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ORIGINAL ARTICLE

Is there Epithelial Tissue in Bone Pate? A Histopathology Study

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Objectives: To evaluate the histology of bone pate collected during mastoidectomy in chronic suppurative otitis media (CSOM) patients with or without cholesteatoma.

Study Design: Prospective controlled study.

Materials and Methods: The study recruited 32 patients (15 males, 17 females; average age 25.3 (range 19–56 years) who underwent mastoidectomy for CSOM: 16 with cholesteatomas (group I) and 16 controls with granulation or polyp tissue (group II). In all patients, bone pate was collected separately from the mastoid cortex (Level 1), subcortical air cells (Level 2), and just before the antrum mastoideum (Level 3). All samples were stained with hematoxylin-eosin (H&E) and immunochemically with a monoclonal antibody against epithelial membrane antigen (EMA). The presence of squamous epithelium in the bone pate was examined under a light microscope.

Results: In Group I, we detected epithelial cells in the bone pate samples obtained from the mastoid subcortex (two of 16 patients) and antrum (four of 16 patients) levels. However, no epithelial cells were observed in bone pate from the mastoid cortex. In the controls (group II), no epithelial tissue was observed in any sample from any level.

Conclusions: Epithelial cells can inoculate bone pate collected from the subcortical and antral areas of the mastoid bone in patients with cholesteatomatous CSOM. To prevent epithelial cell inoculation, we recommended that bone pate be collected only from the cortical bone of the mastoid.

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Introduction

Temporal bone surgery requires the drilling of bone, producing large quantities of bone dust. This dust is called bone pate and has been used as graft material for many different purposes in various ear surgery techniques. Gersdorff and Robillard first described the use of autogenous bone pate as reconstruction material in ear surgery [1]. Many otologists use bone pate to fill mastoid defects and reconstruct the posterior canal wall in chronic otitis media surgery [2-5].

A cholesteatoma is a destructive lesion containing layers of keratin in a cavity lined by squamous epithelium that often requires complex ear surgery. During cholesteatoma surgery, the surgeon may need to collect bone pate as a reconstruction material. The bone pate should be collected from healthy bone in chronic suppurative otitis media (CSOM) patients with cholesteatomas. However, the criteria for defining healthy bone and collecting and using bone pate in these patients are not clear. We suggest that the reconstructed area may at risk of inoculation with squamous epithelium inside the bone pate.

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Many studies have reported the long-term anatomical and functional results of reconstruction with bone pate[2-4]. To our knowledge, however, no study has examined the histology of this autogenous material. Therefore, this study evaluated the histology of bone pate collected from different levels of diseased temporal bones.

Material and Methods

The study was approved by the local research and ethics committee of the Medical School, Dicle University (20.10.2011/225). Patients' written informed consents were obtained.

The study recruited 32 patients (15 males, 17 females; average age 25.3 (range 19–56 years) who underwent mastoidectomy for CSOM: 16 had cholesteatomas (Group I), and 16 controls had granulation or polyp tissue (Group II).

Surgical Procedure

All of the patients were operated under general anesthesia. Once the patient was prepped and draped, 2% lidocaine with 1.25:100,000 dilution epinephrine (Jetocaine amp, Adeka, Turkey) was injected to postauricular region for local vasoconstriction. A postauricular incision was made about 1 cm behind the postauricular crease and a plane developed between the subcutaneous tissue and temporalis muscle and periosteum of the mastoid. Monopolar cautery was used to bleeding control. A periosteal incision was made down through the periosteum to the temporal bone. The periosteum was elevated until the mastoid cortex was exposed. The surgical field was washed with saline to prevent squamous epithelium inoculation from skin. A large cutting burr was used to make the first cuts in the mastoid cortex. Bone pate was collected at this level using a large curette (Level 1). The surgical field was washed with saline after the finishing cortical mastoidectomy. The bone was dissected through the antral cell using standard mastoidectomy procedures. Bone pate was collected during dissection of the subcortex and mastoid air cells (Level 2). The surgical field was washed with saline again and bone pate was collected again once the antral air cell was identified (Level 3). Then, the ear surgery appropriate to the pathology was performed. In each patient, chronic otitis media with or without cholesteatoma was confirmed by final specimen histopathologic result.

Histopathologic examination

Three different bone pate specimens were collected from each patient in separately numbered tubes and fixed with 10% formalin. The tubes were centrifuged for 5 min at 4000 rpm. Then, the samples were embedded in paraffin. The tissue blocks were 1-2 mm in thick. All blocks were cut in 5-□m-thick sections with a microtome. The sections were stained with hematoxylin-eosin (H&E) and a monoclonal antibody against epithelial membrane antigen (EMA). All blocks were cut and stained until the entire bone-pate sample was examined. The presence of squamous epithelium in the bone pate was examined under a light microscope (Nikon, Eclipse, 80i, Japan) by the same pathologist, who was blind to the study groups. In our study, the proof of cholesteatoma in the bone-pate mixture was defined as presence of any epithelial cell or fragments of cholesteatoma. The histologic appearance of granulation tissue is characterized by the proliferation of blood vessels and fibroblasts. The polyp tissue is a fibrous type of scar tissue composed of dense collagen and inactive-appearing fibroblasts with few vessels. However these structures can be seen in bone tissue. Because of that, in our study there is no any clear proof for presence of granulation tissue or polyp in the bone pate mixture.

The squamous epithelial cell/fragment counting was done as follows :

On microscopic examination, for all cases, it was defined as positive inoculation if there is any EMA (Epithelial Membrane Antigen) -positive cells in the sections from paraffin blocks of bone pate mixture. In the hematoxylin-eosin (H&E) stained sections, the presence of any squamous epithelial cell was considered positive whereas the absence of any epithelial cell was considered as negative. All blocks were scored as containing or not containing squamous epithelium in all sections at magnifications of ×100 and ×200.

Results

The presence of squamous epithelium in the bone pate is summarized for both groups in Table 1. In group I, no epithelial cells were observed in any level 1 sample

(Figure 1). Epithelial cells were observed in level 2 samples from two patients (Figure 2) and epithelial cell groups and epithelial tissue fragments were observed in level 3 samples from four patients (Figure 3). We did not observe any epithelial tissue in any sample from the control patients (Group II).

Table 1. The presence of squamous epithelium inside bone pate collected at different levels. Group I, chronic suppurative otitis media patients with cholesteatoma; group II, control; level 1, mastoid cortex; level 2, subcortical air cells; level 3, antrum mastoideum.

Level	Group I	Group II
1	0/16	0/16
2	2/16	0/16
3	4/16	0/16

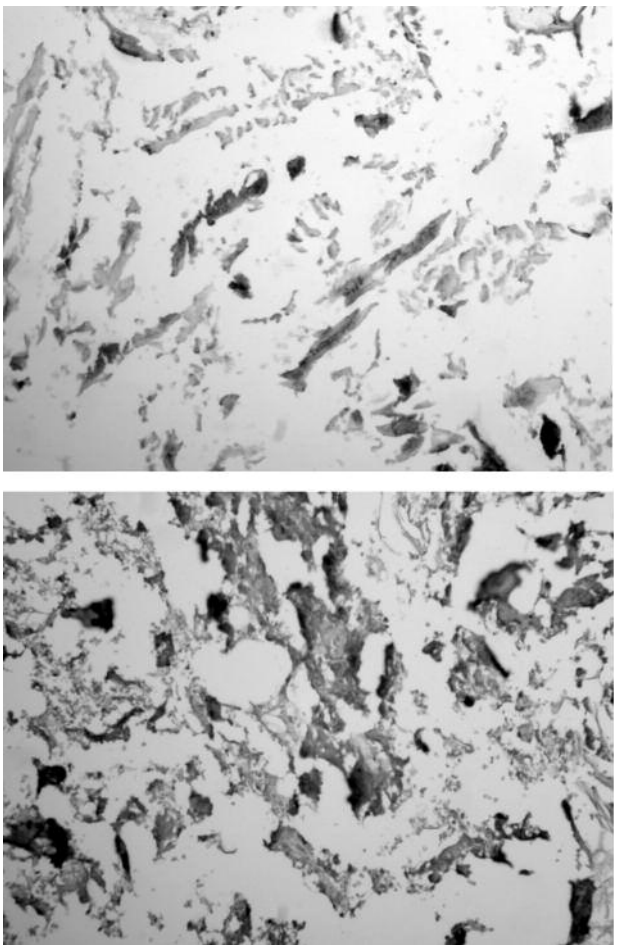

Figure 1. The study group (Level 1): a) bone dust and spicules containing no epithelial cells (H&E stain, 100); b) no epithelial cells or EMA is seen (Immunoperoxidase, 200)

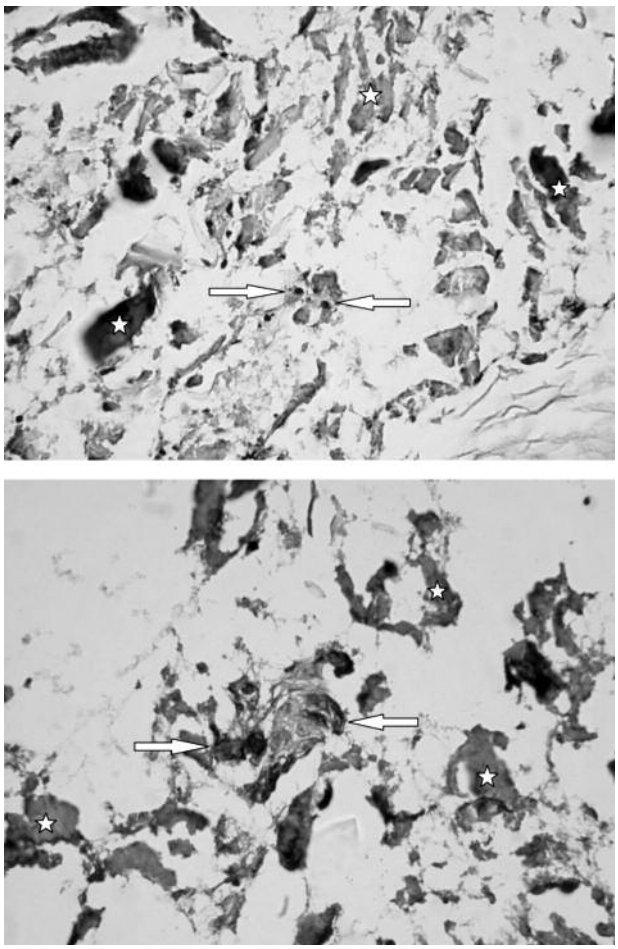

Figure 2. Study group (Level 2): a) epithelial cells among bone spicules (H&E stain, 200); b) EMA-positive cells among bone spicules (Immunoperoxidase, 200)

Discussion

Cholesteatomas can be classified into two broad classes: congenital and acquired. The etiopathogenesis of acquired cholesteatoma is controversial. There are several hypotheses regarding the mechanism of cholesteatoma development [6,7]. One is the implantation theory, which proposes that squamous epithelium becomes implanted in the middle ear as a result of surgery, a foreign body (ventilating tubes), or blast injury. Wullstein and Wullstein [8] reported that some cases of acquired cholesteatoma follow surgery for noncholesteatomatous conditions (*e.g.*, myringoplasty) or temporal bone injury. There are case reports of epithelial cyst formation on the same side as otological surgery [9]. McKennan and Chole [10] presented three cases of post-traumatic cholesteatomas of which one case had undergone previous otological

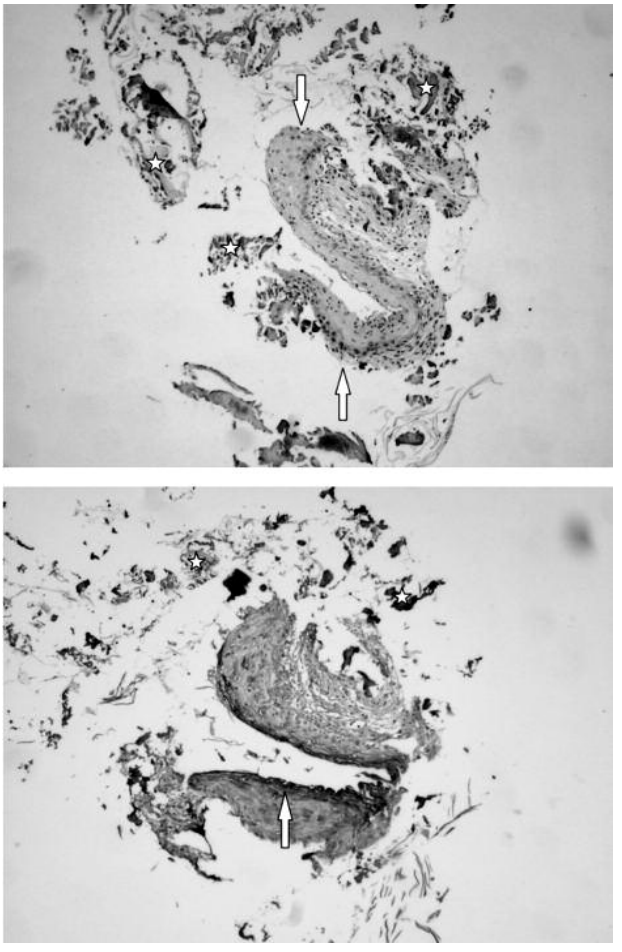

Figure 3. Study group (Level 3): a) epithelial tissue fragment among bone spicules (H&E stain, 100); b) EMA-positive epithelial tissue among bone spicules (Immunoperoxidase, 100) (arrows, epithelial cells; stars, bone dust and spicules)

surgery. These reports postulated that the cholesteatoma arose from the implantation of epidermal elements in the middle ear at the time of the prior surgery. In an animal study, Magalhaes *et al*.[11] demonstrated that an epidermal cyst (cholesteatoma) can grow after the implantation of the auricular skin of a mouse next to its femur. Epithelial tissue implantation with bone pate could account for this type of acquired cholesteatoma.

The ideal material for mastoid obliteration, scutumplasty, or posterior canal wall reconstruction should be easy to handle, ready available, stable over an extended period, tolerated, and inexpensive and should resist resorption and retraction. Thus, bone pate is a valid material for these kinds of reconstruction. It has a wide range of uses in various surgical procedures. Its effectiveness in middle ear surgery for repairing labyrinthine capsule defects and in skull surgery for filling calvarial defects has been reported[5,12]. Bacciu *et al*.[13] reported good long-term anatomical and functional results for scutumplasty with bone pate in patients with cholesteatomas. They also examined the histopathology of the bone pate, biopsying areas during the second-look operation where bone pate had been initially placed, and found well-structured bony tissue with signs of active tissue remodeling. They reported that the rate of residual cholesteatomas at the second-look surgery 12 months after the first surgery was 13.4%, and recurrence was seen at follow-up in 5.2% of the operated ears. However, it is difficult to determine whether the recurrence occurs due to the inoculation of squamous epithelium inside the bone pate.

In the mastoidectomy procedure, the mastoid cortex, mastoid cells, and antrum mastoideum are drilled together with diseased soft tissues. Consequently, squamous cells in the choleste7atoma or external auditory canal skin can be inoculated into the reconstructed area with bone pate. Subsequently, these epithelial cells may act like a cholesteatoma and continue to grow in the new location, producing keratin and forming a cyst. We did not observe any epihtelial tissue in any sample from the group II patients. These patients had non-cholesteatomatous CSOM which confirmed by final histopathologic result. Our study demonstrated that bone pate can be harvested from all levels of the mastoid bone in non-cholesteatomatous CSOM patients. In patients with a cholesteatoma, however, the mastoid cortex was the only safe area for collecting bone pate. The subcortical air cell and mastoid antrum carry a high risk of epithelial cell inoculation in these patients. Yanagihara *et al*.[14] described a mastoid cortex plasty using bone pate collected only from the mastoid cortex. They did not observe any recurrence. Althaus[5] harvested bone pate from the mastoid cortex near the tip of mastoid. Moffat *et al*.[3] also collected bone pate from the mastoid cortex. Our study provides histological support for this technique for collecting bone pate. Additionally, we suggest that the surgical field must be irrigated with saline to eliminate bone particles and epithelial remnants.

Lee *et al*.[15] successfully grew cholesteatoma in cell cultures. However, can the cholesteatoma fragments in the bone pate mixture grow? Further studies with bone pate are needed to answer this question.

Conclusions

Epithelial cells may be inoculated into the bone pate mixture. Therefore, bone pate may be a factor in the etiology of squamous epithelium implantation. We recommend that bone pate be collected only from the mastoid cortex in cholesteatomatous CSOM patients. The use of bone pate collected from the subcortical and antral levels in patients with cholesteatoma bears a risk of squamous epithelium implantation in the recipient area.

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