International Journal of Pediatric Otorhinolaryngology 86 (2016) 213–217

Contents lists available at ScienceDirect

International Journal of Pediatric Otorhinolaryngology

journal homepage: www.elsevier.com/locate/ijporl

Histological analysis of retraction pocket pars tensa of tympanic membrane in children

M. Urík [a,*], P. Hurník [b,c,d], D. Žiak [b,d], J. Machač [a], I. Šlapák [a], O. Motyka [e], J. Vaculová [b,c], J. Dvořáčková [b,c,d]

[a] Department of Paediatric Otorhinolaryngology, Faculty of Medicine, Masaryk University and University Hospital Brno, Czech Republic
[b] Department of Pathology, University Hospital Ostrava, Czech Republic
[c] Department of Pathology, Faculty of Medicine, University of Ostrava, Czech Republic
[d] CGB laboratory, Ostrava, Vítkovice, Czech Republic
[e] Nanotechnology centre, VŠB-Technical University of Ostrava, Czech Republic

ARTICLE INFO

Article history:
Received 1 April 2016
Received in revised form 1 May 2016
Accepted 5 May 2016
Available online 12 May 2016

Keywords:
Retraction pockets
Histological analysis
Cholesteatoma
Children

ABSTRACT

Aims: Histological and histochemical analysis of retraction pocket of pars tensa of tympanic membrane in children. Identification of morphological abnormalities in comparison with a healthy tympanic membrane as it is described in standard textbook. Identification of signs typical for cholesteatoma and support for a retraction theory of cholesteatoma formation.
Study design: A prospective study analysing 31 samples of retraction pockets taken during surgery.
Departments: University Hospital, Children's Medical Centre
Methods: Samples of retraction pockets were processed by a standard process for light microscopy, stained by haematoxylin-eosin. Van Gieson's stain was used for differential staining of collagen, Verhoeff's stain for elastic fibre tissues, Alcian blue for acidic polysaccharides and PAS (Periodic Acid Schiff) method for basement membrane polysaccharides.
Results: The following findings were observed in the samples of retraction pockets: hyperkeratosis (100%), hypervascularisations (100%), subepithelial fragmented elastic fibres (96%), myxoid changes (87%), subepithelial inflammatory infiltration (84%), rete pegs (71%), papilomatosis (71%), intraepithelial inflammatory cellularizations, (48%), intraepithelial spongiosis (16%) and parakeratosis (3%). No basement membrane continuity interruptions were observed. Thickness of retraction pocket, thickness of epidermis, occurrence of rete pegs and frequency of fragmented elastic fibres was higher in a Grade III stage RP than Grade II stage RP (according to Charachon).
Conclusion: Morphological abnormalities in the structure of retraction pockets in comparison with a healthy tympanic membrane were described. The changes are typical for a structure of cholesteatoma (these changes are common in matrix and perimatrix), supporting retraction theory of its origin. Our observations show that it is inflammation that probably plays a key role in the pathogenesis of retraction pocket. The frequency of some of the changes increases with the stage of retraction pocket (II–III according to Charachon). Basement membrane continuity interruptions are not typical for retraction pockets.

1. Introduction

Tympanic membrane retraction pocket (RP) is a localised area of tympanic membrane retracted into tympanic cavity. Unlike a healthy tympanic membrane, which is described as a relatively tough, elastic membrane without a tendency to collapse, RP is flexible and tends to collapse into the tympanic cavity. RP is characterised by atelectasis of a part of tympanic membrane which is retracted into the area of bony anatomical structures, such as incisura Rivini, scutum or malleus [1]. RP can be located in all quadrants of pars tensa of tympanic membrane; pars flaccida can also be affected. Both parts can be affected simultaneously. RP is a result of a long-term or relapsing negative middle ear pressure which is caused mainly by a dysfunction of Eustachian tube [1–8]. On the other hand, there are theories and hypotheses claiming that pathogenesis of RP includes the presence of mesenchyme (as a residue of intrauterine development) or an inflammatory reaction that followed otitis media, mainly in a posterosuperior quadrant of pars tensa and in the area

Declaration: This article did not receive sponsorships and there are no competing interests.
Supported by a project: MUNI/A/1266/2015.
* Corresponding author at: Department of Paediatric Otorhinolaryngology, Faculty of Medicine, Masaryk University and Faculty Hospital, Černopolní 9, 61300 Brno, Czech Republic. Tel.: +420532234225; fax: +420532234440.
E-mail address: docttor.urik@gmail.com (M. Urík).

http://dx.doi.org/10.1016/j.ijporl.2016.05.007
0165-5876/

of pars flaccida, the two places typical for RP and atelectasis. Some authors claim that without inflammation, retraction does not occur [9,10]. These two areas are generally very fragile parts of the eardrum and therefore tend to retract in the presence of negative middle ear pressure.

Some histological studies describe interruptions of basement membrane continuity in cholesteatoma, supporting retraction theory of cholesteatoma, and only one paper (in the last 5 years by PubMed) described the histological structure of RP or signs leading to formation of cholesteatoma [11]. Sadé studied ear drums with atelectasis and observed degeneration of middle collagenous fibrous layer of ear drum caused, in his opinion, by an inflammatory process in this area [7,9]. In their works, Sudhoff and Toss describe disruptions of basement membrane of cholesteatoma. These disruptions of basement membrane are localised in areas of growing rete pegs and subepithelial inflammatory infiltration focuses [12,13]. Based on these and other observations the authors support retraction theory of cholesteatoma based on the mechanism of tympanic membrane invagination followed by epithelial proliferation resulting in hyperplasia, impairment of cell migration and production and accumulation of keratin, all these being the basis for cholesteatoma [12,13]. Their observations dealt only with cholesteatoma and up till now only a few works have studied retraction pockets as a possible pre-stage of cholesteatoma.

Our study presents a histological and histochemical analysis of retraction pocket of pars tensa of tympanic membrane (RP) in children. It describes morphological abnormalities of RP in comparison with a structure of a healthy ear drum and signs typical for cholesteatoma. Also, it aims to prove or disprove hypothesis that RP involves disruptions of basement membrane.

2. Methods

We performed a prospective study during which 31 RPs of pars tensa of tympanic membrane were taken during standard planned surgeries in children. These were RPs grade II and III according to Charachon, with no signs of progression to cholesteatoma. The indications for surgery were: hypoacusis related to RP, recurring infections or otomicroscopically observed for disease progression. The criteria for exclusion included RPs with clinical signs of cholesteatoma which could not be totally extracted. In all cases, after RP excision, tympanic membrane was reconstructed using chondroperichondrial graft from tragus.

All patients underwent pre-operative assessment including history, otomicroscopy examination, tympanometry and pure tone audiometry.

RPs were divided into two groups according to Charachon classification (II – RP controllable, fixed, III – RP uncontrollable, fixed). RPs stage I (controllable, not fixed) are not operated [5].

After being taken, all samples were oriented and put on a stripe of a foam fixing material and put into a histologic chamber so that the outer (ear canal) part was upwards and the inner (middle ear) part downwards in the block. The samples were then placed into 4% buffered formalin. After fixation (24–48 hours) the samples were cut into max. 5 mm wide blocks. Fragments were divided into halves and positioned on the cutting line. All tissue blocks were then dehydrated in a Tissue – Tek® VIP® 6 Vacuum Infiltration Processor (Sakura). After being placed into paraffin wax, 2–4 μm thin sections were prepared. All sections were stained with a basic histological dye – haematoxylin and eosin. Van Giesson's stain was used for differential staining of collagen, Verhoeff's stain for elastic fibre tissues, Alcian blue for acidic polysaccharides and PAS (Periodic Acid Schiff) method for basement membrane polysaccharides. The samples were processed into 31 paraffin blocks and preparations stained with H-E and 31 preparations stained with Alcian blue, PAS, Van Giesson and Verhoeff methods. With an optical microscope Olympus BX 45 with magnification 100× and 200×, HPF 400× was used for the assessment. Two pathologists cooperated to assess the histological preparations. Epithelium width was measured at ten different places, then an arithmetic mean was calculated.

3. Results

The mean age of patients with RP was 10 years and we operated patients from 6 to 18 years old.

The average sample length was 4955.4 μm. A bigger length of grade III RPs was observed compared to grade II ($p = 0.01293$, two sample T-test), which correlates with otomicroscopic findings. The average sample thickness was 333.5 μm. With two extreme values excluded, the grade III thickness is bigger than in grade II group ($p = 0.001005$, two sample T-test).

3.1. Epidermis: outer epithelial layer

Outer epithelial layer of tympanic membrane composing of keratinized stratified squamous epithelium was present in 31 samples. The average thickness of epidermis was 46.3 μm. A difference in thickness of epidermis was observed between grade II and III, the average thickness of epidermis in grade II was 33.4 μm, and in grade III it was 54.4 μm, which is a statistically significant ($p = 0.001469$, two sample T-test). All 31 RP samples showed increased keratinization (hyperkeratinization) of the outer layer of tympanic membrane (Figs. 1 and 2). Two types of keratinization were observed – lamellar (Fig. 1) and basket-weave (Fig. 2). Lamellar hyperkeratosis was observed in all samples; 26 samples showed signs of basket-weave hyperkeratosis. Parakeratosis (nuclei in keratinized layer, indicates increased cell turnover) was identified in only one case. Papilomatosis was observed in 71% of all samples.

3.2. Epidermis: Inner basement layer

In 22 cases we observed extensions of stratified squamous epithelium (epidermis) in a form of rete ridges – rete pegs (Fig. 3) – rete pegs projected into the middle layer of tympanic membrane. These epithelial extensions were more frequent in RPs grade III (18 out of 18 patients) compared to grade II (4 out of 13 patients) ($p = 3.547e\text{-}05$, Fischer exact test).

Table 1 provides detailed descriptions of the observed abnormalities of epidermis depending on the grade of RP.

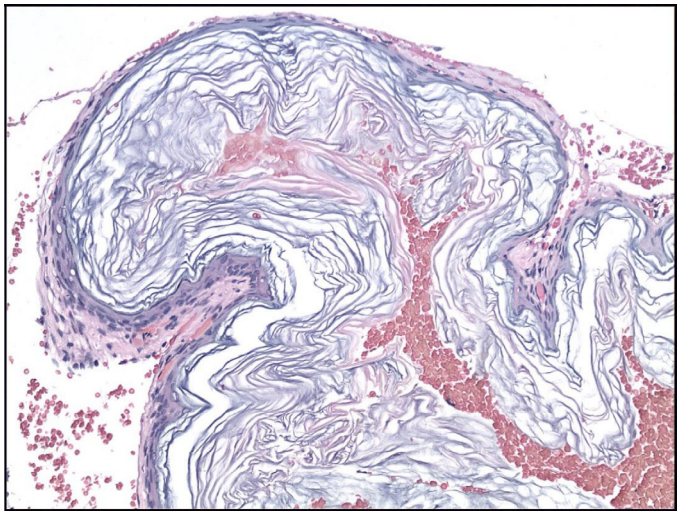

Fig. 1. Basket-weave hyperkeratosis (HE, 200×).