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Comparative histology aspects of the gingiva of children and adults in the University Dental Clinics

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Abstract: *To study the gingival morphology of children and adults, characterizing and comparing them. After approval by the Ethics Committee and signing of the Informed Consent Term, the gingival tissue of 4 children and 5 adults with surgical needs were collected and stored in 10% formaldehyde solution (pH 7.2). The histological processing was performed with increasing alcohol battery, diaphanization in xylol, embedding, 5 µm microtome cuts and Blade mount and coverslip. The tissues were stained with hematoxylin-eosin and toluidine blue, the slides were analyzed under light microscopy (Leica DM 2000) and photodocumented. In the gingival tissue of children and adults, epithelium of the keratinized pavement stratified type was observed. The four layers of the epithelium were identified. In the corneal layer in children, however, the keratin thickness was higher, 27µm on average, when compared to adults, 13µm. The connective tissue was similar in both age groups. A larger number of mast cells were found in the gingiva of children. The gingiva of children and adults were similar in composition and cell types. The keratin thickness of the stratum corneum, however, was lower in adults which may facilitate the installation of microorganisms and consequently of gingival and periodontal diseases.*

Keywords: *Age, Gingival tissue, Morphology.*

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I. INTRODUCTION

The periodontium is a structure of the stomatognathic system composed of the gingiva, alveolar bone, cementum and periodontal ligament. The gingiva is the periodontium of protection. It has main function is the mechanical and biological protection of alveolar processes and teeth [22,26]. Histologically it consists of a stratified squamous epithelium and connective tissue. In the epithelium, keratinocytes represent the major cellular component. In connective tissue the predominant cellular element it is fibroblastos [22, 4, 15, 11].

Liable to suffer modifications with age, the gingiva is also subject to morphological and functional changes through interaction with the environment [16,27]. The morphological analysis it is the first step, being fundamental in the evaluation and study of the tissues. Research aimed at identifying the morphological aspects of gingival tissue in different age groups is indicative of the health and regenerative power of this tissue.

The gingival tissue has a capacity for recovery and repair, even though it is constantly exposed to mechanical, chemical and biological aggressions of the buccal environment [9, 16], however these abilities vary according to age [24]. Morphological studies have been carried out with the aim of improving knowledge about gingival tissue and applying its skills in repairing oral structures and other parts of the body in tissue engineering research [19,18,10,28,7].

Gingival diseases affect children and adults differently, altering tissue morphology [2]. Adults are commonly the most affected, with prevalence of gingiva disease and chronic periodontitis, often cumulative [14]. In children and adolescents the gingivitis and aggressive periodontitis are the major aggravations [25, 1, 2]. Differences in the gingival tissues of children and adults involve not only pathological aspects. Studies suggest

that the periodontal structure of children may present a thinner corneal layer, a more vascularized connective tissue, and less organized collagen fibers [30,19].

The physiological mechanisms and changes involved in the reparative potential of gingival tissue, at different ages, are not yet fully understood. Research aimed at identifying the cellular types of the gingival tissue, with a view to morphological analysis, are elucidative regarding the regenerative power and cellular potential. Thus, this study aims at a morphological study of the gingiva of children and adults, characterizing and comparing them.

II. METHODOLOGY

•Ethical aspects

The study complied with the ethical requirements of the Ethics and Research Committee according to the National Council of Health's 466/12 CONEP Legislation for the development of human research. The patients were informed about the research and those who agreed to participate were asked to sign the Free and Informed Consent Form (FICF). In the case of the children, the signing of the Term of Assent (TA) and their parents was requested the Free and Informed Consent Term (FICF). The project was sent to the Research Ethics Committee of the Federal University of Piauí and is approved with the opinion 055310/2015.

• Inclusive criteria:

The participants included in the study were children from 2 to 9 years of age and adults aged 20 to 59 years who sought dental care at the Dentistry Clinics of the Federal University of Piauí (UFPI) and received the indication of surgical procedures for the removal of gingival tissue. Individuals who had systemic diseases, compromised surgical beds, smokers, and patients on medication were excluded.

The age groups were determined by the researchers, based on the criteria of the World Health Organization (Ministry of Health, 2011).

• Samples

Were selected four children and five adults who sought dental care at the UFPI clinics and given an indication to perform ulectomy procedures in the case of children and crown increase and gingivectomy for adults. Surgeries were performed under local anesthesia, under conditions of asepsis and antisepsis. After the procedures, gingival tissue samples were immersed in buffered 10% formaldehyde solution (pH 7.2), for a minimum period of 24 to 48 hours and taken to the Laboratory of Histotechnical and Embryology, Department of Morphology, Center for Health Sciences of the UFPI for histological analysis.

• Histological Analysis

After the adequate period of fixation, the gingival tissues were submitted to routine histological processing and staining by Hematoxylin and Eosin (HE) and Toluidine Blue (TB). The procedure started with the dehydration in batteries of increasing alcohols with submersion of 30 minutes each. The samples were then dipped in alcohol-xylol and diaphanized in increasing Xilol baths, also for 30 minutes. The biological material was impregnated with liquid paraffin, kept in the oven at 60ºC for 30 minutes and embassies. The blocks were submitted to microtomy at a thickness of 5 μm and fished on histological slides. The cuts were taken to the oven at 60 ° C for deparaffinization for a period of 10 minutes and then immersed in xylol I, II and III for about 5 minutes each. Subsequently, they underwent rehydration with decreasing sequence of alcohol for about 3 minutes, finishing the hydration with a bath in running water for 10 minutes. Some slides were stained with Hematoxylin-eosin and immersed in Hematoxylin for 45 seconds followed by a wash in running water for 5 minutes until removal of excess staining and staining by eosin for 30 seconds. The other slides were stained with Toluidine Blue for about 20 minutes. Then washed with running water and dipped six times in 70% ethyl alcohol. They were subjected to 5 minute baths in 100% ethyl alcohol, then two xylol baths for 5 minutes each to diaphanize. Finally, the slides were mounted on lamina laminula using ERV-MOUNT (Easy-path). They were taken under the light microscope (Leica DM 2000) for observation and analysis in Leica Image Manage® software program and photodocumented.

III. RESULTS AND DISCUSSION

In the gingival tissue, squamous epithelium with flattened cells was observed (Fig. 1). According to cell characteristics, the epithelium is divided into basal, thorny, granular and corneal layers (Fig. 1A). The basal layer, closer to the conjunctiva, has cuboid cells adhered to the lamina propria by hemidesmosomes; It has function is the protection and synthesis of keratinocytes. In the thorny layer the cells are irregular and with numerous cytoplasmic filaments, whereas in granulosa they are flatter with granules in their cytoplasm. The corneal layer is the most differentiated, loss of the nucleus of the cells remaining only keratin [17].

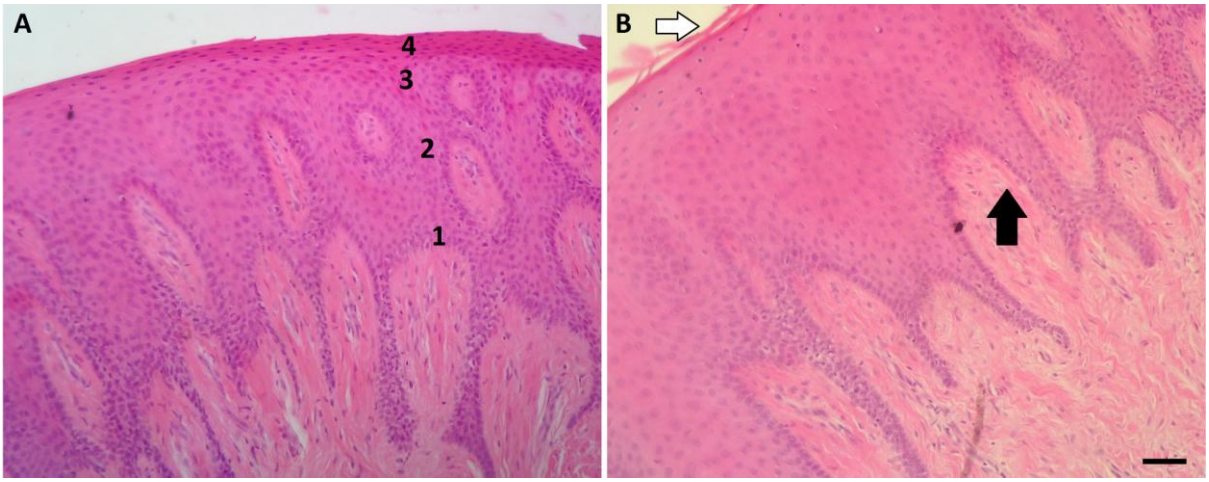

Figure 1- Human and adult gingiva photomicrograph of adult and child stained with HE (A and B). Stratified squamous epithelium (A) divided into four layers: Basal (1), Thorny (2), Grainy (3) and Cornea (4). (B) Projections of connective tissue in the epithelium forming the connective tissue papillae (black arrow). Color: HE. Obj. 50x. Bar:50µm.

The oral epithelium that lines the outer surface of the gingiva may be orthokeratinized or parakeratinized (Fig. 2) [17]. The gingiva is a functional unit that presents shape and contour variation; The presence of keratin on the epithelium is due to an adaptation of the tissue to the situations that are submitted, offering protection against mechanical trauma, tooth eruption and bacterial invasion [22].

The keratin layer in the child's gingival tissue was thick, presenting a mean of 27µm (Fig. 2A), while in the adult it presented a mean of 13µm (Fig. 2B). With age, the thickness of this layer decreases and sharpens on the epithelium, increasing the risks of bacterial antigens entering, thus increasing the chances for the appearance of periodontal diseases [23,17]. Bimstein et al. (1993), however, found no significant differences in the thickness of the keratinized layer of the oral epithelium of children and adults.

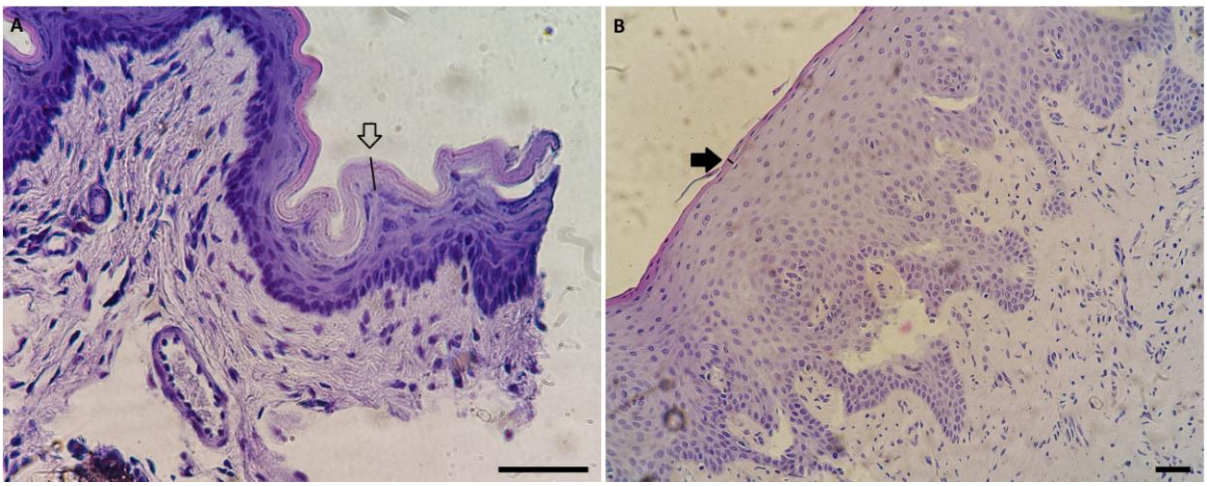

Figure 2 - Human and adult human gingival photomicrograph (A and B). (A) Presence of a thick layer of keratin on the epithelium (black line). (B) A thin layer of keratin on the adult gingival epithelium (black arrow). Color: HE. Bar: 50 µm (50x) and 20 µm (20x).

The connective tissue is composed mainly of collagens, proteoglycans, fibronectin, osteonectin, tenascin and elastin [26]. The connective tissue of children and adults did not present significant differences. (Fig. 3). Zappler (1948) and Ruben et al. (1971) studied the periodontal structure of children and observed that the deciduous dentition presented more vascularized connective tissue and less organized collagen fibers.

Parts of the connective tissue protrude into the epithelium forming the papillae of the connective tissue; no differences were observed regarding the papillae between the two age groups (Fig. 1B). Adjacent to the epithelium, the connective tissue exhibits dense unmodified fibroblasts and collagen fibers (Fig. 3). The lamina propria may also be loose and in deeper areas may be dense disorganized with thick collagen fibers [3].

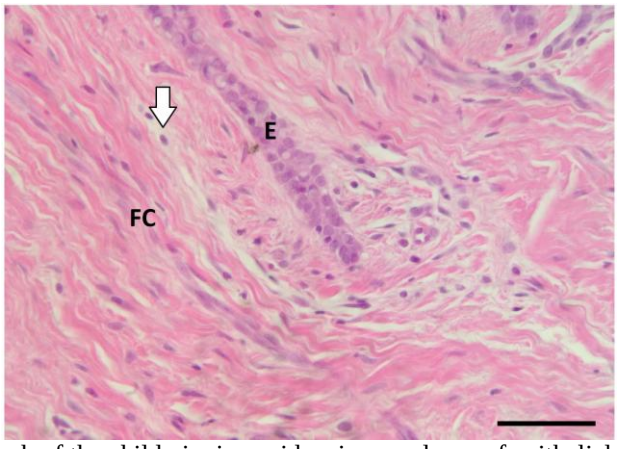

Figure 3 - Photomicrograph of the child gingiva evidencing a column of epithelial cells (E) in the unformed dense connective tissue with predominance for fibroblasts (white arrow) and collagen fibers (CF) present. Color: HE. Bar: 50 um (50x).

Mast cells are resident cells of fibrous connective tissue and participate in the immune system [13]. The lamina propria harbor several cellular elements necessary for host protection against invasion of bacteria and promote tissue regeneration and repair [22]. In the human gingiva, mast cells are numerous and ubiquitous, usually observed around blood vessels and associated with nerve fibers [29], being found both in the gingival tissue of the child and in the adult tissue (Fig 4). These cells exhibit various morphological types of cytoplasmic granules with characteristic subgranular content, varying in shape and density being observed in both normal and non-inflamed periodontals [8, 6].

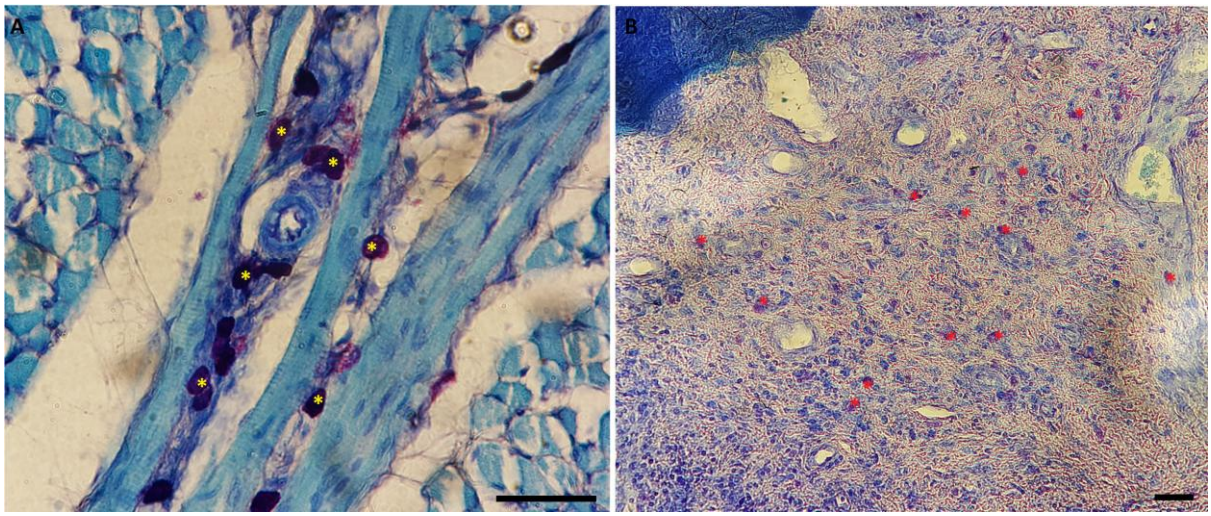

Figure 4 - Child and adult human gingival photomicrograph (A and B). (A) Presence of mast cells in muscle tissue (yellow asterisks). (B) Mast cells in the connective tissue (red asterisks). Color: Toluidine blue. Bar: 50 µm (50x) and 20 µm (20x).

The number of mast cells present in the gingiva with chronic periodontitis may be higher when compared to tissues with gingivitis and healthy tissues, indicating the importance of these cells in the defense mechanism [12]. In the healthy gingiva, no significant differences were observed in the amount of mast cells in the gingival tissue of the child and the adult. Sanché et al. (2011) performed a descriptive analysis of infiltrating inflammatory cells in the junctional epithelium and showed that 70% of the children's samples, as well as 60% of the adult samples, did not have these types of cells.

IV. CONCLUSION

Studies on the morphology of gingival tissues translate and elucidate physiological and pathological aspects that are of great importance for the construction of knowledge on the subject. Structurally, the gingiva of children and adults were similar in composition and cell types. The keratin thickness of the stratum corneum was lower in adults, which may be related to an easier installation of microorganisms and, consequently, of gingival and periodontal diseases.

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