

ORIGINAL ARTICLE

Evaluation Study of the Fungal Flora of the External Ear Canal in Healthy People and Patients with Chronic Otitis Ear Referred to Central Hospital of Zahedan-Iran

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ABSTRACT

Otomycosis is a fungal infection. This study aimed to find any fungi sp. caused the ear fungal infection. This case - control study 250 cases involving 125 persons were healthy subjects and 125 persons were patient that were selected using questionnaire and clinical examinations. Samples were taken and cultivated in SCC, S and CMA culture. In this study of 125 healthy persons 21 individuals (17.6%) had positive cultures and 104 cases had negative culture. In the patients group of 125 persons, 70 persons (55.2) had positive culture and 55 cases had negative cultivation which there is a significant differences between two groups ($P < 0.01$). Saprophytic fungi were the most common contaminant (60%). In the subgroup of Saprophytic fungi *Aspergillus niger* (37%) was the most common fungi. The most common subtype of dermatophytes in healthy and patients subjects was *Trichophyton mentagrophytes* (58.38%). The rate of positive fungal culture in the external ear canal of patients with otitis media was significantly higher than control group and due to the special role of fungi in disease process and also prolonged middle ear infection. We can prevent indiscriminate use of antibiotics and lessen the chance of antibiotic resistance in the community and shorten the period of illness and also reduction the stability of disease process.

Keywords: Fungal Flora, Chronic Otitis of Middle Ear, External Ear Canal, Ear Fungal Infection, South-East Of Iran.

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INTRODUCTION

According to studies, in 10-20% of the external ear infections (Otitis external) fungus were the generating factors and usually in acute and chronic state of disease, several bacterial agents added secondary to the primary causative agent and may cause to production of pus with an unpleasant odor, inflammation and pain and sever redness in ear canal [1]. Otomycosis is a chronic or acute fungal infection in external ear canal that is accompanied with secretion, inflammation, itching and dry shells [2].

Chronic middle ear infection (chronic otitis media), is an inflammation of mucous membrane that is one of the main factors in the incidence of otitis media. Predisposing factors of otitis media include of anatomic abnormalities cleft palate, allergic rhinitis, trisomy 21 and cystic fibrosis. Otomycosis usually caused by risk factors such as entry of foreign objects into the ear canal, ear scratching and manipulation of ear canal, leaving in dusty areas or in a region with warm and humid weather [3,4]. Saprophytic yeast fungi such as *Candida albicans* and other *candida spp*, saprophytic yeast-like fungus with endogenous origin, saprophytic mold transparent fungi with exogenous origin such as different types of *Aspergillus sp*, *Penicillium sp* and various type of *Mucorales*, *Scopulariopsis*, *Epicoccom* and rarely *Dematiaceous sp* are considered as pathogens. Diagnosing techniques of fungal infection are based on history, physical examination, sampling and direct examination to see fungal factors and also the

cultivation in appropriate culture medium [5-10]. According to surveys that were conducted in different countries, different statistics of ear fungal infections have been reported that shows the incidence of fungal infections is higher in tropical areas, especially in East Asia [11]. Currently, based on its prevalence, treatment of patients with chronic otitis of middle ear most reported as bacterial treatment and not respond to prescribe antibiotics is considered as resistant otitis media. Indiscriminate use of antibiotics raises the chances of antibiotic resistance in the general population. Therefore, in this study if the evidence of the high prevalence of infection was obtained we want to reduce the risk of antibiotic resistance and decreased the length of treatment and also proof the important role of fungi in pathogenesis and stability of disease process.

MATERIAL AND METHODS

This is the control-case study. The study population composed of patients with chronic otitis middle ear (COM) and healthy persons referred to ENT clinic of Khatam hospital of Zahedan which included 125 patients with COM and 125 healthy subjects. After getting informed consent from patients (eligible patients include having hospital records and history of otitis) using interview, questionnaire, clinical examination methods required data were collected. Frequency distributions tables were used to describe information and chi-square test was used to analyze the data (SPSS, version 18). To separate fungi and disease diagnosis, sampling should be done from right and proper place with complete and accurate process. Thus, samples were taken from external ear using sterile swabs that moistened in normal saline. Then samples were put in sterile tubes containing saline and transported to the laboratory. Part of collected samples placed on a sterile slide and after adding a drop of KOH 10% put on a sterile coverslip then slide of sample gently heated over flame. Finally, we will wait ten minutes for transparent samples. Under the microscope, the conidia mycelium chains and sometimes conidiophores and or swollen vesicles of fungi such as *Aspergillus* spp can be seen. Another part of the clinical samples were inoculation and cultured in three periods in Sabourauds dextrose agar and Sabourauds dextrose agar + Chloramphenicol (Sc) culture medium and were maintained at room temperature (RT) for fungal growth and colonization. In case of the fungal growth in culture medium, genus and species composition fungal agent colonies were determined using macroscopic characteristics of colonies, wet smear were prepared from colonies and if necessary, provide a view of culture slides (Slide culture). The diagnosis of fungal agents involved in causing the disease, adaptation of direct test results with the results of fungal cultures and also Growth of fungal agents in the same series of culture media was considered. Corn meal agar medium (Corn meal agar (CMA)) is the exclusive environment for *Candida* spp to be used for the detection of *Candida albicans*

RESULTS

Study was conducted on 125 patients with chronic otitis and 125 healthy individuals (in terms of susceptibility to chronic otitis). In the patient group 67 persons were men (54%) and 58 persons were female (46%). In the control group, 70 individuals were male (51%) and 55 persons were females (37%). No significant relationship was found between gender and test results ($P > 0.01$). Of the control group, 21 cases (17.6%) had positive fungal cultures, and 104 cases (82.4%) had negative fungal cultures, and in group of chronic otitis media in 70 cases (55%) had positive fungal cultures in 55 cases (45%) had negative fungal cultures and this statistical results were significant ($P < 0.01$). Total of 90 positive fungal cultures (73%), 60 cases (48%) had single fungal infection, 17 cases (14%) had two fungal infection and 12 cases (9.6%) had three fungal infection (Table 2). In both groups, the most common fungal incidence was in the age group 10-20 (Table 1). Chi-square test showed that there is no statistically significant difference between age groups and those who their result test were positive ($P > 0.01$). Prevalence of fungal infection of each sub groups of patients and healthy persons. Saprophyte group is composed of 24 cases *Mucor* sp (27.5%), 10 cases *Penicillium* sp (11.5%), 33 cases *Aspergillus* sp (37.2%), 2 cases *Scopulariopsis* sp (2.24%), 11 cases *Alternaria* sp (13%) 5 cases *Fusarium* sp (6%), 1 cases *Acromonium* sp (1.15%), 1 cases *Helminthosporium* sp (1.15%). Dermatophytes group are composed of 3 persons *Epidermophyton floccosum* (12.5%), 14 cases *Trichophyton mentagrophytes* (58.38%), 1 cases *Trichophyton schoenleinii* (4.17%), 4 cases *Trichophyton violaceum* (17%), 1 cases *Trichophyton verrocosum* (4.17%), 1 cases *Trichophyton rubrum* (4.17%). Fungal yeast group were formed of 18 cases *Candida* spp (66.6%), 1 cases *Candida albicans* (3.7%), 8 cases *Rhodotorula rubra* (29.6%). Of all the subjects 13 cases (10.4%) had two types of saprophyte and dermatophytes fungi and 3 cases (2.4%) had two dermatophytes and yeast fungi and 1 cases (0.8%) had two yeast and saprophyte fungus and 2 cases (1.6%) had three types of fungus such as dermatophytes, yeast and saprophyte in their culture medium (table 3). In healthy subjects, 12 persons (19.2%) in right ear and 9 cases (14.8%) in left ear had positive culture ($P=0.49$). in patients group, 35 cases (56.5%) in right ear and 35 cases in left ear (57.4%) had

positive culture (P=0.917). a significant relationship was not observed between positive or negative culture and right or left ear (table2). It should be noted that none of the patients with chronic otitis had history of drug use.

Table 1: Frequency of fungal culture results and the age of patients with chronic otitis media and healthy

| Age (year) | Results of culture | Groups | Number (percent) | p-value |
|--------------|--------------------|----------|------------------|---------|
| Less than 10 | positive | Patients | 8 (6) | 0.0212 |
| | | Normal | 1 (1) | |
| | negative | Patients | 1 (1) | 0.0212 |
| | | Normal | 9 (7) | |
| 10 - 20 | Positive | Patients | 21 (17) | 0.0393 |
| | | Normal | 5 (4) | |
| | Negative | Patients | 20 (17) | 0.0393 |
| | | Normal | 24 (19) | |
| 20 - 30 | Positive | Patients | 15 (12) | 0.0412 |
| | | Normal | 6 (4) | |
| | Negative | Patients | 15 (12) | 0.0412 |
| | | Normal | 33 (27) | |
| 30 - 40 | Positive | Patients | 7 (6) | 0.0214 |
| | | Normal | 5 (4) | |
| | Negative | Patients | 7 (6) | 0.0214 |
| | | Normal | 20 (16) | |
| More than 40 | Positive | Patients | 16 (15) | 0.0384 |
| | | Normal | 5 (4) | |
| | negative | Patients | 12 (10) | 0.0384 |
| | | Normal | 17 (14) | |

Table 2: The frequency of positive fungal cultures of the external auditory canal

| Ear | Group | Number (percent) | p-value |
|-----------|----------|------------------|---------|
| Right ear | patients | 36 (57) | 0.49 |
| | Normal | 13(19) | |
| Left ear | Patients | 36 (57) | 0.917 |
| | Normal | 52 (85) | |

Table 3: Relationship between the subjects (patients and healthy subjects) with fungal contamination of them

| Type of contamination | Patients group | | Healthy group | |
|-------------------------|----------------|-----|---------------|-----|
| | Number | % | Number | % |
| Single fungal infection | 49 | 45 | 11 | 8.8 |
| Two fungal infection | 11 | 8.8 | 6 | 4.8 |
| Three fungal infection | 8 | 6.4 | 4 | 3.2 |
| Four fungal infection | 1 | 0.8 | 0 | 0 |

Table 4: Relationship between the subjects (healthy and diseased) with mushrooms group

| | Healthy group | | | | Patients group | | | | Total | |
|---------------|---------------|-----|--------|-----|----------------|-----|--------|-----|--------|----|
| | number | % | number | % | Number | % | number | % | number | % |
| Saprophytes | 3 | 5 | 4 | 6 | 22 | 37 | 7 | 12 | 36 | 60 |
| Dermatophytes | 2 | 3 | 1 | 1.5 | 5 | 8.5 | 3 | 5 | 11 | 18 |
| Yeast | 1 | 1.5 | 0 | 0 | 7 | 12 | 5 | 8.5 | 13 | 22 |

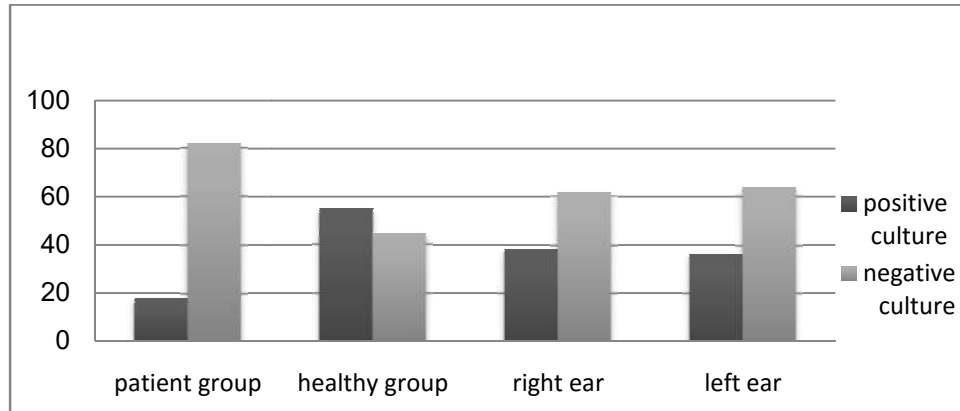


Fig 1: Graphs of the results of fungal cultures in both groups and both ears

DISCUSSION AND CONCLUSION

Evaluation of fungal culture results in healthy and patient subjects showed that in patients 55.2% of cultures was positive that this frequency compared with frequency of healthy subjects (17.6%) showed that significant differences between healthy subjects and patients for the presence of the fungus in the ear discharge. Otomycosis study in different countries like India, Russia, Iraq, Spain, and the results of current study showed a significant difference in the incidence of fungal species in two groups of healthy and infected ear discharge. Common findings of these studies have shown that saprophytic mold fungus species, notably *Aspergillus niger* and the fungus *Candida albicans* are the main generating factor of this infection. In a study that was conducted on 94 suspected patients, the causative factors were reported: *Aspergillus niger* (36.36%), *Aspergillus flavus* (36.36%), *Candida glabrata* (18.18%) and *Candida albicans* (9.09%). In another study was done on 87 patients by Ozcan and colleagues in 2003 in Turkey have been reported that *Aspergillus sp* is the main factor that isolates from 44.8% of patients [13]. A study in Nepal took place on the Otomycosis as a common illness showed that 87 of 100 patients were suspected to otomycosis [14]. The prevalence was similar in both men and women and types of *Aspergillus sp* have been identified as a major factor of infection that is in consistency with our finding [14]. It should be noted that recent studies conducted in India have reported a higher incidence in men [15,16]. The most common fungal infection was in age group 10-20 years with 21% frequency (Table 1). It is probably due to the occupational activity in this age group and also patient employment in occupations such as agriculture and also presence of winds with dust. Also, due to the definite impact of climate on growth, sporulation and survival of various saprophytic fungi this subject is considered as an expected issue. Meanwhile personal and social hygiene level and types of jobs in different populations of patients lead to make differences in the results, in other countries, including Iraq 16-30 years [17], Spain at age 30 [18] and some other studies have reported age of 50 [19]. Identify various types of mold fungus and saprophyte yeasts such as *Aspergillus sp* with endogenous origin and *Candida* with exogenous origin of studied patients in this study are the risk factors for mycosis because *Aspergillus sp* has more than 600 species with rapid growth strength, excellent compatibility with a variety of different climates and abundant production of spores. Large-scale airborne spores of *Aspergillus sp* are present in human environment. *Candida* species, particularly *Candida albicans* remains the most abundant component of stable microflora of skin [22] so that in two groups of patients, is the most common fungal saprophyte group among the three groups and then housed two groups of yeasts and dermatophytes, respectively. In similar studies in Brazil, the most common species, is *Aspergillus sp* (75%), and then *C. albicans* (70%) [20]. Well as in Russia, most common yeast is *candida* (19.2%), in Poland the most common Saprophyte have been reported *Aspergillus sp* (37.1%) and then *candida* (22.9%) [22]. Given that the ear fungal infection is related to the environment and living conditions, therefore, the underlying risk factors should also be taken under control and then proportional to generator disease factor and different sensitivity of fungi to various antifungal drugs considering the appropriate therapy. We can prevent indiscriminate use of antibiotics and lessen the chance of antibiotic resistance in the community and shorten the period of illness and also reduction the stability of disease process.

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