Study of fungal infection in Iraqi patients suffering from chronic cough and antifungal sensitivity test in vitro for the fungi isolated from these patients

Heba F. Hassan,1 Nada S. Hassain,2 Zainab A. Aldhaher,1 Rasha M. Shaker,1 Jamal A. Tawfeeq1 and Ghada Ibraheem1

1Department of Basic Sciences, College of Dentistry, University of Baghdad, Iraq.
2Ministry of Health, Medical City, Teaching Laboratory, Baghdad, Iraq.
3Department of Animal Production, Faculty of Agriculture, Baghdad University, Baghdad, Iraq

ABSTRACT

Aim and Objective: This study was undertaken to determine isolation of Aspergillus species and candida species from sputum samples in patients suffering from chronic cough and used antifungal sensitivity test in vitro for the fungi isolated from these patients and for the purpose of access to the remarkable progress in the field of fungal treatment.

Methods: Sputum samples were collected from 62 patients and controls for isolation of Aspergillus species and yeast and in vitro antifungal sensitivity test.

Results: A total of forty sputum samples from the patient with chronic cough were isolated and identified, the result of fungal growth of sputum samples, the isolates are Aspergillus fumigatus 14 (35%) and Candida albicans 12 (30%). It was revealed that Amphotericin B 24 (60%) was more sensitive and effective antifungal drugs while Ketoconazole 27 (67.5%) was more resistant and poor antifungal drug.

Conclusion: The present study revealed that Aspergillus fumigatus was he present study revealed that Aspergillus fumigatus was more species isolates from sputum samples from the patients with chronic cough and Amphotericin B was more sensitive and effective antifungal drugs for the fungi isolated from sputum samples.

Keywords: Chronic cough, sputum, antifungal sensitivity test, Aspergillus fumigatus, Candida albicans

INTRODUCTION

Cough is defensive reflex mechanism that clears secretions from the upper airways of the respiratory tract by stimulation of a complex reflex arc due to respond to chemical or mechanical irritant stimulation [1]. Cough can be classified as acute, subacute, or chronic depend on duration. Acute cough lasts up to 3 weeks, subacute cough lasts 3-8 weeks, and chronic cough lasts longer than 8 weeks. The prevalence of chronic cough is estimate between 11 and 20% [2,3]. This type of cough is observed commonly in women and in obese individuals [4]. Chronic cough that cannot be controlled after basic evaluation is a common reason for referral to respiratory outpatient clinics [5]. Despite heedful diagnostic evaluation and numerous treatment regulations [6], a diagnosis cannot be made in a subgroup of chronic cough patients. This condition is termed by chronic idiopathic cough (CIC) [7,8], which is defined as an uncontrolled cough that difficult treated by any current treatment [9]. Recently encountered a groups of patients with allergic fungal cough (AFC), which is difficult and is characterized by sensitization to Bjerkandera adusta [10], among a new clinical disease concept termed fungus associated chronic cough (FACC) with the following manifestations: chronic cough, the presence of environmental fungi or in the sputum and good clinical response to antifungal drugs [11]. In the last three decades, fungal infection especially pulmonary aspergillosis has become important cause respiratory tract infection. The increase in frequency is mainly due to use of broad spectrum antibiotics, intensive cytotoxic therapy, corticosteroids and Immuno-suppressants [12]. Diagnosis of pulmonary aspergillosis is usually missed as test of their detection cannot carry out in routine diagnostic laboratories [13]. Aspergillus species are ubiquitous fungi, usually occurring in water, soil and decaying vegetation. Route of
infection by inhalation of fungal spores. From lungs, spread takes place leading to systemic  
spergillosis. Appearance of aspergillus infection in  
association with chronic lung disease like  
pulmonary TB, asthma, lung abscess,  
bronchopneumonia, and lung malignancy has been  
documented. There are 185 species of genus  
aspergillus, only 20 can cause human infection.  
Aspergillus fumigatus is the most common species  
causes human infection all over the world  
[14]. Antifungal therapy have provided a new treatment  
strategy for chronic difficult cough, included in  
unexplained chronic cough the efficacy of  
antifungal treatment for chronic idiopathic cough  
has not been evaluated yet [15]. Now in vitro  
antifungal susceptibility tests are mainly used for  
epidemiological study, determination of the degree  
of antifungal activity, and the prediction of clinical  
outcome depend on an optimization of antifungal  
treatment [16].

METHODS AND METHODOLOGY

Patients and controls: Forty patients attending  
the respiratory outpatient clinic at medical city in  
Baghdad suffering from chronic cough from  
October 2013 till April 2014 were eligible in this  
study; they were all diagnosed by specialist  
physician. In addition thirty apparently healthy  
volunteers were considered as control group their  
age and gender matched with patients group were  
enrolled in current study.

Collection of the samples: Sputum sample was  
collected from each patient and control in sterile  
cup then this sputum samples was separated from  
saliva by used centrifuge then streaked in duplicate  
plates on Sabouraud Dextrose Agar (SDA) media  
with chloramphenicol , one plate of inoculated  
incubated at 25 °C and the other at 37 °C for 2-5  
days. The isolates were transferred in vial contain  
distilled water (D.W) and stored at room  
temperature.

Fungal growth: Fungal growth was identified by  
colony morphology and lacto phenol cotton blue  
stain while yeast growth was differentiated by use  
of API for candida species diagnosis.

Antifungal susceptibility testing: Disc diffusion  
method by using different antifungal disks at the  
following potencies: Clotrimazole (10mcg/disk),  
Fluconazole (25mcg/disk), Ketoconazole  
(10mcg/disk) and Miconazole (10mcg/disk),  
Amphotericin B (20mcg/disk), Nystatin (100  
IU/disk), Econazole(10mcg/disk). The isolates  
were transferred from D.W stocks to SDA agar to  
enhance sporulation. Then 0.1ml was taken of  
growth suspension (10⁶ cells / ml) was put on  
surface of Sabouraud Dextrose Agar (SDA) media  
and spread by using glass spreader L-shaped , left  
to dry for (10-20) minutes, then antifungal disks  
were applied to the plates, after which the plates  
were incubated at (25°- 37)C° for (24-48) hr. after  
the colonies grew, the activity (susceptibility&  
resistance) of each antifungal disks were measured  
of diameter of the inhibition zone around each  
disks using the ruler [17].

Statistical analysis: The statistical analysis of this  
prospective study performed with the statistical  
package for social sciences (SPSS) 21.0 and  
Microsoft Excel 2013. Categorical data formulated  
as count and percentage. Chi-square test used to  
describe the association of these data.

RESULTS AND DISCUSSION

Distribution of Patients and Controls According  
To Age and Gender: The mean age of patients  
with chronic cough was 39.8 ± 13.37 years, there  
was statistically significant difference (p>0.05) in  
gender between both studied groups (figure 1), but  
there was male predominance among patients, there  
were 24 (60%) of chronic cough patients males,  
while only 16 (40%) of patients were females  
(table 1, figure 2). The present result showed that  
chronic cough is more prevalent in patients with  
with mean age (39.8) and this result is consistent  
with other study reported by Shrimali  
[12]. Also the current results denoted a predominance  
of chronic cough among males than females which is  
comparison with other study conducted by Rai who  
indicated that chronic cough occurs more  
commonly in females [9].

The isolation: A total of forty sputum samples  
from the patient with chronic cough were isolated  
and identified, the result of fungal growth of the  
sputum samples are shown in table (3) , the isolates  
are Aspergillus fumigatus 14 (35%) , Candida .  
albicans 12(30%) , mixed infection with  
Aspergillus fumigatus & Candida . albicans  
17 (42.5%) and no growth 31(77.5%) in patients  
samples and no growth in healthy controls as  
clearly shown in Table 2. these results agree with  
Shrimali et al.[12]. Also Daleine et al. was  
observed that prevalence of Aspergillus species  
was 15.1% in sputum specimens [18].Pursell and  
Paredes were found that 4.8% had been colonised  
with Aspergillus species depend on positive culture  
of the sputum samples [19]. Johnson concluded that  
fungal growth and Candida in respiratory
secretions can be associated with chronic sputum production, bronchiectasis and mucous plugging. Fungal bronchitis is typically ignored, and lead to significant morbidity and sometimes mortality. Antifungal treatment for Candidal bronchitis led to improvement in patient symptoms and clinical status, while early stopping of treatment lead to recurrence. Controlled clinical experiment are needed to determine whether therapy guidelines should be modified to support effective identification and antifungal treatment for such patients [20], and used the corticosteroids cause impaired clearance and killing of Aspergillus fumigatus conidia, and has also been associated with enhanced growth of Aspergillus species[21]. The spectrum of pulmonary fungal infection is wide. While invasive fungal infection and aspergillosis are known, chronic bronchitis and mucous plugging association to fungal infections are not. Most studies on pulmonary fungal infection refused fungi isolated from sputum, requiring fungus from tissue or pleural fluid culture [22]. There is some recognition that fungi in sputum has adverse health effects. Aspergillus tracheobronchitis characterize aspergillosis in sputum with thick mucous plugs [23]. C. albicans cultured from sputum of cystic fibrosis patients is related with increased hospital-treated exacerbations [24]. Many patients had prior bacterial bronchitis or pneumonia. Fungal bronchitis may be a risk factor for bacterial infection. C. albicans is a risk factor for pseudomonas pneumonia in the intensive care unit, prolonged glucocorticoid, long-term antibiotics and diabetes use have increased colonization with C. albicans and other fungi [25].

The susceptibility to antifungal drugs: Regarding the data, it was revealed that Amphotericin B 24(60%) , Econazole 19(47.5%), Clotrimazole17(42.5%) respectively were more sensitive and effective antifungal drugs while Ketoconazole 27(67.5%), Fluconazole 26(65%) , Nystatin 26(65%) respectively were more resistant and poor antifungal drug activity table 3. Amphotericin B has a wide spectrum of activity that included the majority of yeast and mould isolates, there have been reports submit that some strains of Candida lusitaniae and Trichosporon spp. are less susceptible to amphotericin B than other yeast species [26]. This results agree with study in India conducted carried out antifungal susceptibility testing showed 16.7% were resistant against Fluconazole and 9.5% resistant strains of Candida against Nystatin[27]. Antifungal susceptibility testing methods are useful to detect antifungal resistance and to define the best therapy for a specific fungus. Clinical microbiology depend on these methods to select the therapy for fungal infection, and to know the local and the international epidemiology of antifungal resistance [28].Fungal infections are a major cause of morbidity and mortality in spite of the latest developments of diagnostic tools and treatment options. Early initiation of the correct antifungal treatment has been demonstrated to have a direct effect on the patient’s [29]. New chronic lung infections have been described with a wide effect on the patient’s quality of life, and a high cost of therapy and care [30]. Candida, Aspergillus, cryptococcus and Pneumocystis are the main etiologic factor of fungal infections [31]. The burden and mortality related with these diseases based on the area and the affected population [32], for the time being, three main families of antifungals drugs are used clinically to treat fungal infections: polyenes represented by amphotericin B , azoles with different derivatives such as itraconazole, fluconazole, voriconazole, posaconazole, isavuconazole; and the echinocandins caspofungin, micafungin and anidulafungin. The increased use of antifungals drugs has encouraged a higher selective pressure on fungal strains and resistance has arisen in two main ways: several species have developed secondary resistance and susceptible species have been replaced by resistant ones, changing the epidemiology of fungal infections [33]. Antifungal resistance is becoming an emerging problem. On the one hand, there is the intrinsic resistance, and on the other hand the development of secondary resistance that should be diagnosed because resistant strains are related with poorer outcomes, intrinsic resistance of C. glabrata and C. krusei to fluconazole is well known [28]. During antifungal treatment acquired resistance in Candida spp. infections has also been reported and C. albicans, C. tropicalis and C. krusei, have also confirmed for developing secondary resistance [34]. Change on genes encoding the target enzymes of these therapy (14 alpha sterol demethylase (ERG11) or up regulation of multidrug efflux transporters for azoles groups [35].

CONCLUSION
The present study revealed that Aspergillus fumigatus was he present study revealed that Aspergillus fumigatus was more species isolates from sputum samples from the patients with chronic cough and in vitro antifungal sensitivity test used to determine antifungal resistance and define the best therapy for a specific fungus, Amphotericin B was more sensitive and effective antifungal drugs while Fluconazole was more resistant and poor antifungal drug activity for the fungi isolated from sputum from the patients with chronic cough.
Figure -1: Distribution of chronic cough Patient and Healthy Control according the age

Table-1: Distribution of chronic cough Patient and Healthy Control According the Gender

<table>
<thead>
<tr>
<th>Gender</th>
<th>Study</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
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<tr>
<td>Male</td>
<td>24</td>
<td>60</td>
</tr>
<tr>
<td>Female</td>
<td>16</td>
<td>40</td>
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<tr>
<td>Total</td>
<td>40</td>
<td>100</td>
</tr>
</tbody>
</table>

Figure-2: Distribution of chronic cough Patient and Healthy Control According the Gender

Table-2-Isolation and identification of fungal culture from sputum samples and controls

<table>
<thead>
<tr>
<th>Species</th>
<th>No. sample</th>
<th>%</th>
<th>Healthy control</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus.fumigatus</td>
<td>14</td>
<td>35</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Aspergillus + Candida albicans</td>
<td>3</td>
<td>7.5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>12</td>
<td>30</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>NO growth</td>
<td>11</td>
<td>27.5</td>
<td>30</td>
<td>100</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>100</td>
<td>30</td>
<td>100</td>
</tr>
</tbody>
</table>

Table-3- the sensitive and resistant of antifungal drugs

<table>
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<th>Resistant</th>
<th>Sensitive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>AMP</td>
<td>5</td>
<td>12.5</td>
</tr>
<tr>
<td>NS</td>
<td>26</td>
<td>65</td>
</tr>
<tr>
<td>KT</td>
<td>27</td>
<td>67.5</td>
</tr>
<tr>
<td>FLC</td>
<td>26</td>
<td>65</td>
</tr>
<tr>
<td>ECO</td>
<td>10</td>
<td>25</td>
</tr>
<tr>
<td>MIC</td>
<td>18</td>
<td>45</td>
</tr>
<tr>
<td>CLO</td>
<td>12</td>
<td>30</td>
</tr>
</tbody>
</table>

*High significant


REFEERENCES