Age influence on periodontal tissues: a histological study

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Abstract
Oral mucosa becomes thinner, smooth and loses stippling aspect with aging. From histological standpoint appears: narrowing and alteration of gingival epithelium, modification of epithelial-connective interface and decreasing of keratinization. However, it cannot be detected significant histological alterations in size, shape or arrangement of epithelial cells that could be endorsed to aging process. Histological studies indicate: decreasing of keratinization, regressive changes in epithelium and fibrosis in underlying connective tissue. Parakeratosis is frequent with aging because of microtraumas, in many cases is expression of permanent inflammation.

Keywords: age-related changes, gingiva, histopathology.

Introduction
The normal structure of teeth and periodontal surrounding tissues undergo important histological and clinical changes with aging [1, 2]. These changes are morphological, functional and changes associated with modification of oral environment [3]. The periodontal condition of seniors are characterized by followings: oral mucosa become atrophic, satin-like and friable, gingiva loses stippling aspect, epithelial tissues becomes thinner and connective tissue reduce its elastic properties, capillaries decrease in number and as consequence the blood supply is reduced. Clinically, gingiva is reduced, migrates to apex, presents a reduced resilience, and becomes more sensitive to external factors [4].

At gingival level, from histological standpoint, are identified the following changes with age: diminished ketatinization, reduced number of cells in connective tissue, increased quantity of intercellular substances and decreased oxygen consumption [5].

Age-related changes in mucosa are still controversial, age alone is not consider a risk factor in developing periodontitis [6-8], but some alterations of gingiva could be caused by nutritional deficiencies or some systemic diseases [9, 10].

Objective
The aim of this study is to investigate the morpho-pathological changes that occur in gingival tissue of senior patients in relationship with histological aspect of gingival tissue of young patients.

Materials and Methods
The gingival tissue samples were collected under local anesthesia during small surgery maneuvers like tooth extraction, wisdom molar surgery or gingivectomy. Gingival samples were taken after obtaining informed consent of patient.

The control group consists of healthy patients of 20 to 29-year-old. Samples were collected during extraction of teeth without prognosis. The senior group consists of patients over 65-year-old without uncontrolled systemic diseases and samples were taken during gingivectomy or tooth extraction. Specimens were from healthy or inflamed gingiva.

Gingival samples were about 3–5 mm in diameter and were washed with sterile saline solution. Tissue samples were fixed in 1/4 diluted formalin solution neutralized with calcium carbonate in glass recipients. The following working technique was applied for fixed specimens: washing in running water, dehydration, clarification, inclusion in paraffin blocks, cutting paraffin blocks into 5–7 μm sections, displayed on glass slides. After dewaxing, specimens were stained with Hematoxylin–Eosin (HE), studied with optic microscope (×10, ×20, ×40 and ×100 magnifications) and then interpreted.
Results

When analyzing microscopic aspects of gingival fibromucosa of the control group that comprises subjects of 20 to 29-year-old, histological examination of gingival samples illustrate healthy aspects of epithelium and connective tissue (Figure 1).

Epithelial tissue is stratified on squamous epithelium made of four layers, sustained by fibrous chorion and basal membrane with a wavy aspect due to papillary prolongations of epithelium. Basal layer is made of one row of cuboidal closed cells with small intercellular spaces. Basal cells are arranged with longitudinal axes perpendicular on basal membrane, namely palisade arrangement. These cells have big nucleus, round or oval. Spinocellular layer is made of polyhedral cells arranged in multiple rows, with many and large intercellular spaces and granular layer made of 2–3 layers of plate cells with pyknotic nuclei and cytoplasm rich in keratohyalin granules. Keratinized layer is made of 10–15 layers of keratinized cells; these cells are plate, without normal contour and nuclei.

In healthy epithelium, there is an equilibrium between continuous epithelial desquamation (keratinization) process and renewing process (cellular proliferation), and when appears gingival inflammation it is shown hyperkeratosis (thickening of keratinized layer), parakeratosis (nucleated cells appear in keratinized layer).

Examination of underlying connective tissue of control group illustrates an organized aspect of cells, fibers, extracellular matrix and neuro-vascular network, and no inflammatory infiltrate when gingiva is healthy. The connective tissue forms papillae that penetrate through epithelium.

When analyzing microscopic aspects of gingival fibromucosa of study group that comprises subjects over 65-year-old, histological aspects of gingival samples illustrated modifications both in epithelium and in connective tissue.

At epithelium level appears acanthosis (enlargement of spinocellular layer) because of adaptation of gingiva to multiple irritative factors (Figures 2 and 3) and atrophy (narrowing of epithelium) (Figures 4 and 5). Lack of balance between keratinization and cellular proliferation is revealed by parakeratosis associated with inflammatory infiltrate in underlying connective tissue (Figures 4 and 6). Biopsies from study group showed dystrophy of epithelial cells with vacuolization and cytoplasmic changes that lead to necrosis (Figure 6).
For the connective tissue, characteristic is fibrotic aspect caused by increased number of fibers organized as bundles and decreased number of cells (Figure 4). Degradation of connective tissue with lymphoplasmocytary inflammatory infiltrate, having diffuse or nodular aspect, sometimes at the perivascular level and associated with capillaries dilatation appears in the presence of periodontitis (Figures 3 and 4).

Discussion

Aging is a complex, continuous, and slow process that gradually involves most if not all organs of the organism, causing their abnormal functioning of in both qualitative and quantitative terms, as well as morphological or structural changes [11].

Oral mucosa becomes thin, smooth and dry with advancing age. Gingiva is composed histologically by epithelium and connective tissue. Changes in epithelium caused by aging are atrophy (thinning of epithelium), diminished keratinization and modification of junction between epithelium and connective tissue [12]. Flattening of rete pegs with age leads to loss of stippling aspect [2]. In connective tissue the number of cell decreases when age increases [13] and in vivo and in vitro studies show morphologic and functional alteration in fibroblasts with aging [14–17] and increased the number of collagen fibers [18, 19]. Densely packed collagen fibers are founded also in relation with altered collagen metabolism due to drugs like Nifedipine, Phenytoin and Cyclosporine A [20–22]. However, in healthy individuals, collagen turnover pathways are similarly affected by Cyclosporin A treatment in young and aging gingival fibroblasts [23].

However, it could not be detected significant changes in size, shape or arrangement in epithelial cells that can be done by aging process. Furthermore, histological alterations of gingival cells are produced by nutritional deficiencies, like iron or B vitamins or systemic disorders [24, 25]. Parakeratosis is frequent with advancing age because of microtraumas. Pambuccian G [26] found that in 50% of cases parakeratosis is expression of permanent gingival inflammation. Gingival inflammation is associated with increased mitotic activity and thickness of epithelium [27]. Anuradha A and Sivapathasundharam B [28] found age-related alterations in normal exfoliated gingival cells, after 40-year-old there is a steady decrease in nuclear diameter, cell diameter and nuclear-cytoplasmic ratio.

Periodontal tissue deteriorates under persistent oxidative stress induced by inflammatory reactions in the microflora of the oral cavity [29]. Fibroblasts are affected by oral bacteria and their product, like lipopolysaccarides. Abiko Y et al. [30] show that lipopolysaccarides induce in fibroblasts releasing of inflammatory cytokines such as prostaglandin E2, interleukin and plasminogen activator, but other study reports that age cannot be identified as a factor that strongly affects the cytokine expression and fluctuations [7].

Aging process declines protein synthesis in gingival fibroblasts [17, 31] and also alteration in the composition of proteoglycans extracellular matrix secreted by fibroblasts [32]. Kletsas D et al. [33] report that fibroblast responses persist during aging, suggesting the involvement of systemic factors in the regulation of the healing process, and endopeptidase-24.11, a metalloproteinase controlling the action of neuroendocrine peptides and also of immuno- cytokine chemotaxis, is overexpressed during aging.

Due to epithelial atrophy and disturbance between keratinization process and epithelial cells proliferation process, gingival defense capacity is decreasing, inflammation develops easier and healing process is slower. Immuno-senescence refers to the gradual deterioration of the immune system caused by natural aging. It involves the host’s capacity to respond to infections and the development of long-term immune memory. The ineffective protection against microbes in the muco-cutaneous barriers, including the breakdown of local immunity in the gingival and oral cavity is age related [34]. Pawelec G et al. [35] have been reported ageing-related immunological alterations and Fransson C et al. [36] have been reported a more rapid and severe development of gingivitis as well as changes in inflammatory response induced by gingivitis in elderly patients. Matsuzaka K et al. [37] have reported age different response in defense of gingival epithelium, results revealed human β-defensin-2 positive cells in spinous cells in the elderly group and in the
parakeratinized layer in the young group. In addition, dente-dine gingival cells, responsible for immune response, are significant lower in older age group than in young group [38].

For this reason, altered tissue response and healing, in seniors periodontal treatment is recommended carefully handling of tissues and periodontal surgery should be performed asatraumatically as possible. Excellent wound healing can be obtained in seniors when tissues are handled properly [39].

S Conclusions

According to this histological study in gingival tissues are modifications, which can be related with inflammation caused by gingivitis or periodontitis than aging process: anacanthosis, parakeratosis, and areas of necrosis. In case of periodontal disease, there is a lymphoplasmocytary inflammatory infiltrate in connective tissue. Epithelial cells proliferation is accentuated in case of plasmocytary inflammatory infiltrate in connective tissue. Necrosis. In case of periodontal disease, there is a lympho-inflammation caused by gingivitis or periodontitis than tissues are modifications, which can be related with handled properly [39].

References


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