

A sample of patients affected with chronic respiratory tract infection

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Abstract

Background and objectives: Cryptococcosis is subacute or chronic respiratory disease caused by yeast like fungus called *Cryptococcus neoformans*. The disease may metastasize to CNS and other parts of the body. The objective of the study is to find the prevalence of this disease in a sample of patients in Erbil.

Method: One hundred and twenty eight patients with chronic respiratory tract infection were included in this study. Early morning sputum sample was collected from each patient and subjected to both direct examination and culture

Results: The study showed 24 positive cases of Cryptococcosis, from the total 128 patients examined; 16 of them (66.6%) gave positive culture for *Cryptococcus neoformans* while 8 (33.3%) gave positive culture for opportunistic species of *Cryptococcus*. The latter were immunocompromised individuals.

Conclusion: It is concluded that looking for *Cryptococcus*, is quite significant in patients with chronic respiratory diseases.

Keywords: Cryptococcosis. Chronic respiratory disease, immunocompromised

Introduction

Species of *Cryptococcus* are usually found in soil, on fruits and vegetables.¹ They are encapsulated yeast like fungi, usually urease positive. *Cryptococcus neoformans* is constant pathogen of the genus; its maximum growth temperature is 39.4°C, which usually possesses a large capsule.² Man acquires infection through inhalation of the yeast causing mild or severe reaction in the lung. It may metastasize to other parts of the body like CNS, to initiate chronic meningitis or cyst formation in hemispheres, skin, long bones, joints, eyes and myocardium may be involved.³ The aim of this study is to find the prevalence of the *Cryptococcus* infection in Erbil area.

Method

A total of 128 patients suffering from different chronic respiratory tract infections, were included in this study. Out of them 83 were males and 45 were females. Their age ranged between 18-90 years old. They were recruited from hospitalized patients in Nanakaly and Rizgari teaching hospitals in Erbil city throughout a seven-month period dating from November 2005 to June 2006. All patients included in the study were having a chronic respiratory disease diagnosed by the consultant. Early morning sputum samples were collected in sterile screw-cup bottles and were immediately transported to the Microbiology Lab, Department of Microbiology, Hawler Medical College. Cultures were made on Sabouraud's slant agar, by taking bacteriological loopful of the sputum and spread out on the surface of the media, then incubated both at 37°C and room temperature.²

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Direct examination of the sputum made by making **Wet preparation:**² A drop of sputum to be mounted on a slide; to this, a drop of 10% KOH which contains DM30 (Dimethyl sulfoxide) was added. The slides were examined under high power microscope to observe any yeast cells. Detection of the capsule was made by adding a drop of India ink to the wet preparation and examined under the microscope with low brightness² Cultures were examined daily; any suspected growth was subjected to direct examination, as described above. Detection of any yeast growth was subjected to biochemical tests by conventional methods.¹ As mentioned in detail in Table (1) which included Nitrate utilization and production of the enzyme phenol oxidase.

Results

Positive culture on sabouraud's agar, both at 37°C and room temperature, showed creamy mucoid growth. Twenty four cultures yielded growth of *Cryptococcus* species. Among these 16 (66.6%) were *Cryptococcus neoformans* and 8 (33.3%) were other species of *Cryptococcus*. Table

(1) shows biochemical tests of different species of *Cryptococcus*, that is to say that *Cryptococcus albidus* was the only species to reduce nitrate, Phenol oxidase gave positive results for all species tested, except *Cryptococcus humicola*. The table also shows profuse growth of *C. neoformans* at 37°C, scanty growth for *C. laurentii* and no growth for other species of *Cryptococcus* at this temperature. Table (2) shows API 20 C code number (biotype) of different species of *Cryptococcus* isolated in this work. The numbers represent different species according to their biochemical reactions. Table (3) shows that 5 out of 16 patients with chronic bronchitis were infected with *C. neoformans*; beside one patient with chronic bronchitis showed saprophytic species. On the other hand *C. neoformans* was isolated from 3 out of 23 patients with chronic obstructive pulmonary disease (COPD) while in acute bronchitis *Cryptococcus* was isolated from 2 patients out of 8 diagnosed as acute bronchitis. It was found that the isolated species of *Cryptococcus*, showed significant correlation with the type of the disease.

Table 1: Growth at 37°C and biochemical tests of isolated species of *Cryptococcus*

| Species | Growth at 37°C | Pellicle in broth | NO ₃ utilization | Phenol oxidase |
|--------------------------------|----------------|-------------------|-----------------------------|----------------|
| <i>Cryptococcus neoformans</i> | + | - | - | + |
| <i>Cryptococcus albidus</i> | - | - | + | + |
| <i>Cryptococcus laurentii</i> | -/+ | - | - | + |
| <i>Cryptococcus humicola</i> | - | - | - | - |

Table 2: Yeast biotypes identified by the API20 C Auxanogram

| Yeasts | Code number (sub-biotype) |
|--------------------------------|---|
| <i>Cryptococcus neoformans</i> | 2747073 2357133 2517073 2577173 |
| <i>Cryptococcus albidus</i> | 427761777 2104271 276273 427761777 |
| <i>Cryptococcus laurentii</i> | 6777773 2753773 |
| <i>Cryptococcus humicola</i> | 6777577 677773 |

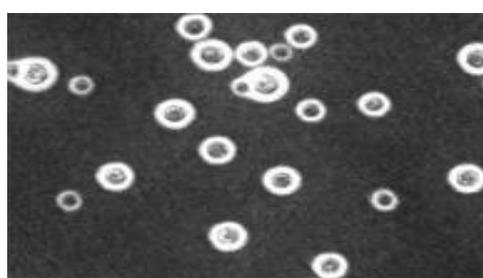


Figure.1: India Ink Preparation of *C.neoformans* shows the capsule around the yeast cells grown at 37 °C.

Figure 2: *C. neoformans* grown on Sabouraud's agar at 37 °C after 48 hrs of growth. Notice the mucoid creamy colonies on the surface of the agar.

Table 3: Prevalence of *Cryptococcus* species by type of respiratory infection

| | | Type of Disease | | | | | | | | % |
|--------|------------------------|--------------------|------------------|-----------|--------|------|----|--------------|-------|-------|
| | | Chronic bronchitis | Acute bronchitis | Pneumonia | Asthma | COPD | TB | Cor-pulmonal | Total | |
| Growth | No growth | 2 | 5 | 4 | 42 | 12 | 3 | 1 | 69 | 53.90 |
| | <i>Cry.neoformans</i> | 5 | 2 | 2 | 0 | 3 | 3 | 1 | 16 | 12.5 |
| | <i>Cry.albidus</i> | 0 | 0 | 1 | 2 | 1 | 0 | 0 | 4 | 3.12 |
| | <i>Cry.humicola</i> | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 2 | 1.56 |
| | <i>Cry.laurentii</i> | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 2 | 1.56 |
| | <i>Candida species</i> | 8 | 1 | 9 | 5 | 6 | 5 | 1 | 35 | 27.34 |
| | Total | 16 | 8 | 16 | 50 | 23 | 12 | 3 | 128 | 100 |

Discussion

To the best of our knowledge this is the first study to be conducted on fungal infections in patients suffering from chronic respiratory illness at Kurdistan region, this study revealed that *Cryptococcus* is not uncommon co-infection in patients with chronic respiratory illnesses, this is an important finding and its significance is substantiated by knowing the fact that 16 cases (66%) of the *Cryptococcus* were caused by *Cryptococcus neoformans* which is well known to be pathogenic and worsen the health status of the afflicted patient. On reviewing the literature our finding quite agrees with similar studies done in other countries.⁵ Interestingly enough the remainder 8 cases of Cryptococcosis were isolated from debilitated patients and were caused by other species of *Cryptococcus* (non-*neoformans*) namely *Cryptococcus humicola*, *Cryptococcus albidus* and *Cryptococcus laurentii*, although no fatality was reported in our study but literature review reveals cases of chronic meningitis caused by *Cryptococcus laurentii* and a fatal case of Cryptococcaemia caused by *Cryptococcus albidus* in a patient with AIDS.⁶ This means that the opportunistic species may cause disease in immunocompromised individuals.⁷ In our study the commonest respiratory illnesses associated with *Cryptococcus* infection were chronic bronchitis, COPD and pulmonary tuberculosis this is in agreement with findings of other workers.⁸⁻¹⁰ What attracts attention is that there was a number of saprophytic species of *Cryptococcus* isolated in these cases. *C. neoformans* yielded a good growth at 37 °C; probably due to synthesis of the heat shock protein(hsp), their definition is inducible by heat shock. This heat shock protein plays a key role in systemic infection.³ From the above data presented, it seems that *Cryptococcus* is an important coincidental event in patients with chronic pulmonary diseases; therefore laboratory investigations should be directed to detect *Cryptococcus* species in such patients.

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