplasia, there are incriminated the following: infectious pathogenic conditions, tumoral pathogenic conditions, both benign and malignant ones, inflammatory pathogenic conditions.

Pseudoepitheliomatous hyperplasia or Heck’s disease represents an epithelial proliferation, as a response to a chronic irritation, the conjunctive proliferation being more reduced and unregulated.

In the literature, there is recorded that there are oral lesions frequently accompanied by pseudoepitheliomatous hyperplasia, sometimes interpreted as epidermoid carcinomas. The most frequent oral lesions accompanied by pseudoepitheliomatous hyperplasia are: myoblastoma, keratoacanthoma, tuberculosis. Sometimes, the fistule epulides are accompanied by a pseudoepitheliomatous hyperplasia. (Crăiţoiu Ş., Florescu M., Crăiţoiu M., 1999).

Pseudoepitheliomatous hyperplasia accompanies and mimes especially the following conditions: infectious conditions, like: tuberculosis, actinomycosis, candidosis; benign and malignant tumors, like the papilloma, epulis (inflammatory fibrous hyperplasia, pyogenic granuloma, peripheral bone fibroma, peripheral granuloma, congenital granuloma), keratoachantoma, granular cell tumor, pleomorphus adenoma, melanoma, base-cell carcinoma, squamous cell carcinoma; inflammatory conditions like the oral lichen planus and pemphigus (Green R., Cordero A., Winkelmann R.K., 1977; Bucur A., Navarro Villa C., Lowry J., et al, 2009; Mitrache C., Benea V., Țovaru M. et al, 2011; Florescu M., Simionescu C., Mârgăritescu C., 2004; Mârgăritescu C., Simionescu C., Surpăţeanu M., 2010; Pătroi G., 2010).

CHAPTER 2
GROWTH FACTORS INVOLVED IN THE ORAL MUCOSA
HYPER GROWTH

Cytokines are molecules with a biological activity that in low concentrations induce a specific response to the sensitive cells. The cytokine activity manifests in vivo and in vitro in very low concentrations, and because of this they were called immune hormones or immune response regulatory hormones. Cytokines are also known as mediators of immunity, inflammation proliferation and differentiation of the cell lines. They are involved in all the pathological aspects, their spectrum of action being quite a large one.
Cytokines may be classified into: interleukins, tumor necrosis factors, colony stimulating factors, growth factors, interferons and chemokines.


PERSONAL CONTRIBUTION

CHAPTER 3

HISTOPATHOLOGICAL STUDY

The histopathological study investigated the main microscopic morphological characteristics of oral pseudoepitheliomatous hyperplasia and the etiopathogenic conditions of these lesions.

The histopathological material was taken from the cases of the Laboratory of Pathological Anatomy within the Emergency Clinical County Hospital of Craiova and it was represented by the archived paraffin blocks. At first, there were selected the blocks and blades for the histopathological diagnosis according to the clinical observation sheets sorted during the clinical and epidemiological examination of the patients hospitalized in the Oro-maxillo-facial Clinic within the same hospital.

The study lasted for 3 years, the cases being selected between 2012-2014, a number of 47 cases of oral pseudoepitheliomatous hyperplasia constituted the object of the histopathological study.

From the observation sheets, there were selected the anamnestic and clinical data, namely: patients’ age and sex, lesion localization, pathological medical history (preexistent conditions, primitive tumor/ tumor relapse), etiopathogenic conditions associated to these lesions, symptoms, etc.

In the histopathological study, we used the classical histological technique of paraffin inclusion, and as staining methods we used the following:

- Hematoxillin-Eosin (HE) for the diagnosis evaluation;
- Alcian Blue - PAS for the evaluation of the basal membrane integrity and the glycoproteic profile of the lesions;
- Masson trichrome with aniline blue for evaluating the fibrosis stage.
In the performed study, oral pseudoepitheliomatous hyperplasias represented only 0.87% of the total of oral lesions diagnosed between 2012-2014 in the Oro-maxillo-facial Clinic within the Emergency Clinical County Hospital of Craiova.

The most frequently affected age groups were the 5th and 6th decades, with 36.2% of cases and 32%, respectively. The average age of oral pseudoepitheliomatous hyperplasia onset in the studied groups was 49 years old. These lesions developed more frequently in males (57.5%), etiopathogenically the lesions associated to granular-cell tumors and to lichen planus tumors were diagnosed especially in females. Topographically, the most frequent localization of oral pseudoepitheliomatous hyperplasia lesions was the lingual one (44.7%), followed by a jugal localization (23.4%) and by the gingival one (19.15%). The Table 3.1 presents the distribution of the investigated cases on age groups.

<table>
<thead>
<tr>
<th>Table 3.1 Age distribution of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age groups</td>
</tr>
<tr>
<td>No of cases</td>
</tr>
<tr>
<td>%</td>
</tr>
</tbody>
</table>

Regarding the sex distribution of the 47 oral pseudoepitheliomatous hyperplasia lesions we recorded the data found in Table 3.2.

<table>
<thead>
<tr>
<th>Table 3.2 Sex incidence distribution of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
</tr>
<tr>
<td>No of cases</td>
</tr>
<tr>
<td>%</td>
</tr>
</tbody>
</table>

The topographic distribution of the oral pseudoepitheliomatous hyperplasia lesions was summarized in the table below (Table 3.3).

<table>
<thead>
<tr>
<th>Table 3.3 Topographic distribution of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lesional topography</td>
</tr>
<tr>
<td>No of cases</td>
</tr>
<tr>
<td>%</td>
</tr>
</tbody>
</table>
Strictly from a histopathological point of view, we were interested in the histopathological changes characteristic to the lesion diagnosis and the histopathological changes of the etiopathogenic conditions of the oral mucosa where oral pseudoepitheliomatous hyperplasia developed.

One of the most characteristic morphological changes of the surface epithelium in pseudoepitheliomatous hyperplasias is represented by the elongation of these epithelial apices that deeply descend into the chorion, acanthosis representing another change present in all the investigated cases (Fig. 3.1, Fig. 3.2).

In the chorion of 14 cases we observed the subadjacent fibrosis of the epithelium hyperplasia consisting of the presence of collagen fiber fascicles of a variable thickness that interchange at various angles, among them coexisting variable quantities of intercellular matrix and fibrocytes, in the cases of inflammatory and infectious cause there was associated an inflammatory infiltrate mainly of the lymphoplasmacytic type (Fig. 3.3, Fig. 3.4).

Fig. 3.1 Pseudoepitheliomatous hyperplasia – elongated epithelial apices. HE staining x40
Fig. 3.2 Pseudoepitheliomatous hyperplasia – acanthosis of the covering epithelium. HE staining x40
Fig. 3.3 Pseudoepitheliomatous hyperplasia – subepithelial fibrosis. HE staining x100
Fig. 3.4 Pseudoepitheliomatous hyperplasia – inflammatory infiltrate. HE staining x100
The 47 lesions of pseudoepitheliomatous hyperplasia in the oral mucosa were diagnosed in association with a large variety of clinical entities, from infectious stomatites (tuberculosis, actinomycosis and candidosis), to chronic inflammatory conditions (oral lichen planus) and neoplastic lesions, respectively (granular-cell tumor and oral squamous carcinoma).

The most frequent associations were with infectious stomatites, diagnosed in 40.42% of cases, of which 17% were associated with tuberculosis, 12.76% with actinomycosis and 25.53% with candidosis (Fig. 3.5, Fig. 3.6, Fig. 3.7). In 7 cases of oral pseudoepitheliomatous hyperplasia it was associated with lichen planus - Fig. 3.8.

Secondly, there were the associations with undiagnosed oral neoplastic conditions in 29.78% of cases, the most frequent associations being with oral squamous carcinoma found in 17% of cases, followed by the associations with granular-cell tumor, diagnosed in 12.76% of cases (Fig. 3.9, Fig. 3.10).

Fig. 3.5 PEH associated with tuberculosis-specific tuberculous granuloma with caseification necrosis. HE staining x 100

Fig. 3.6 PEH associated with actinomycosis-specific actinomycotic granuloma. HE staining x100

Fig. 3.7 PEH associated with candidosis–chronic inflammatory infiltrate in the chorion with lymphocytes, plasmocytes and epithelioid cells with presence of candidosis spores and hyphae. HE staining x200

Fig. 3.8 PEH associated with oral lichen planus – hydropic degenerescence of the basal stratum and an abundant subepithelial lymphoplasmocyte inflammatory infiltrate. HE staining x200
CHAPTER 4
IMMUNOHISTOCHEMICAL STUDY

In the immunohistochemical study, we used the paraffin blocks from which there were performed the sections needed for the classical histopathological processing, on a smaller group of cases, namely on 20 cases of lesions. We used the LSAB technique (Labelled Streptavidin-Biotin2 System) and the Dako kit (Redox, Romania - K0675). In the immunohistochemical study, we used concentrated antibodies developed in rats or rabbits, directed against humans, whose main characteristics are given in Table 4.1.

Table 4.1 Antibodies used in the study of pseudoepitheliomatous hyperplasias

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Clone/ Manufacturer</th>
<th>Dilution</th>
<th>Antigen Demasking</th>
<th>Positive Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGFβ</td>
<td>3C11/ SantaCruz Biotechnology</td>
<td>1:250</td>
<td>Citrate, pH 6</td>
<td>Kidney</td>
</tr>
<tr>
<td>TGFβ R3</td>
<td>Polyclonal/ SDIX</td>
<td>1:200</td>
<td>Citrate, pH 6</td>
<td>Salivary gland</td>
</tr>
<tr>
<td>EGF</td>
<td>Polyclonal / SDIX</td>
<td>1:100</td>
<td>Citrate, pH 6</td>
<td>Salivary gland</td>
</tr>
<tr>
<td>EGFR</td>
<td>384/LEICA</td>
<td>1:100</td>
<td>Citrate, pH 6</td>
<td>Squamous Carcinoma</td>
</tr>
<tr>
<td>FGF7</td>
<td>Polyclonal / Sigma-Aldrich</td>
<td>1:40</td>
<td>Citrate, pH 6</td>
<td>Appendix</td>
</tr>
<tr>
<td>FGFR2</td>
<td>Polyclonal / Sigma-Aldrich</td>
<td>1:50</td>
<td>Citrate, pH 6</td>
<td>Salivary gland</td>
</tr>
<tr>
<td>Beta-Catenin</td>
<td>B-Catenin-1/ Dako</td>
<td>1:100</td>
<td>Citrate, pH 6</td>
<td>Urothelium</td>
</tr>
<tr>
<td>Vimentin</td>
<td>SP20 / Thermo Fisher Scientific</td>
<td>1:200</td>
<td>Citrate, pH 6</td>
<td>Skin</td>
</tr>
<tr>
<td>MMP9</td>
<td>2C3/ SantaCruz Biotechnology</td>
<td>1:50</td>
<td>Citrate, pH 6</td>
<td>Appendix</td>
</tr>
<tr>
<td>CXCR4</td>
<td>Polyclonal / Acris Antibodies</td>
<td>1:1000</td>
<td>Citrate, pH 6</td>
<td>Tonsil</td>
</tr>
</tbody>
</table>

In the performed study, the TGF-b1 reactivity was recorded in all the strata of the oral hyperplasia epithelium, (Fig. 4.1), the TGF-bR3 reactivity was mainly in the basal stratum and in
the superficial lines of the spinous stratum (Fig. 4.2), the EGF immunoreactivity was observed in only 6 cases (30%) - (Fig. 4.3) and for EGFR there was also a reduced number of cases (15% of the investigated cases) - (Fig. 4.4).

We observed the presence of an immunomarking for FGF7 in 80% of the investigated cases, the maximum of intensity being observed in the cases associated with inflammatory conditions (Fig. 4.5), but FGFR-2 immunoreactivity was highlighted especially in the areas of acanthosis and dyskeratosis, a little lower in the epithelial apices (Fig. 4.6). In the pseudoepitheliomatous hyperplasia areas, the membrane pattern of the beta-catenin expression is preserved in the areas of acanthosis and inside the epithelial apices and networks (Fig. 4.7). Immunoreactivity for Vimentin was observed only in the areas of epithelial apices and epithelial networks (Fig. 4.8). The MMP9 immunomarking was low in some areas of acanthosis and dyskeratosis, but moderately intense in the areas of epithelial apices and networks (Fig. 4.9),
while the CXCR4 reactivity was observed mainly in the areas of acanthosis and dyskeratosis and in the epithelial apices, respectively (Fig. 4.10).

**Fig. 4.5** HPE – strongly intense positive FGF7 reaction in the spinous and superficial stratum cells. FGF7 IHC staining x 40

**Fig. 4.6** HPE - strongly intense positive FGFR2 reaction in the acanthosis area. FGFR2 IHC staining x40

**Fig. 4.7** HPE – membrane reactivity for beta-catenin down into the superior strata. Beta-catenin (brown)/ vimentin (red) IHC stainings x100

**Fig. 4.8** HRE – cytoplasmatic immunomarking for vimentin in the apices and epithelial networks. Beta-catenin (brown)/ vimentin (red) stainings x100

**Fig. 4.9** HPE – MMP9 reactivity especially in the basal and parabasal strata of epithelial apices. MMP9 IHC (brown)/ CXCR4 (red) IHC stainings x100

**Fig. 4.10** HPE – cytoplasmic reactivity for CXCR4 highlighted in the areas of acanthosis and dyskeratosis. MMP9 (brown)/ CXCR4 (red) IHC stainings x40
CHAPTER 5
GENERAL CONCLUSIONS

Pseudoepitheliomatous hyperplasia or Heck’s disease represents an epithelial proliferation as a response to a chronic rash, the conjunctive proliferation being more reduced and irregulated.

Oral pseudoepitheliomatous hyperplasia is a rare lesion, its incidence in oro-maxilo-facial being under 1%.

The association of this entity with a multitude of etiopathogenic conditions was represented by: oral candidosis (25%), oral tuberculosis with oral disseminations (17%), oral squamous carcinoma (17%), oral lichen planus (15%), actinomycosis with oral determinations (13%) and granular cell tumor (13%).

The existence of this kind of etiopathogenic associations requires the performance of a thorough histopathological diagnosis necessary both for establishing a certain etiology, and also for avoiding some diagnosis errors with a negative impact on the health of these patients.

The fact that, on the one side, there are important sources for growth factors, and, on the other side, other sources of matrix metaloproteases in the subadjacent chorion, with a strong inflammatory character, also explains the frequent association of this pseudoepitheliomatous hyperplasia with pathogenic inflammatory conditions.

SELECTIVE REFERENCES


