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Original Research Article

Mycological Study of Aspergillus Infections in Otomycosis in Eastern Part of Maharashtra

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ABSTRACT

Background: The incidence of mycotic infections and the diversity of pathogenic fungi have increased dramatically in recent years. Otomycosis is a sub-acute or chronic superficial fungal infection of the external auditory canal. It is found throughout the world but is more prevalent in hot, humid, dumpy and dusty climate.

Aim: The purpose to determine the quantum of aspergillus infections in otomycosis and to determine the predisposing factors associated with of these isolates.

Methodology: In this study a total of 132 cases clinically suspected having chronic ear infection were enrolled. Specimen collected with the help of clinicians and diagnosis confirmed by direct mycological analysis.

Results: Prevalence of Aspergillus infection was seen in 25 % cases of chronic otitis externa in eastern part of Maharashtra and most common fungal pathogen isolated was Aspergillus niger (62%). Agriculturists constituted the largest group (67%), history use of prolonged antibiotic and steroid drops was present in 76% of the patients.

Conclusion: Hot and dry climate of eastern Maharashtra may be responsible for more prevalence. In this study we observe that otomycosis is unilateral condition more common in young to middle age agriculturists particularly during summer and rainy season. Aspergillus niger (62%) was found to be the major etiological agent causing otomycosis.

Key words: Otomycosis, Aspergillus niger, Chronic infection, Antibiotic and steroid drops.

INTRODUCTION

Among the various mycoses of man, aspergillosis is one of the earliest fungal disease to be recognized. Aspergillus spp. are ubiquitous fungi, commonly occurring in water and decaying vegetation. Aspergillus spp. have been cultured from

unfiltered air. ventilation system, contaminated dust dislodged during hospital renovation and construction, horizontal surface, food and ornamental plants. [1] Otomycosis is a superficial, sub-acute or chronic infection of the external auditory meatus and ear canal, usually unilateral

characterized by inflammation, pruritus and scaling caused by opportunistic fungi. [2]

It is estimated that otitis externa make up 5 to 20% of ear-related visits to ENT OPD, most of them caused by bacteria, and from the latter approximately 5 to 25% are caused by fungi, called fungal otitis or otomycosis. ^[3,4] It is more prevalent in warm, humid climate and among peoples belongs to lower socio economic status with poor hygienic conditions. ^[3]

The present study was performed to define the prevalence of various species of Aspergillus and predisposing factors for Aspergillus infection in patients of otomycosis.

METHODOLOGY

The present study was carried out for two years in the department of Microbiology, Government Medical College and Hospital, Nagpur. A total 132 cases were studied including in the study.

The typical presentation is with inflammation, pruritus, scaling and severe discomfort. The mycosis results in superficial epithelial exfoliation, masses of debris containing hyphae and suppuration. Pruritus is more marked than with other forms of ear infections and discharge is often a marked feature. Ear discharge material was collected by sterile swab stick and loop.

All specimens were subjected to 10% KOH wet mount preparation and Gram stain to detect the fungal elements. A small amount of specimen was mixed with 10% Potassium Hydroxide (KOH) and was examined under magnification of 40 objectives for the presence of fungal elements. KOH gradually dissolves the human material and makes the fungal cell easier to see. These samples were inoculated on two slopes of Sabouraud's dextrose agar (SDA) with Chloramphenicol. Slopes were incubated at room temperature. The slopes

of SDA were observed every alternate day for appearance of growth up to 20 days before release as negative [Figure:1]. The growth was identified by standard procedures ^[5] as the identification of isolates was done by macroscopic examination of culture tubes; the characteristics considered in fungal identification were texture, color and growth rate. The slide culture technique with Lactophenol cotton blue (LPCB) shows characteristics such as mycelium, conidia types and hyphae more clear [Figure:2].



Figure 1: Showing the Culture of Aspergillus on Sabouraud's dextrose agar.

A: Aspergillus fumigatus; B: Aspergillus niger;

C: Aspergillus flavus.

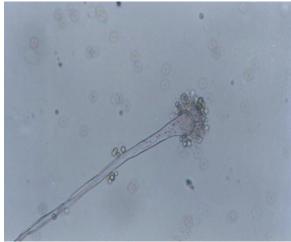


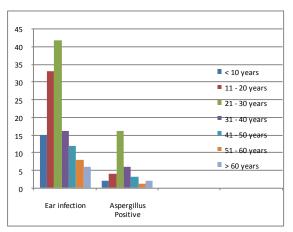
Figure 2: Lactophenol Cotton Blue Tease Mount Showing Aspergillus Sporing Head.

Statistical analysis

Statistical analysis was performed by 'Fisher exact' test and Chi-square (' X^2 ') test done by using the Statistical Package Social Sciences (SPSS) with p < 0.05 taken as statistically significant.

RESULTS

The study enrolled 132 cases clinically suspected of chronic ear infection out of whom seventy three (55%) were male and 59 (45%) were female. Chief presenting complaints was ear discharge, earache, deafness and itching either bilaterally or unilaterally. Out of 132 specimens 37(28%) were positive for fungal infection. Typical fungal hyphal elements aspergillus sporing head were detected in 35 cases and culture were positive in 37 cases; but those were positive in both microscopy and culture included in the study [Table:1]. Aspergillus spp. was isolated in 33 (25%) cases (nineteen were male and 14 were female) and other fungi in four cases. We observed 8 (24%) cases bilateral and 25 (76%) cases had unilateral aspergillus infection. The incidence of aspergillus infection in otomycosis was more in the age group of 21-40 years (22/33 i.e.67%). From 0-20 years it was 18% whereas, in 50-69 years it was 6%. [Graph:1].



Graph:1 Prevalence of Aspergillus infection according to age in aspergillus otomycosis.

Aspergillus niger was the predominant species isolated in twenty three cases (62%) fallowed by Aspergillus fumigatus in ten (27%). Maximum cases were agriculturists (cultivators, farmers and laborers) constituted the largest group i.e. 67%. and from rural area (64%).

Table: 1 Correlation between microscopy and culture in aspergillus infection.

Microscopy (10% KOH)		Culture Positive for Aspergillus		
	Positive	Negative		
Positive	33 *	2	35	
Negative	4	93	97	
Total	37	95	132	

^{*} Included in the study.

Table: 2 Showing different predisposing factors associated with Aspergillus infection in otomycosis

Predisposing factors	Ear infection	Aspergillus positive	Aspergillus negative	Odds	X^2	P value
	cases (132)	(33)	(99)	ratio		
Age group	58 (44%)	22 (67%)	36 (36%)	-	-	-
(21-40 years)						
Farmers	63 (48%)	22 (67%)	41(41%)	-	-	-
Rural area	61(46%)	21 (64%)	40 (40%)	-	ı	-
Trauma	10 (8%)	3 (9%)	7 (7%)	0.2	1.27	>0.05
Topical steroid/	73 (55%)	25 (76%)	48 (48%)	3.16	6.90	<0.05*
antibiotic drops						
Herbal juices	5 (4%)	0 (0%)	5 (5%)	-	F**	>0.05
Oil drops	19 (14%)	2 (6%)	17 (17%)	0.88	F**	>0.05
Chronic Symptoms	37 (28%)	17 (52%)	20 (20%)	2.60	1.69	>0.05
Diabetes Mellitus	13 (10%)	1 (3%)	12 (%)	-	F**	>0.05
No specific factor	33 (25%)	2 (6%)	31(31%)	0.63	F**	>0.05

X²: Chi -square test; **: Fisher Exact test; *: Statistically significant.

The incidence of 26% of aspergillus otomycosis in males and 24% in females was recorded in our study (Male: female ratio is = 1.1:1). Predisposing factors leading to aspergillus infections was found to be prolonged used of topical steroid and antibiotic ear drops [Table:2].

DISCUSSION

Otomycosis are frequent infection in tropical countries, because of humidity and heat.

The fungal agents responsible for this clinical entity are found as saprobes of the environment. It is mainly produced by yeast & filamentous fungi that affects squamous epithelium of external auditory canal. [4,6] In the present study fungal elements by microscopy were seen in 39 cases (30%). Gugnani et al [7] reported 23% cases were positive by microscopy. Incidence of otomycosis was common among age groups of 20-35 years. In present maximum percentage aspergillus infection of ear was seen in age group of 21 - 40 years. Same reported by Paulose et al. [8] Occupational incidence mainly involves agriculturists, because of unhygienic practice of self cleaning of ear canal with dirty fingers, hair pins and match sticks hastens the deeper invasion of fungus. [6,9] Those bad habits associated with trauma that favors the colonization of opportunistic fungi. In this study out of 33 aspergillus otomycosis twenty two cases agriculturists and maximum (21) cases from rural area (64%).

Otomycosis is commonly one sided disease. In our present study unilateral otomycosis was found in twenty five cases(76%) and 8 cases had otomycosis in both side. Paulose et al ^[8] & Desai KJ et al. ^[6] who also reported that otomycosis is predominantly a unilateral disease in percentage of 85% & 100% respectively. As far as the side of ear is concerned studies

could not find any differences. possibility may be; otomycosis occur due to more risk factors works together as vegetative trauma by putting dirty objects into the ear for cleaning (changes in coating epithelium that failure in the ear's defense mechanisms), using unsterile lightly warm oils for pain relief (traditional method), prolonged use of antibiotic and steroid ear drops and environmental conditions. [10] Published data in different studies are extremely varied when we analyze the species of fungi identified in patients with otomycosis. There is a large number of and an important geographical variance of its prevalence. The most frequently reported genders are Candida Aspergillus, and Penicillium. [4,6,9,10] Prevalence of aspergillosis clinically suspected otomycosis was 25% in present study. Oliveri et al [11] reported 49% suffering from aspergillus patient otomycosis, Paulose et al reported 70%, [8] Gugnani et al reported 13%, [7] Zaror et al reported 68%, Sarvan et al [10] reported 68%, Pontes ZB et al [4] reported 7% and ChandraPrasad S. et al [9] reported 53%. Higher prevalence 84% also reported by Desai et al [6] [Table:3]. The incidence of a determined gender depends a lot on geographical and climatic conditions in which the patient lives, because the presence of a specific fungus in the environment is related with environmental conditions of temperature and air relative humidity. The pattern of organism varies from place to place and depends upon age, habitual of the inhabitants, their immune status and the clinical factor. [6]

In this study the most common isolated species was *Aspergillus niger* (62%), followed by *Aspergillus fumigatus* (27%). Paulose KO et al, ^[8] Desai KJ et al (2012), ^[6] Prasad ZB et al (2014) ^[9] isolated predominantly *Aspergillus niger* from cases

were 55%, 76%% and 38% respectively

[Table:3].

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Table 3: Con	inarison of	commonest	tungal	species.	callsing	otomycosis

Study	Total	Prevalence of	Commonest	%
	Cases	Aspergillus infection	Species	
Paulose et al (1989) [8]	193	136 (70%)	A. niger (106)	78%
Kaur et al (2000) [15]	95	71 (74.5%)	A. fumigatus (30)	58 %
Sarvan RR et al (2012) [10]	100	68 (68%)	A. niger (39)	45%
Desai KJ et al (2012) [6]	100	84 (84%)	A. niger (69)	76%
ChandraPrasad S.et al(2014) [9]	150	80 (53%)	A. niger (38)	38%
Present study	132	33 (25%)	A. niger (23)	62%

Because Aspergillus niger grows on cerumen, epithelial scales & debris deep in the external auditory canal & resulting in plug of mycelium. There are many predisposing factors of otomycosis like chronic infection of ear, use of unsterile oils, broad spectrum antibiotic and steroid ear drops, excessive accumulation of cerumen, swimming in dirty water like lake, fungal infection elsewhere in the body like dermatomycosis, poor hygiene, putting dirty into the for sticks ear cleaning, malnourishment in children & hormonal changes precipitating flaring up of the infection as seen during pregnancy or menstruation. [13] All of the following factors have been considered to encourage infection like changes in epithelial covering. Excessive sweating during summer dilutes wax & reduces the protective sleeve property. High humidity gives suitable condition for fungi. The relative high humidity & temperature in external auditory canal which approximate that of the body are the prime factors. [14] In our study the maximum risk for aspergillosis was found with use of topical broad spectrum antibiotic and steroid ear drops. Odds ratio for use of prolonged steroid drops is 3.16 means that, if the ear infection patient used topical steroid drops then there is 3.16 times risk for development of aspergillus infections.

In our study, we tried to define the prevalence of Aspergillus infection in chronic ear infection. The knowledge of these facts is important in the clinical

suspicion and investigative approach of ENT patients with chronic ear complaints in our daily practice.

CONCLUSION

Otomycosis is commonly unilateral condition more common in young- middle age particularly during summer and rainy season with predisposing factors as using dirty objects to cleaning the ear and unnecessary use of broad spectrum ear drops. Most of the patients were agriculturists, belong to low socio economic conditions and with bad habits. Basic health education to them may reduce the prevalence of otomycosis.

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