

CLINICO-MICROBIOLOGY OF CHOLESTEATOMA OBTAINED DURING MASTOID SURGERY IN PATIENTS WITH CHRONIC SUPPURATIVE OTITIS MEDIA

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Abstract

Chronic Otitis Media (COM) is a long standing inflammation of the middle ear and mastoid, it is a common health problem. The evidence of microbiology in COM in different studies showed variable frequencies, but little studies showed the role of fungal colonization.

This study aimed to identify the microbiology of cholesteatomatous COM concentrating on clarification of fungal colonization. A prospective study was done in otolaryngology department of Basrah Teaching Hospital from June 2018 till July 2019. This study was carried on a randomly collected data of 25 patients diagnosed as COM by a specific designed questionnaire including history and examination. The specimen was collected during mastoid surgery which included any cholesteatoma in the middle ear and mastoid and sent for bacteriological and mycological examination. Statistical study was done by using SPSS v.23

Bacteria were isolated in 84% of the cases in which *Pseudomonas aeruginosa* was the commonest one (33.3%). Fungi were isolated in 28% cases, in which the Aspergillosis was the commonest (19.3%). There is no recognizable pattern of significant association between the fungi and the reported complications or the stage of the disease.

In conclusion, there is significant percentage of fungal colonization in COM with cholesteatoma so it may has a role in inflammatory response of perimatrix, but there is no clear mechanism of this interaction, so may need larger sample and longer duration of study with postoperative follow-up and trail of combined antibiotic-antimycotic treatment.

Key words: *clinical, microbiology, cholesteatoma, mastoid surgery, otitis media.*

Introduction

Entire forms of otitis media are considered as a common health problem. Chronic suppurative otitis media (CSOM) is defined as a long standing inflammation in the middle ear and mastoid¹. The most frequent causative organisms in CSOM are; *Pseudomonas aeruginosa*, Methicillin sensitive *Staph. aureus* (MSSA), Methicillin resistant staphylococcus aureus (MRSA) and coagulase negative staphylococci with a variable frequency according to different studies²⁻⁵. Other bacteria cultured from chronic draining ears are: *Enterobacter*, *Acinebacter*, *Proteus*, *Streptococcus*, and *Klebsiella*. There is evidence of microbiology changing overtime, a study showed

increasing in MRSA in CSOM from 0.7% to 11.4% in a period between 1998–2006⁶. In chronic diseases such as chronic rhinosinusitis as a classical example in ENT, this shows susceptibility for fungal colonization⁷. The usage of topical steroids as a combined topical treatment for CSOM is considered as an important predisposing factor for fungal colonization. Cholesteatoma itself is regarded as an ideal medium for saprophytic fungi because of its keratinous debris content⁸⁻¹².

This study was designed to identify the microbiology of cholesteatomatous CSOM by using appropriate mycological and bacteriological methods in a group of operated upon patients, and concentrated

on clarification of the clinical effects of the fungal colonization of cholesteatoma.

Patients and methods

This prospective study was done in the otolaryngology department at Basrah Teaching Hospital in the period from June 2018 to July 2019. The study sample composed of 25 patients of either gender, their ages ranged from 8–58 years old, the mean is 25 years. The data was collected according to a specific questionnaire. All patients were diagnosed as chronic otitis media by history of the most presenting symptoms and its duration, any complications, congenital anomalies such as cleft palate, chronic medical disease or immunocompromising disease, and preoperative treatment (before 1 week or less). Ear examination included; preoperative auroscope and otomicroscopy to classify the type of COM, fistula test, hearing assessment by tuning fork, PTA, and CT scan. Informed consent was mandatory. The type of surgery performed for patients was determined according to the disease extension and the opinion of the surgeon between mainly; Canal wall down (CWD) and canal wall up (CWU). For staging of cholesteatoma, EAONO/JOS system was used⁹ which applies to four

types of cholesteatoma in the middle ear: Localized in the primary site, involving 2 or more sites, associated with extracranial complications, and associated with intracranial complications.

During mastoid surgery, a sterile micro forceps was used to collect the cholesteatoma and maintained in a sterile containers of normal saline, the sample was transported immediately to the mycology laboratory, Collage of Science, University of Basrah. Some samples were sent to the central laboratory, Basrah Teaching Hospital for processing. Fungal analysis was done by direct study of sample onto sterile glass slide containing a drop of 10% KOH, and culture onto sabouraud dextrose agar (SDA) containing 0.05 g/l chloramphenicol. For analysis of bacteria, specimen was inoculated onto nutrient agar, blood agar, and MacConkey agar.

Results

A 25 patients diagnosed as CSOM with cholesteatoma were recruited in this study, 15 (60%) were males and 10 (40%) were females. All patients complained from otorrhoea, 11 of them (44%) were associated with hearing defect, 3 cases have vertigo, and earache in one case (table I).

Table I: Presenting symptoms number and percentage

Symptoms	No.	Percentage
Otorrhoea	10	40 %
Otorrhoea with hearing defect	11	44%
Otorrhoea with vertigo	3	12%
Otorrhoea with earache	1	4%
Total	25	100 %

Extracranial complications were demonstrated in 28% of the cases, mostly was labyrinthine fistula in 4 (16%), facial palsy in 2 (8%), and post auricular abscess in one.

Table II: Complications number and percentage

Complications	No.	%
No complication	18	72%
Facial palsy	2	8%
Labyrinthine fistula	4	16%
Post auricular abscess	1	4%
Total	25	100%

According to EAONO/JOS system for cholesteatoma staging, we found 6 cases (24%) of stage I, 12 cases (48%) of stage II, and 7 cases (28%) with stage III (Fig.1).

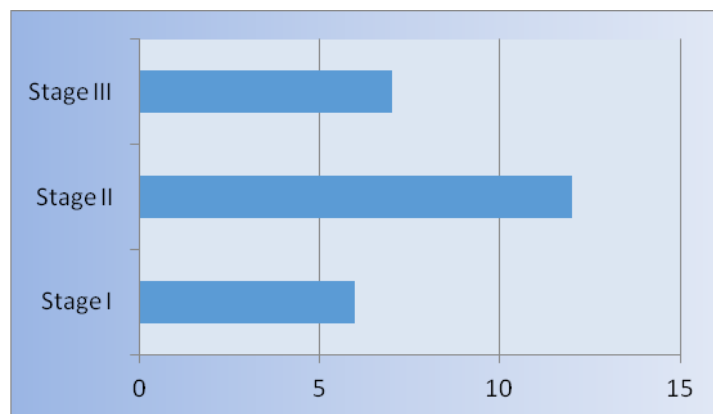


Figure 1: Number of disease stage according to EAONO/JOS system

Out of the suspicious 25 patients; 21 (84%) confirmed to have bacterial cholesteatomatous CSOM with a positive culture. A total of 23 isolates representing eleven species of bacteria were obtained from the 21 patients. The most prevalent species was *Pseudomonas aeruginosa* (9 isolates, 39.13% of total positive sample), followed by *Enterobacter* (4 isolates, 17.39%), *Escherichia coli* (2 isolates, 8.69%), and no bacterial growth in four patients (16% of the total sample) as shown in table III.

Table III: Types of the isolated bacteria

Bacterial species	No. isolates	Percentage
<i>Acromobacter denitrificans</i>	1	4.34
<i>Enterobacter sp</i>	4	17.39
<i>Escherichia coli</i>	2	8.69
<i>Klebsiella pneumonia</i>	1	4.34
<i>Proteus sp</i>	1	4.34
<i>Providencia struati</i>	1	4.34
<i>Pseudomonas aeruginosa</i>	9	39.13
<i>Ralstonia sp</i>	1	4.34
<i>Staphylococcus aureus</i>	1	4.34
<i>Staph. Lugdunensis</i>	1	4.34
<i>Stenotrophomonas maltophile</i>	1	4.34
Total	23	100

There is no significant association between bacterial infection and the occurrence of complications (P-value 0.999), neither with stage of disease (P-value 0.378) (table IV).

Table IV: Summary of association of bacterial infections with complications

		Complications		
		Yes	No	Total
Bacterial infection	Yes	7	14	21
	No	0	4	4
	Total	7	18	25

Fisher's exact test value: 0.242, P-value 0.999.

Regarding fungi, out of the 25 clinically diagnosed cholesteatomatous CSOM patients, only 7 cases were mycologically positive by direct microscopy and culture, which represent 28% of the total sample. In the current study, three genera were isolated; *Aspergillus* (3 species) that represent mold, *Candida* (1 species), an ascomycetous yeast,

and *Naganishia* (1 species), basidioid yeast. The commonest species isolated in this study was *Aspergillus niger* (3 species, 37.5% of total positive sample) followed by *Candida albicans* (2, 25%). While *A. flavus*, *A. fumigatus* and *Naganishia albida* were represented by one isolate (12.5 %) for each species. Five cases were associated with bacterial isolation, and 2 cases as pure growth of fungi as demonstrated in table V.

Table V: The isolated fungi number of isolates and their percentage

Fungal species	No. isolates	Percentage %
<i>Aspergillus flavus</i>	1	12.5
<i>Aspergillus fumigatus</i>	1	12.5
<i>Aspergillus niger</i>	3	37.5
<i>Candida albicans</i>	2	25
<i>Naganishia albida</i>	1	12.5
Total	8	100

Regarding association between fungi and the reported complications, there was no recognizable pattern of significant association (P-value 0.786)(table VI).

Table VI: Summary of association of fungal infections with complications

		Complications		
		Yes	No	Total
Fungal infection	Yes	3	4	7
	No	4	14	18
	Total	7	18	25

Chi squared value: 1.064, P-value 0.302

Discussion

In this study, the sample size was 25 patients of either gender with male preponderance, which is smaller than Singh¹¹ (40 patients) and more than Effat¹³ (18 patients), with nearby same age groups in both studies.

In this study all patients complained from otorrhoea, in 11 patients associated with hearing loss, 3 patients with vertigo which are similar to Singh study. Because a comparison was done in 1999 between recovered micro-organism by conventional ear swab and by middle ear specimen, only 51% of middle ear aspirate micro-organisms were found in conventional ear swab, so we choose cholesteatoma as a middle ear or mastoid specimen for microbiology.

The proportion of different isolated micro-organisms varies from one study to another, but in general the *Pseudomonas aeruginosa* is the commonest isolated organism in about 39.13% cases in this study, (31% Ricciardiello et al¹⁰, 28.6%

Singh¹¹, and 51% Attallah¹². There was no bacterial growth in 16% of the specimen in this study which is corresponding to Attallah¹². Regarding mycology, its incidence in cholesteatomatous CSOM is variable as low as 1.4% in Ricciardiello study¹⁰ to 89% in Effat¹³.

In this study, the fungal infestation was detected in 7 of our 25 cases (28%), 8 fungal species were isolated, *Aspergillus* is the commonest one (62.5%) followed by *Candida* in 25%. Six of fungal isolates was associated with bacterial growth, while 2 isolated as pure nature.

The route of fungal infection to cholesteatoma¹¹ may be from external auditory canal or Eustachian tube. It can be induced by; topical steroid which is used in COM treatment, long term of antibiotics use, and chronic ear discharge which can cause meatal epithelium maceration which act as a good

environment for colonization of fungi. It is difficult to distinguish whether fungal infestation in cholesteatoma has a clinical relation or just colonization. Capability of fungi to be opportunistic pathogens depends on the virulence factor which determined by: Toxin production, Size of the organism, Ability to grow in 37 centigrade at natural PH. Hall & Fariior¹⁴ divided *Aspergillus* infestation in temporal bone into: localized, noninvasive, bony invasion by fibrosis and granulomatous respond, fulminant tissue and angioinvasion in immunocompromised persons.

The cholesteatoma is a sac of keratin surrounded by matrix and peri-matrix. The bacterial infection cause a chronic inflammation in a peri-matrix, so by the presence of pathogenic fungi can cause progression of COM by one of these mechanisms: mycotoxicosis of a toxic metabolites of fungi, fungi and bacteria interaction against antimicrobial and the host defense, pro-inflammatory cytokines which are released by common

saprophytic fungi, hypersensitivity reaction type I & III, and epithelial cell desquamation by fungal products. In this context, extracranial complications were recorded in 7 cases and fungal infection was detected in 3 cases with a P-value 0.75 which is found to be not significant. This study fail to find a significant relationship between fungal infestation and complications and this result is similar to Singh study (P-value 0.68), however 18 patients of the sample were not having a complication, 14 (77.78%) of them were not infected with fungi.

Conclusion; This study showed Gram-negative bacteria mainly *Pseudomonas aeruginosa* is significantly associated with COM. Also there was no insignificant percentage of fungus infection in cholesteatomatous COM which may play a significant role in an inflammatory response in the perimatrix and disease progression, but there is no clear mechanism of interaction between chronic inflammation and fungal infection.

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