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RESEARCH ARTICLE

Diagnosis of Pulmonary Cryptococcosis in Respiratory Specimens from Immuno Compromised Iraqi Patients

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Abstract

Background: *Cryptococcus neoformans*, the causative agent of cryptococcosis, is a common encapsulated fungus that can cause a scope of sicknesses. Inhalation of organisms is the standard course of the disease that may stay spread to the lungs or experience hematogenous disseminations and reach to any organ through the body. Objective: The goal of this study was to determine the pulmonary Cryptococcosis in a specimen of immune compromised patients by utilizing traditional and molecular strategies. Methods: This study included a total of two hundred (n=200) clinical samples from 100 immuno compromised patients and control group included 100 samples from immune competent individuals were enrolled in this study during the period from the January2015 to February 2016. Result: Among those 100 immune compromised patients 43 (43%) were males and 57 (57%) were females , their mean ages was 54.56 ±16.46 years which ranged from 16 to 90 years, compared with 100 samples from apparently healthy individuals used as a control group. Out of 20 patients were PCR positive for pulmonary Cryptococcus's 12(60%) were females and 8 (40%) were males. Five (5%) samples out of 100 immuno compromised patients were nigrosine stain positive; Three out of 100 samples were culture positive for *C. neoformans.* Conclusion: diagnosis and treatment of pulmonary cryptococcosis are as yet difficult. In any case, with early diagnosis and appropriate management, most non-AIDS patients with pulmonary cryptococcosis have a good prognosis

Keywords: *Pulmonary cryptococcosis, Cryptococcus neoformans, Bronichoalveolar lavage (BAL), Pleural effusion (PE), Nigrosin stain.*

Introduction

Cryptococcus neoformans, the causative agent of cryptococcosis, is a common encapsulated fungus that can cause a scope of sicknesses including a lethal infection of the central nervous system (CNS).Inhalation of organism sis the standard course of the disease that may stay spread to the lungs or experience hematogenous disseminations which reach to any organ through the body [1].

The major ecological wellsprings of *C. neoformans* have been appeared to be either soil contaminated with pigeon droppings if there should be an occurrence of *C. neoformans* or Eucalyptus trees and rotting wood framing hollows in living trees if there should arise An*c. gattii* [2]. Cryptococcus disease can happen in people with normal immunity but is more common in the immune compromised host. Predisposing factors include acquired immunodeficiency syndrome (AIDS), hematological malignancy, organ transplantation, and corticosteroid and other crippled disease [2]. Be that as it may, pulmonary cryptococcosis in subjects with no identifiable immunologic imperfections may resolve spontaneously and not require antifungal treatment [3] despite the fact that it can every so often be extreme and savage [4].

Radiographic features and laboratory investigations of pulmonary cryptococcosis are generally non-specific, so it might effortlessly be misdiagnosed or under diagnosed.. Manifestations of Pulmonary Cryptococcosis in immune-compromised hosts

are quite variable; seldom subclinical, to extreme condition with substantial mortality [5] In symptomatic patients, the clinical features are nonspecific and can simulate a wide range of other respiratory distress diseases [6]a definitive diagnosis of Pulmonary Cryptococcosis requires identification of the organism by culture or India ink staining of samples obtained by Bronchoalveolar lavage (BAL) or tissue biopsy[7].

PCR offers an amazing option for the early finding of cryptococcosis contrasted with customary strategies, as it is quick, can recognize low fungal load, and can be utilized for a little sample size [7]. The goal of this study was to determination of Pulmonary Cryptococcosis in a specimen of immune compromised patients by utilizing traditional and molecular strategies.

Material and Methods

Patient's Selections

Two hundred clinical samples (N= 200) were collected from 100 immuno compromised patients (43 men, 57 women, average age 16-90 years) including 70 samples Bronichoalveolar lavage (BAL), 21 sputum and 9 plural fluid with underlying 22 leukemia, 17 solid tumor, 15 lymphoma, 12 chronic pulmonary obstructive, 10 asthmatic steroid therapy, 10 Rheumatoid arthritis under cytotoxic therapy, 8 solid organ transplantation and 6 multiple myeloma with clinical and radiological suspension of pulmonary Cryptococcosis. And 100 from apparently healthy individuals as control group. Samples were collected from admitted and out patients who attended of different hospitals in Baghdad during the period from January2015 to February 2016.

Sample Collections

Bronchoalveolar Lavage (BAL)

Were collected by specialized physician about 5-10 ml from fluid were dispends in to sterile test tube and transferred in cold container.

Induced Sputum Sample (IS)

Were obtained by induction in patients involved in this study and collected by using sterile screw cup and separated into two portions then treated with normal saline

Pleural Effusion Samples (PE)

After collection of 5-10 ml of PE in sterile container immediately transferred to the laboratory for processing

Sample Processing

All samples were treating with 0.9% NACL and mixed vigorously by using vortexes then centrifuged at 3000 rpm for 5 minutes[8] the supernatant aspirated with a sterile pipette into a sterile tube, then was tested by Latex agglutinations assay (LAT) for Cryptococcal antigen, sediment was re-suspended and separated into two portion first one for direct diagnosis by negative stain (nigrosin stain) and indirect by inoculated one to two drops of the sediments in Sabouraud dextrose agar incubation temperature was 30 ˚C times for 2 days, while the second portion was stored in -20ºC till DNA extractions.

All samples were inoculated and processed in class II biological safety cabinets (BSC) under sterile conditions. DNA was extracted from each sample using a freezing–thawing technique [9] to cell lysis, and the Wizard genomic DNA purification kit (Promega) to purify DNA. The concentration and purity of the purified DNA was quantified by the use of nano drop instrument following the instruction of the manufacturer, Two primers were designed according to internal transcribed spacer regions of ribosomal DNA found in *Cryptococcus neoformans* were used Primer sequence (5' to3') URA5 gene Forward (5'-ACGGTGAGGGCGGTACTATG -3') Reverse (5'-AAGACCTCTGAA CACCGTAC -3') (Bio Corp Canada) to produce a DNA fragment of 345 bp (10).

The thermo cycling conditions with a cleaver scientific thermal cyclers (TC 32/ 80- UK) were as follows: After initial denaturation at 94°C for 5 min, the 35-cycle amplification profile consisted of 94 °C for 30 s, 50°C for 30 s and 72°C for 1 min. Final elongation was at 72 °C for 5 min.PCR products were evaluated with a 1.5% (wt/vol) agarose gel (Merck- Germany) at 120 mV for 30 min. A molecular marker (1-kb DNA ladder; Bioneer) was run concurrently. The DNA bands were visualized and photographed under UV light after the gel was stained with ethidium bromide.

Results

Conventional Methods

This study included a total of two hundred (n=200) samples of (Bronchoalveolar lavage (BAL), sputum and pleural fluids) were collected from 100 immune compromised patients with underlying different diseases; As 22 (22%) leukemia, 17(17%) solid tumor, 15(15%) lymphomas, 12(12%) chronic obstructive pulmonary disease(heavy smoker), 10(10%) asthma(steroid therapy),10(10%) rheumatoid arthritis(cytotoxic therapy), 8(8%) solid-organ transplantation and 6(6%) Multiple myeloma, all patients were HIV-negative by serological test. Control group included 100 samples from apparently healthy individuals. Among those100 immune compromised patients 43 (43%) were males and 57 (57%) were females (Table 1), their mean ages was 54.56 ±16.46 years which ranged from 16 to 90 years, compared with 100 samples from apparently healthy individuals used as a control group. Out of 20 patients were PCR positive for pulmonary Cryptococcus's 12(60%) were females and 8 (40%) were males.

Table 1: Gender distribution among patients and control group

Gender type	Patients	Control
Female	57(57.0%)	50 (50.0%)
Male	43(43.0%)	50(50.0%)
Total	100(100.0%)	100(100.0%)
p value	0.198*NS	

*NS: No-significant difference was demonstrated

Five (5%) samples out of 100immunocompromised patients were nigrosine stain positive which appeared as oval to spherical in shape surrounded by capsule. Three out of 100 samples were culture positive for *C. neoformans* which were creamy colored and mucoid colonies on Sabouraud agar incubation temperature was 30˚Ctimes for 2 days Figure (1) regarding results of Cryptococcal Antigen Latex Agglutination System 12 (12%) samples out of 100 samples were positive for latex agglutination test. All control group (n=100) were negative by staining, culturing and latex system. Amplification of URA5 gene by conventional PCR for detection of *C. neoformans* revealed that 20out of 100 samples were positive with amplifications product size (345)bp Figure (2).All Cryptococcus Antigen Latex Agglutination System 12 samples were positive by PCR. All control group were negative by conventional PCR.

Figure 1: Sabouraud dextrose agar with cream coloured and mucoid colonies of Cryptococcus

Figure 2: Gel electrophoresis (2% agarose,7v/cm²,1.5hrs) of the PCR products, lane1(MW): One hundred base pairs DNA ladder; lane(3,6,8-10): Postive sample for Cryptococcal neoformans gene (345bp); lane2, 4-5: Negative sample; lane 7: Negative control

Discussion

Pulmonary cryptococcosis is an opportunistic infection. Most cases are caused by Cryptococcus neoformans or Cryptococcus gattii infection [11, 12] the current study found that pulmonary cryptococcosis is notrare in patients with chronic debilitated disease because this conditions lead to weaken of cell-mediated immunity and the resistant to cryptococcosis disease depends primarily on it [13] among those 100 immune compromised patients were enrolled in this study, the mean age of the patients was 54.56 ±16.46 years which ranged from 16 to 90 years.

This finding agrees with those published by Yuan et al [14], Hassan F et al [15] it was found that, Out of 20 patients were PCR positive for pulmonary Cryptococcus's 12(60%) were females and 8 (40%) were males, This result was in agreement with the finding Jin-Quan Yu et al [16], Zhang H.[17] and this might reflect the fact that housewives in our society are more prone to exposure to cryptococcal infections as a result of home business that requires cleaning animals and poultry houses, Although pulmonary cryptococcosis is generally an airborne disease, exposures to soil or poultry prior to onset are common. In this study, regarding diagnosis of pulmonary Cryptococcusnigrosine staining method was performed, five samples were positive by staining three of them were confirmed later by Sabouraud dextrose agar culture. Positive culture result is very important to the diagnosis, but is less sensitive than latex agglutination test, because culture techniques requires viable organisms, and this is a problem in partially treated patients and is also influenced by the collection and processing of the specimens may be due to paucibacilliary nature Cryptococcus [17].

The current study the sensitivity and specificity of latex agglutination test for Pulmonary Cryptococcus were 100 %.compared with PCR, Negative and positive predictive values (NPV&PPV)) were 100 % for each values. These results corresponded with these mentioned by Willem, et al [18]; Kiska, et al [19].There was 100% concordance between latex agglutination and PCR in all the samples, demonstrating that both tests are comparable and this was accordance with finding of Shah Hetal[20].In conclusion, diagnosis and treatment of pulmonary cryptococcosis are still challenging. However, with early diagnosis and appropriate management, most non-AIDS patients' withpulmonary cryptococcosis has a good prognosis

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Conflict of Interest

The author declares that they have no competing interests.

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