ULTRASTRUCTURAL ANALYSIS OF GLANDS LOCATED IN THE WALL OF THE CONGENITAL FISTULAE OF THE LOWER LIP OF PATIENTS WITH VAN DER WOUDE SYNDROME

ANÁLISE ULTRA-ESTRUTURAL DAS GLÂNDULAS LOCALIZADAS NA PAREDE DA FÍSTULA CONGÊNITA DE LÁBIO INFERIOR DE PACIENTES COM A SÍNDROME DE VAN DER WOUDE

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The objective of the present study was to evaluate the glands of wall of congenital fistulae of the lower lip with the transmission electron microscope in order to characterize their microstructural pattern. Thin section of Araldite resin embedded congenital fistulae of the lower lip of four patients with Van der Woude syndrome from the Hospital de Reabilitação de Anomalias Craniofaciais da Universidade de São Paulo, Bauru, SP, were analyzed with a transmission electron microscope. The results showed that the glands were mostly made by typical mucous acini exhibiting, with certain frequency, myoepithelial cells surrounding them. In some of lobules, a few acini smaller than the typical mucous, showed granules of moderate electron density or containing a dense core or exhibiting small dense spherule and predominance granular material. These granules resemble to described recently by others in various human minor salivary glands. We concluded that glands associated with congenital fistula of lower lip of patients with Van der Woude syndrome, in spite of being located in vermilion border of the lip, showed at the transmission electron microscope characteristics of labial minor salivary gland, i.e, are mostly mucous with a few seromucous units, while typical seromucous demilunes are not present.

UNITERMS: Fistula, congenital; Lip diseases, congenital; Lip diseases, pathology; Van der Woude syndrome.

INTRODUCTION

In the Van der Woude syndrome, the cleft lip or palate and the lower lip fistulae occur simultaneously in the patient's mouth[1,11,25]. Its general frequency in the population is 1:75,000 – 1:100,000[3,17] and 1:200,000[14].

The importance of diagnosing the presence of lower lip congenital fistulae in cleft lip or palate patients or even in normal family members of these patients is that its presence grealy increases the probability of its carrier to have a cleft lip or palate child[11].

For that reason, it is important to know the most varied aspects of this lesion. The present paper shows the results from ultrastructural analysis performed in glands of wall of congenital fistulae of lower lip of four patients carrying the Van der Woude syndrome. This paper will complement other works about the same subject performed under a light and electron microscope in our laboratory[13,21].

MATERIAL AND METHODS

The four patients carrying Van der Woude syndrome and bilateral symmetrical fistulae with narrow and flattened openings located on the border of the lower lip vermilion and exhibiting an exaggerated prominence, admitted to the Hospital de Reabilitação de Anomalias Craniofaciais da Universidade de São Paulo – Bauru were submitted to lower lip cosmetic surgery under general anesthesia. The fistulae were removed through an elliptical cut applied horizontally in the lower lip, with the removal of the fistulo-glandular complex, following the other phases of the surgical procedure.

From the surgical piece, one of the fistulae was separated for processing directly to the ultrastructural analysis and the other, counterlateral, for the other parallel work already published[13].

Small fragments of proximal, median and fundic sites from each fistula were subjected to the following process: a) fixation in glutaraldehyde (LAAD Laboratories) at 2% in 0.09M, pH 7.3 phosphate buffer for 3 hours; b) post fixation at 1% osmium tetroxide plus 106mg of sacarose for 2 hours; c) in block contrasting with uranyl acetate at 5% plus 106mg of sacarose/ml for 18 hours; d) ethanol and propylene oxide dehydration and e) embedding in Araldite resin[8]. The total of eight Araldite-embedded blocks of the each site of each fistula were cut under a Porter – Blum MT2 ultramicrotome. Thick-sections of 0.5 mm were obtained from each block, placed on a glass slide and stained with methylene blue plus azure II. Examining the sections, the blocks containing glandular structures were selected for ultrastructural study. The thin (gray) sections were obtained and contrasted with uranyl acetate [28] and lead citrate[15]. The sections were examined and photographed under a Zeiss EM-9S and Philips EM-301 electron microscopes.

We inform that the present study was carried out according to norms for medical research established by the World Medical Association in the Helsinki Declaration in 1964.

RESULTS

The glands, which open mainly at the end of the fistula, were present in varying numbers for each analyzed case.

In the light microscope preliminary analysis the glands did not show distinct capsule, but presented lobular arrangement with the lobules varying in number and size between the fistulae.

By using light microscopy criteria, in the interior lobules of the four analyzed cases, were identified mucous acini and mixed acini showing seromucous demilunes, with apparent predominance of the mucous acini.

Under the electron microscope, the majority of the acini showed mucous characteristics. The mucous acini were formed by cells filled with electrolucent granules, showing wide intercellular spaces with a large amount of membrane interdigitations (Figure 1). Surrounding these acini, myoepithelial cells were frequently observed with cellular extensions filled with myofilaments (Figure 2). Typical mixed acini showing seromucous demilune were not observed .

However in some lobules, the presence of minor acini was observed with cells showing more electrodense granules, unlike typical mucous (I in Figure 3). In the same acinus, and/or in neighboring acini, cells exhibiting other forms of granules are seen. Thus , cell with dense cored secretory granules (II in Figure 3) and containing granules with small dense spherule and granular material (III in Figure 3) were detected.

DISCUSSION

Congenital fistulae of lower lip are malformations in the shape of a glove finger, their depth varying from 0.5 to 2.5 cm, with greater bi-lateral frequency in the vermilion border of lip of patients with Van der Woude syndrome[11].

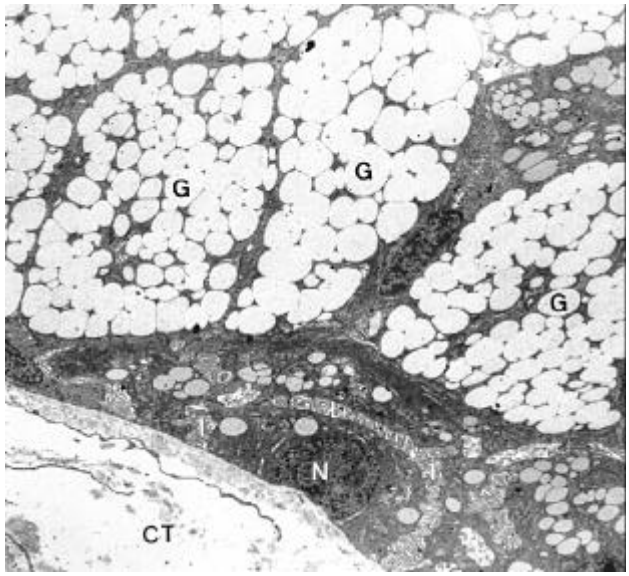

FIGURE 1- Mucous acinus. Observe: nucleus(N), mucous secretory granules(G), intercelular spaces with interdigitations(I) and interacinar connective tissue. x 7,000.

The histopathological studies under light microscope have shown that the fistula's wall is covered in all its depth, with few exceptions, by stratified nonkeratinized squamous epithelium[2,4,6,7,13,16,20,21,24,27].

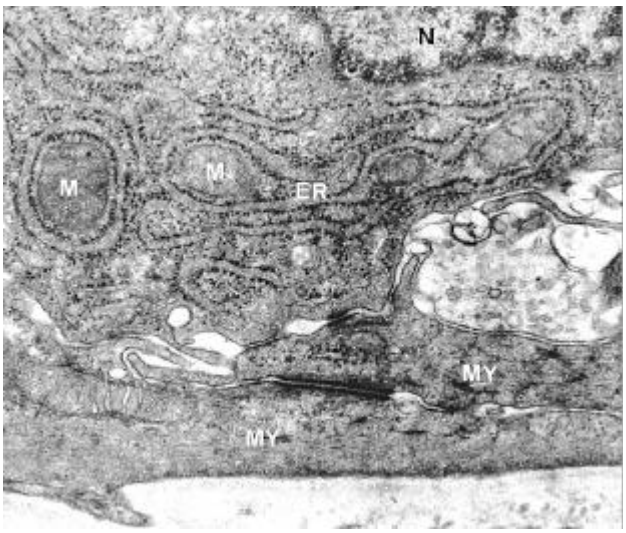

FIGURE 2- Base of mucous acinar cell and myoepithelial cell prolongation's. Observe: nucleus(N), endoplasmic reticulum(ER), mitochondria(M) and myoepithelial cell prolongations(My) exhibiting myofilaments. x 38,500.

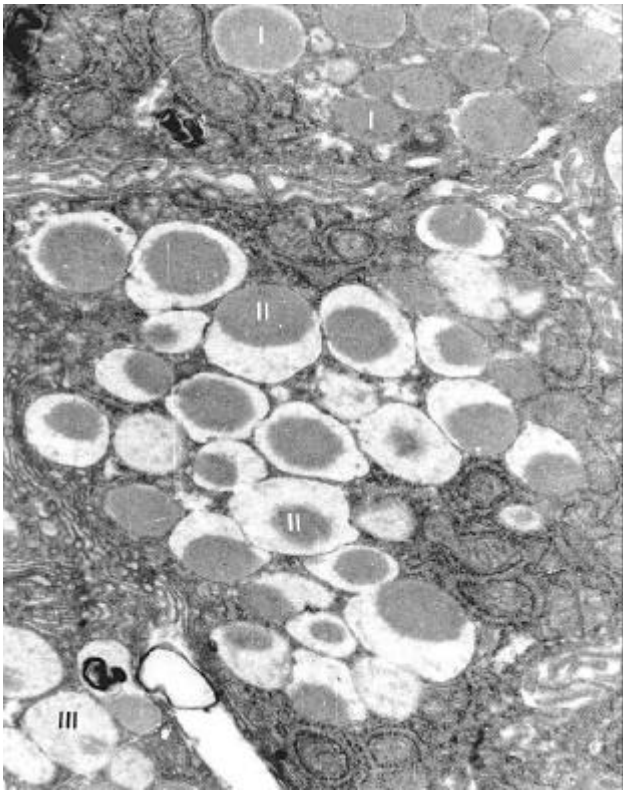

FIGURE 3- Acinus exhibiting cells with differents seromucous-like granules (I, II and III in the figure). x 38,500.

Regarding the glands which open through their excretory ducts mainly at the fundic site of the fistula, these exhibit, under light microscope, all the characteristics of the minor labial salivary glands, that is, mixed with the presence of mucous and mixed acini, containing seromucous demilune with predominance of the mucous[10,13].

In the present research, mixed typical acinus showing seromucous demilunes could not be detected under the electron microscope in the examined glands. The majority of the acini showed pure mucous characteristics. A few acini, smaller than the typical mucous, showed cells with granules of moderate electron density suggesting seromucous secretion (according to definition of Tandler and Phillips[23]). In the same acinus and/or in neighboring acini, we observed cells containing seromucous-like granules with dense core similar to described in cell located between mucous cells of the human posterior lingual glands-Weber's glands[18] and others cells exhibiting granules with small dense spherule and predominance granular material resembling to observed by same cited authors in the human anterior lingual glands.

Tandler, et al.[22] studying innumerous cases of labial salivary gland under the transmission electron microscope, verified that all acini, in spite of some showing characteristics of mixed acini under the light microscope, showed typical mucous ultrastructure. Histochemical studies using lectin reveal differences in carbohydrate composition between two types of secretory cells[26]. However, on these studies the authors suggested that in human labial glands, the images that appeared as seromucous cells represent solely maturative stages of an unique cell type, i.e. the mucous cell.

It should be pointed out that from the beginning, we considered the occurrence of this possibility in the our glands associated to lower lip fistulae, i.e., the few cells exhibiting differents seromucous-like granules could be mucous cells in distinct phases of their functional cycle.

Nevertheless in contrast with these reports, more recent studies using scanning and transmission electron microscope showed that human labial glands were mostly mucous, but there are a few mixed and seromucous units. The seromucous cells, of both mixed and seromucous units exhibited secretory granules that vary greatly in shape, density and substructures[18], some of them alike to that we observed in the fistula wall. In another study, Riva et al.[19] using osmium maceration method confirmed the presence of the typical seromucous cell in these glands. On the other hand, experiments of molecular biology using

in situ hybridization techniques proved that human labial gland have seromucous cells expressing gene for low molecular mucin, i.e., a substance characteristic of seromucous secretion[9].

Therefore, it was concluded that glands associated with congenital fistulae of the lower lip of patients with van der Woude syndorme, in spite of being located in vermilion border of the lip, a place where usually minor salivary glands not exist, show the characteristics under the transmission electron microscope of the minor salivary gland present in the lower lip mucosa, i.e., are mostly mucous with a few seromucous units, but seromucous demilunes are absent.

RESUMO

O objetivo do presente estudo foi avaliar a ultraestrutura de glândulas da parede de fístula congênita de lábio inferior ao microscópio eletrônico de transmissão para caracterizar seu padrão microestrutural. Deste modo, as fístulas congênitas de 4 pacientes com a síndrome de Van der Woude do Hospital de Reabilitação de Anomalias Craniofaciais da Universidade de São Paulo, Bauru, SP, foram processadas para inclusão em resina Araldite e os cortes finos foram analisados no microscópio eletrônico de transmissão. Os resultados mostraram que as glândulas estavam constituídas por ácinos mucosos típicos exibindo com certa freqüência células mioepiteliais ao seu redor. Em alguns lóbulos, foram observados em pequeno número, ácinos menores que o mucoso típico, exibindo células com grânulos de moderada eletron-densidade contendo um corpo denso ou uma pequena esférula densa no interior de um material predominante granular. Estes grânulos lembravam os descritos recentemente em glândulas salivares labiais humanas. Em vista dos resultados obtidos concluímos que as glândulas associadas com a fístula congênita de lábio inferior de pacientes com a síndrome de Van der Woude, apesar de estarem localizadas no vermelhão do lábio, mostraram ao microscópio eletrônico de transmissão características de glândula salivar labial, i.e., são predominantemente mucosos com poucas unidades seromucosas, mas semiluas seromucosas típicas não estão presentes.

UNITERMOS: Fístula, congênita; Doença labial, congênita; Doença labial, patologia; Sindrome de Van der Woude.

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