

Etiologic Agents of Otomycosis in the North-Western Area of Iran

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Background: Otomycosis is a superficial fungal infection often involves the pinna and external auditory canal. It is a pathologic condition, with *Candida* and *Aspergillus*, the most common fungal species. It is common worldwide but more prevalent in tropical and subtropical countries

Objectives: The aim of this study was to determine the etiologic agents and local epidemiologic pattern of otomycosis in northwest Iran.

Patients and Methods: A series of 140 patients with clinically symptomatic otomycosis were studied in 21 cities, towns, and villages throughout northwest Iran between 2009 and 2011. Clinical samples were collected by swabs and then assessed by mycological investigation.

Results: Otomycosis was diagnosed in 129 cases (92%, 76 male, 53 female) with the highest prevalence of cases occurring in males between 21 - 40 years of age. From an etiological point of view, 116 patients (90%, 21 - 40 years old) were infected by saprophytic mold and 9 patients (7%) were infected by yeast. Three cases (2%) involved dermatophytes, and in one case (1%) the subject was infected with *Eurotium* (the perfect stage of *Aspergillus fumigatus*). *Aspergillus niger* was the most common mold that was isolated, followed by *A. flavus*, *A. fumigatus*, *Penicillium* spp., *Fusarium* spp., and *Rhizopus* spp. A total of 2 yeasts belonging to genus *Candida*, *C. albicans* and *C. tropicalis*, were isolated.

Conclusions: Our study showed a high prevalence of otomycosis in the northwestern area of Iran. As such, proper diagnosis and treatment by aseptic techniques for this disease is urgently needed.

Keywords: Otomycosis; Fungi; Yeasts; *Aspergillus niger*

1. Background

Otomycosis, or fungal ear infection, is a superficial fungal infection of the ear that may include the pinna, external auditory canal, and tympanic membrane (1). This fungal infection has been reported in both human and animals (2, 3). The fungus is not the actual cause but merely a secondary contaminant in cases of otitis external, therefore otomycosis can be seen in mixed bacterial and fungal infections (3). The causative agents of otomycosis involve various types of fungi, such as hyaline saprophytic mold, dematiaceous saprophytic mold, yeasts, and, rarely, pathogenic molds like dermatophytes (4-8). It is notable that the most common causative agents of otomycosis are from the genus *Aspergillus*, particularly *Aspergillus niger*, which has been established by many studies (9-12), but some authors have collected more than 50 causative fungi species in their studies. These agents include species from the genera *Penicillium*, *Fusarium*, *Mucoraceae*, *Scopulariopsis*,

Alternaria, *Malassezia*, and *Candida*, as well various dermatophytes (13-18).

Based on various studies, the current opinion is that fungi may cause otomycosis when the ear is in poor health. As such, otomycosis is more common amongst people of lower socioeconomic levels or who live under poor hygienic conditions (19-21). The main risk factors for otomycosis include moisture; minor inflammation; the use of broad spectrum antibiotics, steroids, chemotherapeutic agents, or topical ear drops; physical injury; living in warm and humid climates; and frequent bathing or swimming (19, 22, 23). In addition, immunocompromised hosts are more vulnerable to otomycosis. Patients with diabetes, lymphoma, HIV, endocrine abnormalities, changes in hormonal balance, or a history of transplantation, as well as patients receiving chemotherapy or radiotherapy, are at a greater risk of contracting otomycosis than healthy individuals. Tortuosity, di-

iameter, and length of the auditory canal are unrelated to the risk of developing infection (24, 25). In otomycosis, fungi may cause either true initial invasion or secondary invasion after tissue abnormality resulting from a primary bacterial infection (26, 27).

Furthermore, there are various antifungal agents that may be used to treat otomycosis. Mercurochrome (1% - 2% solution), clotrimazole, miconazole, amphotericin B ointment, meta cresol acetate (Cresatin), thymol (1%) in meta cresol acetate, and phenylmercuric acetate (0.02%) in sterile water or as borate ointment (0.005% and 0.04%) can be used to treat patients after cleaning the ear using Burow's solution, urea-acetic acid solution, or aluminum acetate solution (5%) (7, 9, 28). Antifungal agents should be chosen after fungus identification by direct microscopy examinations and culture, because various types of causative fungi are sensitive or resistant to specific types of antifungal agents.

2. Objectives

The present study was carried out to ascertain the etiologic agents and epidemiologic patterns of otomycosis in northwestern Iran.

3. Patients and Methods

The study involved 140 patients who presented with symptoms of otomycosis from 21 cities, towns, and villages in northwest Iran between 2009 and 2011. Patients reported to the ear, nose and throat (ENT) department of Tabriz university of medical sciences and were investigated, prospectively. First of all, clinical details such as chief complaint, name, age, gender, suspected risk factors, hygiene status, occupation, history of infection, address, and other relevant information were recorded. Informed written consent was obtained from all subjects. After establishing a clinical diagnosis, specimen and clinical materials from the external auditory canal were collected from all patients by means of sterile cotton swabs. Materials were divided into two samples for mycological processing. One sample of external auditory debris was clarified with a 10% potassium hydroxide solution on a glass slide. The slide was then heated gently over a low flame and examined directly using a microscope. The morphology, amount of fungi (yeast and mold), and other relevant characteristics (spores, arthroconidia, septate and non-septate hyphae, etc.) were identified.

The second sample of the material was inoculated into two sets of three different media tubes. One tube contained Sabouraud Dextrose agar (BioMerieux, Marcy-1, Etoile, France) with 0.5 mg/mL cyclohexamide (AppliChem GmbH, Germany) and 0.05 mg/mL chloramphenicol (AppliChem GmbH, Germany). A second set contained Sabouraud's Dextrose agar and 0.05 mg/mL chloramphenicol. The third tube contained only Sabouraud's Dextrose Agar. The media were incubated at

room temperature (25°C) and observed for 3 weeks. Cultures were examined every day to determine the probable growth of fungi colonies and their identification.

The identification process of the isolated fungi was carried out through traditional methods, including macromorphological and micromorphological studies. Lactophenol cotton blue preparations were made from the cultures and then examined microscopically. The slide culture technique was also used when a study of the morphological details of various fungi was necessary for exact identification. The isolated yeast species were identified using classic laboratory methods including morphology germ tube production, the production of different spores on corn meal agar (Oxoid Ltd., UK) with Tween 80, and the API 20C AUX identification system based upon the assimilation of carbohydrates (bioMerieux, Marcy L'Etoile, France). All mycological investigation was carried out in the medical mycology department of the Tabriz university of medical sciences. These criteria were used to identify the pathogenic fungus and to begin treatment with an appropriate antifungal agent. The patients were called in for a regular follow-up after four weeks for clinical and mycological reinvestigation, including direct microscopy examinations and repeated cultures.

4. Results

Out of 140 cases submitted for investigation, 129 results were otomycosis-positive (92%, 76 male, 53 female). From an etiological point of view, 116 patients (90%, 21 - 40 years old) were infected by saprophytic mold, and 9 patients (7%) were infected by yeast. Three cases (2%) involved dermatophytes, and in one case (1%), the subject was infected with *Eurotium* (the perfect stage of *A. fumigatus*). In our results, correlations between the pathology of otomycosis with etiology, gender, and age were emphasized. The patients belonged to all age groups, but the highest prevalence of otomycosis (73 cases) was found in patients between 21 - 40 years of age (57% of positive cases). Among all 129 positive cases, 79 subjects (59%) were male and 53 patients (41%) were female, and nearly all were agricultural workers from a low socioeconomic status who exhibited it was the result of a subjective assessment by the clinician. No patients presented with fungal infection elsewhere in the body. However, the patients in whom dermatophytes were isolated also presented with tinea capitis. Additionally, two of the patients infected with *C. albicans* also presented with candidal onychomycosis in their fingernails.

Before seeking medical attention, the majority of patients attempted to self-treat their symptoms with different types of eardrops, including various oils, garlic juice, antibiotics, antiseptics, and steroids, among other unknown types of materials based on traditional public medicine, herbal medicine, and domestic ad-

vice. The most common complaint among patients was an itch in the external auditory canal and tympanic membrane (100%), followed by aural fullness in 68 subjects (53%), ear discharge in 17 cases (13%), and ear ache in 14 patients (11%). Infection of the left ear was seen in 51 cases (39.5%), while the right ear was involved in 54 cases (42%). Bilateral infection was diagnosed in 36 patients (18.5%). The most commonly isolated source of infection was *A. niger*, followed by *A. flavus*, *A. fumigates*, various *Penicillium* species, various yeasts (specifically, a number of *Candida* species), various *Fusarium* species, *Eurotium* (the perfect state of *A. glaucus*), and finally, a species of *Rhizopus* (zygomycetes). Isolated yeasts included *C. albicans* (7 cases) and *C. tropicalis* (2 cases). Isolated dermatophytes included *Trichophyton mentagrophytes* (1 case), *T. verrucosum* (1 case), and *Microsporum canis* (1 case) (Table 1).

Foreign bodies, including dead insects and pieces of cotton cloth, were seen in the external auditory canals of 9 patients (7%). Also, wax was present in the external auditory canal in 8 cases (6%). Of all 129 patients, three were diabetic, but none had a history of systemic corticosteroids, immunosuppressant drugs, endocrine abnormalities, or external auditory canal anatomical abnormalities. The patients were called in for a regular follow-up after four weeks for clinical and mycological reinvestigation, including direct microscopy examinations and repeated cultures. All patients were symptom-free at that point, and no fungal elements were found by otoscopy, direct microscopy, or repeated culture.

Table 1. Etiologic Agents of Otomycosis ^a

Fungal Species	Incidence
<i>Aspergillus niger</i>	73 (62.9)
<i>A. flavus</i>	24 (20.7)
<i>A. fumigates</i>	11 (9.5)
<i>Penicillium</i>	4 (3.4)
<i>Fusarium</i>	2 (1.7)
<i>Rhizopus</i>	1 (0.8)
Unknown	1 (0.8)
<i>Candida albicans</i>	7 (5.4)
<i>C. tropicalis</i>	2 (1.7)
Dermatophytes	3 (2.3)
<i>Eurotium</i>	1 (0.8)

^a Values are presented as No. (%).

5. Discussion

During recent years, there has been a dramatic increase in the incidence of mycotic infections and in the diversity of pathogenic fungi. Otomycosis, a fungal infection of the ear, is found throughout the world (3). However, it is more prevalent in tropical and subtropical countries because a high degree of humidity, hot weather, and the presence of dust in the environment favor the dissemination and growth of molds. Pruritus, otalgia, hearing loss, and ear fullness are the most common features of this infection (29). Otomycosis studies in patients with suspected mycosis have been conducted in many different countries, including the USA (30), Spain (18), Bahrain (31), Brazil (32), Turkey (33), Russia (20), Nigeria (20, 34), Nepal (35), Gabon (32), and Iraq (36). The results of these studies suggest that otomycosis has a global prevalence; in addition, findings from these studies demonstrate that the saprophytic fungus *Aspergillus*, especially *A. niger*, *A. fumigatus*, and *C. albicans*, are the main agents of this infection. In our study, the prevalence of otomycosis was 92%, which is higher than the results found in some other studies, including work by Kumar (37), who found otomycosis in 75.9% of patients; Pardhan et al. (35), who found otomycosis in 79.4% of patients; Kaur et al. (38), who found the disease in 74.7% of patients; Chin and Jegathesan (39), who found it in 74.6% of patients; Aneja et al. (19) who found otomycosis in 78% of patients, and Ozcan et al. (33), who reported that 65% of their patients were positive for otomycosis.

A higher incidence of otomycosis has been observed in males, most of whom are active in the agriculture industry, are of low socioeconomic status, and who exhibit poor personal hygiene. This finding does not agree with results from a study by Aneja et al. (19) in which the prevalence of otomycosis in females was higher than in males. In addition, our study also reported that 57% of all positive cases were found in patients between 21 - 40 years of age, which is similar to findings by Ologe and Nwabuisi (34). A high prevalence in this age group may be associated with occupational activity, as a significant number of individuals in this age range hold jobs involving agriculture, horticulture, harvesting, farm work, and seasonal construction work that often takes place in small cities and dusty environments.

The etiologic agents responsible for otomycosis may be classified into three groups: saprophytic molds, yeasts, and dermatophytes. Furthermore, saprophytic molds may be grouped into ascomycetes and zygomycetes (1, 4, 26). As Table 1 demonstrates, saprophytic molds were the most common etiologic agents, responsible for 116 (90%) cases of otitis external, followed by the yeasts belonging to *Candida* species (9 cases, 7%). The third group, dermatophytes, was observed in 3 cases (2%). Thus, in this study, the rate of saprophytic mold infection was approximately 13 times greater than that of other types of fungi. Other authors have also postulated that saprophytic molds are

the major etiologic agents in otomycosis (18, 19). *Aspergillus niger* was the most common etiological agent in our study. This finding is in accordance with results from Aneja et al. (19) and Fasunla et al. (20). However, a study by Barati found that *A. flavus* was the predominant etiologic mold in cases of otomycosis in central Iran. Since *Aspergillus* includes more than 600 different species of rapid growth, and because it exhibits excellent compatibility with a variety of different climatic conditions as well as the ability to produce an abundance of spores, it is not surprising that airborne *Aspergillus* spores are present in every day life on a large scale. More so, *Candida*, especially *C. albicans*, is one of the most common and adaptable microorganisms in persistent skin microflora. As such, infection with one of these microorganisms in susceptible individuals is expected. After examination and identification of the infectious agent, the patients received antifungal therapy. Patients were instructed not to allow water to enter their ears during bathing or swimming in order to limit fungal growth. After four weeks, all patients were symptom-free with no evidence of fungal elements.

In conclusion, our study showed a high prevalence (92%) of otomycosis in northwestern Iran, a result that is considerably higher than those found in other studies. These results suggest that some strategies must be developed for the proper diagnosis and treatment of otomycosis in this area in order to prevent unnecessary use of antibiotics.

Authors' Contributions

Abdolhassan Kazemi: study design. Abbasali Jaafari: data collection. Seyyed Amin Ayatollahi Mousavi: study design. Ali Zarei Mahmoudabadi: data analysis. Hossein Alikhah: writing article. Maryam Majidinia: data analysis, data collection, article writing, and examination of samples.

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