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MAIN ARTICLE

Mycological study on cholesteatoma keratin obtained during primary mastoid surgery

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Abstract

Objective: Established middle-ear cleft cholesteatoma is associated with keratinous debris, which is likely to be an ideal medium for saprophytic fungal colonisation. This prospective case study aimed to explore the incidence and nature of fungal elements in cholesteatoma keratin samples obtained during primary mastoid surgery.

Methods: All cases of middle-ear cleft cholesteatoma treated with primary mastoid surgery at the El-Sahel Teaching Hospital over a seven-month period were included. Keratinous debris obtained from the mastoid antrum was subjected to mycological analysis at the Department of Medical Microbiology and Immunology, Faculty of Medicine, Cairo University. A literature search was performed to determine the clinical and pathological relevance of fungal colonisation in cholesteatoma.

Results: Eighteen patients underwent primary mastoid surgery for cholesteatoma (nineteen ears in total) in a seven-month period starting 30 March 2013. Patients included 13 males and 5 females, with an age range of 9 to 45 years (mean 23 years). Fungal cultures were obtained from 17 keratin samples (89 per cent). Of these, five fungal isolates belonged to the dermatophyte group (21 per cent).

Conclusion: Fungal colonisation in middle-ear cleft cholesteatoma probably plays a significant role in disease progression. Moreover, saprophytic fungal colonisation in cholesteatoma keratin may be responsible for the fetor commonly associated with the ear discharge.

Key words: Cholesteatoma; Microbiology; Fungi

Introduction

Fungi are a diverse group of saprophytic (i.e. derive nourishment from dead organic matter) and parasitic eukaryotic organisms. Fungal pathogenicity is determined by the host's immune response and virulence factors specific to the fungus. Fungal features that are not present in other microorganisms are a rigid cell wall and the formation of large hyphae, which are frequently present in clinical specimens. Both features enable fungi to resist the host's defence mechanisms. In normal hosts, fungal isolation commonly reflects colonisation rather than infection. However, in patients with an abnormal immune response, a wide spectrum of fungal-related diseases can develop.[1]

Fungal infection of the ear is most commonly restricted to the external auditory canal.[2] Middle-ear cleft cholesteatoma is a gradually expanding, destructive epithelial lesion. Established cholesteatoma contains keratinous debris, which is probably an ideal medium for saprophytic fungal colonisation.[3]

In this prospective study, we performed a fungal analysis of keratinous debris samples obtained during primary mastoid surgery from patients with cholesteatoma presenting to El-Sahel Teaching Hospital, Cairo. We determined the frequency and nature of fungal elements associated with cholesteatoma. A PubMed and Medline search from 1980 until 2013 was performed using the search terms 'cholesteatoma' and 'fungi'. The literature was reviewed to determine the clinical and pathological relevance of fungal colonisation in middle-ear cleft cholesteatoma. As far as we are aware, this is the first prospective mycological study to be specifically performed on cholesteatoma keratin samples obtained during primary mastoid surgery.

Materials and methods

Approval from the local ethics committee and informed consent from the patients or guardians of children participating in the study were obtained. Patients with cholesteatoma who presented consecutively during the 7 months from 30 March 2013 onwards and were operated on at the El-Sahel Teaching Hospital in Cairo were included.

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Cholesteatoma was clinically diagnosed by otomicroscopy in patients with chronic otitis media. Microsuction of keratin flakes from the tympanic membrane defect or retraction pocket confirmed the diagnosis. Patients presenting with occluding aural polyps had the polyps excised under general anaesthesia, and keratin was detected in the tympanic membrane defect. Pure tone audiometry was performed before and after mastoid surgery; however, audiometric data will not be reported in this work. All patients diagnosed with cholesteatoma underwent computed tomography imaging of the temporal bone to determine disease extent and the associated bone destruction. All patients with cholesteatoma encountered during the study period had advanced disease, requiring canal wall down mastoidectomy for extirpation of the disease. No cases of congenital cholesteatoma were encountered. Only patients that underwent primary mastoid surgery were included in the study.

During the procedure, keratinous debris was removed from the mastoid antrum using sterile microcup forceps, placed in a sterile container and transported promptly to the Medical Microbiology and Immunology Department in the Faculty of Medicine, Cairo University. Initially, a portion of each specimen was examined under high power microscopy after treatment with 10 per cent potassium hydroxide to degrade the proteinaceous components. This enabled the early detection of any fungal elements present in the specimen. The remainder of the specimen was cultured on Sabouraud's dextrose agar medium at 25°C for up to 21 days for fungal growth assessment. Fungal identification was based on colony morphology and pigmentation. Microscopic identification of fungi was performed after lactophenol cotton blue staining. If yeasts were encountered, the germ tube test was used to assay for *Candida albicans*.

Results

Clinical data for patients in the study group and the results of the mycological analysis are shown in Table I. There were 18 patients with middle-ear cleft cholesteatoma (19 ears were operated on): 13 males and 5 females. Patient age ranged from 9 to 45 years (mean 23 years). The right ear was affected in 10 patients, the left ear in 7 patients, and 1 patient had bilateral disease. Patients most frequently presented with hearing loss and offensive purulent aural discharge. One patient presented with a left-sided Bezold's neck abscess and a right-sided Luc's zygomatic abscess. Another patient presented with a left mastoid subperiosteal abscess, while one patient presented to the neurosurgical service with a cerebellar abscess. All patients presenting with abscesses required drainage of the pus one week before mastoid surgery. Four patients had occluding aural polyps at presentation; polyps were excised permeatally one week before mastoid surgery. Fungal cultures were obtained from cholesteatoma keratin from 17 ear samples (89 per cent; fungal isolates shown in Table I). Five fungal isolates belonged to the dermatophyte group (21 per cent).

Discussion

Middle-ear cleft cholesteatoma is an aggressive form of chronic otitis media that requires surgery for extirpation. Clinically, an infected cholesteatoma is associated with a malodorous ear discharge which adversely affects the patient's quality of life.[4] The cause of fetor has been attributed to anaerobic bacterial infection.[5] However, anaerobic bacteria have rarely been cultured from mastoid granulations from ears with cholesteatoma.[6] In a previous study, we postulated that the fetor associated with primary atrophic rhinitis was caused by saprophytic fungal colonisation of the nasal cavities of affected patients.[7] We therefore hypothesised that the fetor associated with infected cholesteatomas might be caused by saprophytic fungal colonisation within the keratinous debris.

In the current study, fungal elements were detected in 89 per cent keratinous debris samples obtained during primary mastoid surgery. In a previous study, fungi were isolated from 25 per cent of discharge samples from ears affected by active chronic suppurative otitis media.[8] In a mycological study on otomycosis, Vennewald and colleagues reported the presence of *Aspergillus* spp. determined histologically in keratinous debris in four cases of cholesteatoma that required mastoidectomy.[9] However, to our knowledge, no prospective mycological study has been previously undertaken in cholesteatoma. The external ear canal can harbour various fungal commensals, and fungi have been isolated from unstable post-mastoidectomy cavities.[10] We chose to investigate fungal colonisation in mastoid antrum keratin obtained during primary mastoid surgery. We propose that the saprophytic fungal colonisation in our patients was a result of germination of fungal spores that entered through the external ear canal. However, an alternative route might have been via the eustachian tube.[11] In the current study, *Microsporum canis* and *Epidermophyton* spp. comprised 21 per cent of the total fungal isolates. Both species belong to the dermatophyte group, which uses keratin as its source of nutrition.[3]

An established cholesteatoma presents pathologically as a keratin-containing sac surrounded by a keratinising epithelial layer (i.e. matrix) and an adjacent subepithelial connective tissue layer with a mucosal epithelial layer boundary (i.e. peri-matrix).[12] Current cholesteatoma studies focus on the peri-matrix–matrix interactions necessary for the progression of this condition. A chronic inflammatory process occurring in the peri-matrix is a cardinal feature in established cholesteatomas that plays a role in disease onset, maintenance, growth and expansion.[12] Bacteria, identified by microbiological cultures from diseased granulations or detected as bacterial biofilms by electron microscopy, have been reported in infected

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TABLE I
MYCOLOGICAL DATA

Pt no	Age (yr)	Sex	Affected ear	Clinical presentation	Otoscopy findings	Date of operation	Fungal isolate
1	36	F	R	Hearing loss + discharge	Pars tensa perforation	30/03/2013	*Penicillium* spp.
2	12	M	L	Hearing loss + discharge	Attic retraction	06/04/2013	*Aspergillus* spp.
3	9	M	R	Hearing loss + discharge	Posterosuperior retraction	13/04/2013	*Candida albicans*
4	21	M	R	Hearing loss + discharge	Posterosuperior retraction	25/04/2013	No growth
5	20	M	R	Hearing loss + discharge	Pars tensa perforation	11/05/2013	*Epidermophyton* spp.
6	13	F	R	Hearing loss + discharge	Aural polyp	23/05/2013	*Epidermophyton* spp.
7	30	M	L	Hearing loss + discharge	Pars tensa perforation	08/06/2013	*Alternaria* spp.
8	29	M	R	Hearing loss + discharge	Aural polyp	20/06/2013	*Aspergillus niger*
9	20	M	L	Bezold's abscess	Pars tensa perforation	04/07/2013	*A niger*
			R	Luc's abscess	Pars tensa perforation	20/07/2013	*A niger* and *Mucor* spp.
10	30	F	L	Subperiosteal abscess	Attic retraction	03/08/2013	*Microsporum canis*
11	32	F	L	Hearing loss + discharge	Pars tensa perforation	15/08/2013	*C albicans*
12	14	M	L	Hearing loss + discharge	Aural polyp	31/08/2013	*Mucor* spp.
13	28	M	R	Cerebellar abscess	Posterosuperior retraction	07/09/2013	*C albicans*
14	45	M	R	Hearing loss + discharge	Pars tensa perforation	19/09/2013	*Aspergillus* spp.
15	15	F	R	Hearing loss + discharge	Attic retraction	28/09/2013	*M canis* and *Mucor* spp.
16	30	M	L	Hearing loss + discharge	Posterosuperior retraction	12/10/2013	*Epidermophyton* spp.
17	12	M	L	Hearing loss + discharge	Posterosuperior retraction	24/10/2013	*C albicans*
18	28	M	R	Hearing loss + discharge	Aural polyp	31/10/2013	No growth

Pt no = patient number; yr = years; F = female; M = male; R = right; L = left

cholesteatomas.[6,13] Bacterial products are responsible for maintaining an inflammatory response in the perimatrix region.[12]

- **Cholesteatoma keratin samples were obtained from 19 ears during primary mastoid surgery**
- **Fungi were isolated from 89 per cent of samples**
- **Fungal colonisation may be related to the clinical and pathological features of cholesteatoma**

In chronic rhinosinusitis, fungal elements have been frequently identified within sinus mucosal bacterial biofilms by fluorescent in situ hybridisation.[14] Common saprophytic and commensal fungi can promote the release of proinflammatory cytokines through orchestrating the innate and acquired immune responses.[15] Fungi and bacteria may interact symbiotically as protection against host defences and antimicrobials.[14] In addition, fungal products were reported to cause epithelial cell desquamation in a cell culture model.[16] Recently, Louw reviewed the role of microorganism-induced alterations in lipid metabolism in matrix cell apoptosis and cholesteatoma deterioration.[17] It is therefore likely that fungal elements associated with cholesteatoma play a significant role in the disease progression.

The major goal of cholesteatoma surgery is to completely remove keratin and matrix debris from the middle-ear cleft to achieve a safe, stable ear.[18] However, fungal diseases have been detected upon operating on a contaminated ear. Invasive fungal disease causing intracranial complications have been described following surgery on an ear with chronic suppurative otitis media.[19] This flare-up was described as a 'forest fire phenomenon', in which the manipulation of previously quiescent fungi leads to activation and invasiveness.[20] In addition, a non-invasive fungal-related entity was recently reported following cholesteatoma surgery. This condition, termed 'allergic fungal otomastoiditis', was attributed to a hypersensitivity reaction to fungal elements in the middle-ear cleft.[21] With increasing numbers of patients receiving immunosuppressants for a variety of illnesses, including the acquired immunodeficiency syndrome pandemic, otologists should be alert to the possibility of fungal diseases in the differential diagnosis of unusual presentations of middle-ear cleft disorders.[22,23]

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Conclusion

The current study identified fungal isolates in 89 per cent of samples of cholesteatoma keratin obtained during primary mastoid surgery. Fungal colonisation in cholesteatoma probably plays a significant role in disease progression through maintaining an inflammatory response in the peri-matrix region. Moreover, saprophytic fungal colonisation in cholesteatoma keratin may be responsible for the fetor associated with ear discharge. Otologists should consider the possibility of fungal infections associated with cholesteatoma and surgery in a contaminated ear.

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