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Choanal atresia: Histochemical, immunohistochemical and ultrastructure study of the nasal mucosa

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ABSTRACT

Objectives: To study the nasal mucosal changes in cases with choanal atresia at the light and electron microscope and to compare the results with the normal side in unilateral cases.
Study design: Cross sectional study.
Setting: Tertiary University hospital, departments of Otolaryngology and pathology.
Methods: Sixteen patients diagnosed to have choanal atresia (seven bilateral and nine unilateral); ranging in age from 3 days to 9 years; were included in this study. During surgical repair, a biopsy of the inferior turbinate mucosa was taken. Biopsy from patent side in unilateral choanal atresia was also taken (as a control). Then biopsies were subjected to histopathological, histochemical, immunohistochemical and ultrastructure studies.
Results: The nasal mucosa in choanal atresia side (weather unilateral or bilateral) showed distorted cilia, marked increase of mucous submucosal glands associated with marked reduction of goblet cell density and lymphocytic cellular infiltration. The patent side (control) showed normal respiratory epithelium with obviously noted intra-epithelial goblet cells. Submucosal glands were less in number and activity than in the atretic side.
Conclusions: Choanal atresia showed a condition of the nasal mucous membrane with characteristic excessive nasal tenacious secretion; mostly actively secreted besides some effect of lack of drainage due to interrupted cilia. Further studies are required to evaluate the impact of atresia repair on detected features.

1. Introduction

Although congenital choanal atresia and stenosis are the most common craniofacial anomalies of the nose, they have a rare incidence of approximately in 7000 births. It is defined as a failure in the development of communication between the nasal cavity and the nasopharynx causing deprivation of nasal airflow and mucus transport [1,2] and one of the features of choanal atresia is accumulated tenacious mucous secretion.

The mucous membrane changes in unilateral choanal atresia is not fully understood, and it is not known whether this mucus is actively secreted due to increased mucus producing elements in the mucosa or just due to simple accumulation, also, it is not known whether this is a feature in bilateral choanal atresia as well or not.

The aim of this work is to study nasal mucosal changes in cases with choanal atresia at the light and electron microscope and to compare the results with the normal side in unilateral cases.

2. Patients and methods

This study was carried out at Otorhinolaryngology–Head and Neck Surgery and Pathology Departments, Faculty of Medicine, Zagazig University in the period between March 2011 and July 2014 on patients diagnosed to have choanal atresia. Patients had syndromic choanal atresia or operated before were excluded from the study.

All patients were subjected to full history taking, general and local examination and routine preoperative laboratory tests. A written informed consent to participate in the study was signed by the relatives of the patients and the Zagazig University IRB approved this study.

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During surgical repair that performed under general anesthesia, a biopsy of the inferior turbinate mucosa (0.5 cm behind its anterior end) was taken. Biopsy of the inferior turbinate mucosa of patent side in unilateral choanal atresi was also taken. Then biopsies were subjected to histopathological, histochemical, immunohistochemical and ultrastructure studies.

2.1. Paraffin blocks preparation

All biopsy specimens were fixed in neutral buffered formalin 10% for 24 h; washed with water and serial dilutions of ethyl alcohol, and embedded in paraffin.

2.2. Hematoxylin and eosin stain

Paraffin tissue blocks were sectioned at 4 μm thicknesses; depraffinized in xylene; rehydrated in descending alcohols; and stained with routine hematoxylin and eosin.

Periodic acid–Schiff stain (PAS) and Alcian Blue (PH = 2.5)/PAS: as described by Bancoft and Gamble [3].

2.3. Results and interpretation

(1) For PAS neutral mucin appears as magenta colored.
(2) For Alcian Blue/PAS stain: acidic mucin appears blue colored, and mixed mucins appear purple blue in color.
(3) Assessment for mucin was done by the two pathologists and expressed as follow: for each stain mucin content was interpreted as absent (−), mild (+), moderate (++), and marked (+++).

2.4. Immunohistochemical analysis

Primary mouse monoclonal antibody (MAb) [anti-CD45 antibody, clone 2B11 + PD7/26 (Dakocytomation, Carpinteria, CA) at a dilution "dil" of 1:20, and anti-NSE antibody, clone BBS/NC/VI-H14, Dakocytomation, Carpinteria, CA) were applied according to Boenisch [4].

All series included positive controls (sections from mouse spleen stained for CD45, and pancreas and brain tissue sections stained for NSE). Negative controls included substitution of the 1ry MAb with non-immune normal mouse sera of same concentration.

2.5. Electron microscopic analysis

From four patients (two had bilateral and two had unilateral choanal atresia), six samples were obtained (four from atresia side; one sample from each case and two from patent side of unilateral cases). The tissue specimens were immersed in a solution of 3% phosphate-buffered glutaraldehyde. After a brief washing procedure in buffer and dehydration, the specimens were incubated in unicryl overnight at 4 °C. After polymerization, semi-thin cuts and staining with toluidine blue for selection of appropriate tissue areas for the electron microscopy. Finally, serial ultrathin sections (70 nm) were cut (Ultramicrotome RMC 6000 XL) and then placed on nickel grids. After double-contrasting with uranyl acetate and lead citrate, ultrastructures were photo-documented with a transmission electron microscope (EM/JEOL 1200 EXII). Special attention was paid to changes of epithelium, seromucous glands, and the vascular system.

Table 1
Patient's data.

Number of patients	16
Gender	
Male	7 (53.6%)
Female	9 (46.4%)
Age at time of surgery and biopsy	
Range	3 days to 9 years
Mean (SD) unilateral	8.7 (2.45) years
Mean (SD) bilateral	6.86 (4) years
Side	
Unilateral	9 (53.6%) – 18 atresia sides
Bilateral	7 (46.4%) – 7 atresia sides
Nature of atresia	
Mixed	11 (68.75%)
Pure bony	5 (31.25%)

3. Results

A total of 16 patients diagnosed to have choanal atresia (seven patients had bilateral choanal atresia, and nine patients had unilateral choanal atresia); ranging in age from 3 days to 9 years; were included in this study (Table 1). All patients had thick tenacious mucous secretions, at the time of surgery, in closed nasal cavity by choanal atresia.

Histopathological study of the 23 biopsies of the mucosa of choanal atresia sides (seven bilateral and nine unilateral) revealed that the covering epithelium was pseudostratified columnar ciliated epithelium with focal mucosal atrophy and ulceration and with intact thickened basement membrane at the choanal atresia side with focal corrugation. Very few goblet cells (0–2 cells per high power field) were detected in the mucosa by light microscope (Fig. 1) with significant difference from normal side (t test = 55.9, P-value < 0.0001). The submucosa showed diffuse gland hyperplasia in most parts and moderately increased lymphocytic infiltrate in the submucosa (Figs. 1 and 2) (Table 2).

Histochemical study of the mucosa of atresia sides revealed that submucosal glands were mainly of the mucous type, highly active

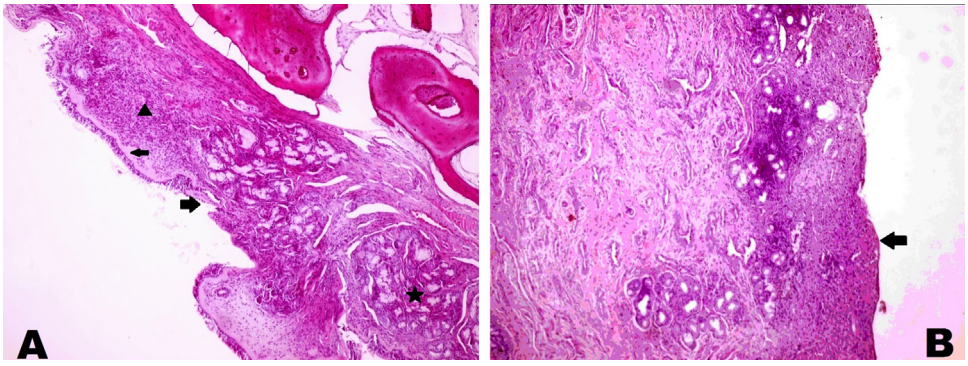

Fig. 1. (A, B) Choanal atresia at the atretic site showing mucosal ulceration (bold arrow) with hyperplastic submucous glands (star) and dense aggregates of lymphocytes (arrow head) with thickened basement membrane (thin arrow), H&E ×100.