Sudan University of Science and Technology

College of Graduate studies

Detection and susceptibility testing of *Aspergillus* spp from sputum of Asthmatic and Cystic fibrosis patients in Khartoum State

الكشف واختبار الحساسية لنوع الرشاشات في عينات التفاف لدى مرضى الازمة والتليف الكيسي بولاية الخرطوم

A thesis Submitted is Partial Fulfillment for the Requirement of M.Sc. Degree in Medical Laboratory Science (Microbiology).

Submitted by:

Israa Khalid Ahmed Mohammed

(BSc. Honor in Medical Laboratory Science (Microbiology), Sudan University of Science and Technology, 2015).

Supervisor by:

Dr. Ehssan Hassan Osman Moglad

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قال تعالى:

(أَمَّنْ هو قانتٌ آناءَ اللَّيلِ ساجداً وقائماً يَحذَرُ الآخرةَ ويَرجو رحمةَ ربِّهِ قلْ هل يستوي الَّذين يعلمونَ والَّذين لا يعلمونَ إنَّما يَتَذكَّرُ أولوا الألباب)

الزمر الآية9

DEDICATION

This M.Sc. thesis dedicated firstly in the memory of my mother to whom I pray god to preserve her place in paradise.

Secondly to my father for his constant love, patience, strength and assistance.

Lastly to my brothers, sisters and cousins without forgetting my dearest friends who gave me endless amount of love, support and encouragement.

ACKNOWLEDGEMENTS

My profound gratitude and appreciation goes foremost to Allah Almighty who enabled me to accomplish this work. My profound gratitude goes to **Dr. Ehssan Hassan‹** for her support, supervision, confidence and her time, wish her a good health. Special thanks to **Dr. Samar Mohamed Saeed** for technical assistance and support.

My profound gratitude also goes to all those who assisted me to carry out this study namable and unnamable.

ABSTRACT

This study was a cross-sectional case study, conducted during the period from February to May 2018 at El Shaab Teaching Hospital. The practical part was carried out in Research laboratory of medical laboratory science collage, Sudan University of Science and Technology. The main objective of this study was to study allergic bronchopulmonary aspergillosis in asthmatic and cystic fibrosis patient and found an effective treatment of it in Khartoum State. A total of 100 sputum specimens were collected from asthmatic (75) and cystic fibrosis patients (25), 43 samples from male and 57 from female. The sputum specimens were processed for isolation of Aspergillus species by standard method. *Aspergillus* species were isolated from 5(5%) out of 100 sputum sample. All positive *Aspergillus* species samples processed for estimation of MIC of voriconazole and itraconazole by E. test. There were no significant association between the prevalence rate of ABPA in Asthmatic and Cystic fibrosis disease (*P value*=0.42).

This study concluded that, the frequency of ABPA in Asthmatic (4%) and cystic fibrosis patient (8%) represent (5%) from all sample and voriconazole is more effective than itraconazole against *Aspergillus*.

المستخلص

هذه الدراسة كانت في الفترة من فبراير الى مايو 2018، تم جمع العينات لهذه الدراسة من مستشفى الشعب التعليمي، الجزء العملي اجري في معمل الأبحاث لكلية المختبرات الطبية، جامعة السودان للعلوم والتكنولوجيا.الهدف الرئيسي لهذه الدراسة عزل فطر الرشاش من مرضي الازمة وتليف الرئة ومعرفة العلاج الفعال من مضادات الفطريات في ولاية الخرطوم.تم جمع مائة عينة نخامة، ثلاثة واربعون عينة من الذكور (43%) وسبعة وخمسون من الاناث (57%). خمس وسبعون من مرضى التليف الرئوي وخمس وعشرون من مرضى الازمة. تمت معالجة العينات لعزل فطر الرشاش باستخدام طريقة العزل المثالية.

أظهرت النتائج ان الفطر موجود في (5%) من العينات. تم معالجة كل عينات الفطر الرشاش لتقدير درجة الحساسية أيضا كانت العلاقة غير مؤثرة بين نسبة انتشار فطر الرشاش بين مرضي ضد الاتراكونازول والفوريكونازول. التليف الرئوي و الازمة.

خلصت الدراسة الي ان نسبة انتشار الفطر قد كان 5%من مجموع العينات الكلي، وكانت نسبة الفطر (4%) من مرضي الازمة (8%) من مرضي تليف الرئة. وان الفوريكونازول هو أكثر فعالية من الاتراكونازول.

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Abbreviation

ABPA	Allergic bronchopulmonary aspergillosis.
LPCB	Lacto Phenol Cotton Blue.
CF	Cystic Fibrosis.
Af	Aspergillus fumigatus.
IgE	Immunoglobulin E.
MIC	Minimum Inhibitory Concentration.
ERCF	Epidemiologic Registry of Cystic Fibrosis.
ISHAM	International Society for Human and Animal Mycology.
BAL	Broncho alveolar lavage.
GM-CSF	granulocyte/macrophage- colony stimulating factor.

CHAPTER ONE

INTRODUCTION

CHAPTER ONE

INTRODUCTION

1.1. Background:

AllergicBroncho Pulmonary Aspergillosis (ABPA) is a complex clinical entity that results from an allergic immune response to *Aspergillus fumigatus*, most often occurring in a patient with asthma or cystic fibrosis. Sensitization to *Aspergillus* in the allergic host leads to activation of T helper 2 lymphocytes, which play a key role in recruiting eosinophil and other inflammatory mediators. ABPA is defined by a constellation of clinical, laboratory, and radiographic criteria that include active asthma, serum eosinophilia, an elevated total IgE level, fleeting pulmonary parenchymal opacities, bronchiectasis, and evidence for sensitization to *Aspergillus fumigatus* by skin testing. Specific diagnostic criteria exist and have evolved over the past several decades. Staging can be helpful to distinguish active disease from remission or end-stage bronchiectasis with progressive destruction of lung parenchyma and loss of lung function (Patterson and Strek, 2010).

The population prevalence of ABPA is not clearly known, but the prevalence in asthma clinics is reported to be around 13% (Agarwal, 2009)and is a major complication in CF patients(De Baets et al., 2018). prevalence of 2% to 15% in people with cystic fibrosis (Perisson *et al*., 2017)

Allergic Bronchopulmonaryaspergillosis (ABPA), an immunologically mediated lung disease, occurs predominantly in patients with asthma. This chronic relapsing disorder ranges clinically from mild asthma to fatal destructive lung disease and is caused by hypersensitivity to colonized *Aspergillus fumigatus* (*Af*) (Shah, 1998).

The disorder was first described by Hinson *et al*., in 1952 from the U.K. whereas the first report from India was published almost two decades later. Despite, six decades of research, the disease remains elusive and is often misdiagnosed as pulmonary tuberculosis, in India. Allergic Aspergillosis clinically presents with poorly controlled asthma (Agarwal *et al*., 2014).

Allergic bronchopulmonary aspergillosis (ABPA) in asthma is a severe, life-affecting disease that potentially affects over 4.8 million people globally. In the UK, ABPA is predominantly caused by the fungus *Aspergillus fumigatus*(Overton *et al*., 2018)

Allergic bronchopulmonary aspergillosis management includes corticosteroids to control the host immune response and antifungal agents to decrease the burden of the organism. Itraconazole is currently the first-line agent for symptomatic ABPA patients based on randomized, controlled clinical trials. Voriconazole is an alternative based on observational data (Jacobs *et al*, 2017)

1.2 Rationale

Allergic bronchopulmonary aspergillosis (ABPA) is a hypersensitivity lung disease caused by bronchial colonization of *Aspergillus fumigatus* that occurs in susceptible patients with asthma and cystic fibrosis (CF). ABPA affects approximately 1–2% of asthmatic patients and 7–9% of CF patients. If unrecognized or poorly treated, ABPA leads to airway destruction, bronchiectasis, and/or pulmonary fibrosis, resulting in significant morbidity and mortality (Knutsen, 2015).

Sensitization to molds in patients with asthma is known to increase the severity of the disease. Patients with asthma, eosinophilia, and history of repeated 'pneumonitis' should be evaluated aggressively for ABPA. This would help avoid diagnostic delay and prevent steady lung damage leading to end-stage fibrosis(Shah and Panjabi, 2016).

Allergic bronchopulmonary aspergillosis (ABPA) in asthma is a severe, life-affecting disease that potentially affects over 4.8 million people globally. In the UK, ABPA is predominantly caused by the fungus *Aspergillus fumigatus*(Overton *et al*, 2018).

Itraconazole is used for the prevention and treatment of infections caused by *Aspergillus fumigatus*. An understanding of the pharmacodynamics of itraconazole against wild-type and triazole resistant strains provides a basis for innovative therapeutic strategies for treatment of infections (Al-Nakeeb *et al*., 2012).

Because of all above mentioned, this is important study for detection of ABPA in asthma and cystic fibrosis setting, also no previous documentation in Sudan about this issue and shedding the light on the association between ABPA infection and Asthma and CF in Khartoum, Sudan

1.3 Objectives

1.3.1General objective

To study Allergic Bronchopulmonary Aspergillosis (ABPA) in asthmatic and Cystic Fibrosis patients among the Sudanese population in Khartoum.

1.3.2 Specific objectives

- To isolate and identify *Aspergillus* in the sputum sample using convenvential culture technique.
- To determines the association between Allergic Bronchopulmonary Aspergillosis with asthma and Cystic Fibrosis.
- To estimate Minimum inhibitory concentration of itraconazole and voriconazole by E.test.

CHAPTER TWO

LITERATURE REVIEW

CHAPTER TWO

LITERATURE REVIEW

2. Literature review

2.1. Aspergillosis

Aspergillus is a fungus that is found throughout the world. Its spores are hardy and ubiquitous, thriving in moist, organic materials. *Aspergillus* can be cultured from outdoor and indoor environments and grows optimally at core body temperature. Spores are tiny and easily aerosolized and deposit in distal and terminal airways, where they germinate if the airway environment is favorable. *Aspergillus* is variably pathogenic in humans. Host characteristics are a major determinant of the type of pulmonary disease that may develop in response to Aspergillus exposure(Patterson and Strek, 2010).

Aspergillosis is a mycotic disease caused by *Aspergillus* species, usually *A. fumigatus*. Aspergillus is a saprophytic, aerobic fungus that develops on dead or decaying organic matter and produces airborne spores that can be inhaled by man. Pulmonary aspergillosis can be subdivided into Aspergilloma, Hypersensitivity reaction (ABPA), Semi-invasive (chronic necrotizing) aspergillosis, Airway-invasive aspergillosis and angioinvasiveaspergillosis. Aspergillus-specific IgE-mediated Type I hypersensitivity reaction and specific IgG-mediated Type III hypersensitivity reactions are believed to play an important role in the pathogenesis of ABPA (Kaur and Sudan, 2014).

2.2. History of ABPA

ABPA was first described in 1952 from the United Kingdom by Hinson et al. Even after five decades of research, this disorder is under diagnosed. In the developing countries, one-third of cases with ABPA are still misdiagnosed as pulmonary tuberculosis. Though asthma is the most common contributing factor, ABPA is also seen in patients with cystic fibrosis and other underlying diseases(Kaur and Sudan, 2014).

2.3. Epidemiology of ABPA

ABPA, one of the many forms of *Aspergillus* disease, results from a hyper-reactive immune response to *A. fumigatus* without tissue invasion. ABPA occurs almost exclusively in patients with asthma or CF who have concomitant atopy. The precise incidence of ABPA in patients with asthma and CF is not known but it is not high. Approximately 2% of patients with asthma and 1 to 15% of patients with CF develop ABPA. Although the incidence of ABPA has been shown to increase in some areas of the world during months when total mold counts are high, ABPA occurs year round, and the incidence has not been

definitively shown to correlate with total *ambient Aspergillus* spore counts. There is no gender predilection noted(Patterson and Strek, 2010).

Data from the Epidemiologic Registry of Cystic Fibrosis (ERCF) on 12,447 CF patients gathered from 224 CF centers in nine European countries were analyzed, The overall prevalence of ABPA in the ERCF population was 7.8% (Mastella *et al.*, 2000).

Since there were no consensus-based guidelines on ABPA so far, the International Society for Human and Animal Mycology (ISHAM), in September 2011, constituted a working group on ABPA complicating asthma. Data on *Aspergillus* sensitization and ABPA published since circa 2000 was collected by the ISHAM Working Group. The prevalence of *Aspergillus* sensitization among patients with asthma ranged from 5.5%-38.5%, and the prevalence of ABPA in asthma varied between 2.5% and 22.3% with a pooled prevalence of 8.4% (Shah and Panjabi, 2016).

2.4. Etiology of ABPA

Aspergillus fumigatus is a saprophytic fungus that plays an essential role in recycling environmental carbon and nitrogen. Its natural ecological niche is the soil, wherein it survives and grows on organic debris. Although this species is not the most prevalent fungus in the world, it is one of the most ubiquitous of those with airborne conidia. It sporulates abundantly, with every conidial head producing thousands of conidia. The conidia released into the atmosphere have a diameter small enough (2 to 3 mm) to reach the lung alveoli. *A. fumigatus* does not have an elaborate mechanism for releasing its conidia into the air; dissemination simply relies on disturbances of the environment and strong air currents. Once the conidia are in the air, their small size makes them buoyant, tending to keep them airborne both indoors and outdoors. Environmental surveys indicate that all humans will inhale at least several hundred *A. fumigatus* conidia per day. For most patients, therefore, disease occurs predominantly in the lungs, although dissemination to virtually any organ occurs in the most severely predisposed (Latgé, 1999).

Identification of *A. fumigatus* is based predominantly upon the morphology of the conidia and conidiophores. The organism is characterized by green echinulate conidia, 2.5 to 3 mm in diameter, produced in chains basipetally from greenish phialides, 6 to 8 by 2 to 3 mm in size. A few isolates of *A. fumigatus* are pigmentless and produce white conidia. The chains of conidia are borne directly on broadly clavate vesicles (Latgé, 1999).

2.5. Pathogenesis

ABPA is the consequence of a chronic inflammatory reaction in the airway to *A. fumigatus*. It is a ubiquitous spore- forming filamentous fungus found in soil and decaying organic matter, and in humans

its port of entry is by inhalation. Its success in colonizing the lungs is related to its size and its ability to evade local clearance mechanisms. *Aspergillus* causes disease by releasing allergens (Asp 1), virulence factors and proteases. Virulence factors released by *A. fumigates* lead to impaired mucociliary clearance; impaired action of fungicidal proteins and complement in the airway lining fluid; inhibition of phagocytosis and the killing capacity of phagocytic cells (macrophages, neutrophils); and degradation of extracellular matrix proteins by *A. fumigates* proteases. The net result is persistence of *A. fumigates* in the airway, which triggers eosinophilic airway inflammation by releasing allergenic peptides. Proteases released by *A. fumigates* also induce the release of interleukin (IL- 8) from the bronchial epithelium, which may also promote cellular influx and eosinophil activation (Wark, 2001).

Airway inflammation in asthma is characterized by an eosinophilic infiltrate orchestrated by the release of cytokines from TH_2 lymphocytes, with thickening of the epithelial basement membrane and airway smooth muscle hypertrophy. Although hypersensitivity to *A. fumigates* is considered to be the underlying pathogenic mechanism, the consequences are clearly different to other allergic airway diseases. The pathological manifestations of ABPA are more widespread than uncomplicated asthma. Broncho alveolar lavage (BAL) of five patients with ABPA demonstrated a mixed inflammatory pattern with eosinophils, lymphocytes and neutrophils and increased levels of granulocyte/macrophage- colony stimulating factor (GM- CSF), IL- 4 and IL- 5. These are cytokines associated with a typical TH_2 response seen in allergic asthma. However, there was also an increase in IL- 2 and IFN- γ, indicating a more complex mixed TH_1/TH_2 lymphocyte response, similar findings are observed in murine models of ABPA. This is consistent with in vitro data, which demonstrate that different peptides from *Af* can stimulate either a TH_1 or TH_2 response. We examined induced sputum in ABPA and compared this to matched subjects with stable asthma. We found an increase in the intensity of airway inflammation with a mixed eosinophil/neutrophil infiltrate, high levels of IL- 8 and marked eosinophil degranulation reflected by elevated sputum eosinophil cationic protein levels. The intensity of inflammation correlated with the degree of disease by computed tomographic (CT) scan. Together, these results suggest that airway inflammation in ABPA is more intense than in asthma, there is a mixed inflammatory response and this may account for the increased symptom severity, the development of the characteristic mucus plugs and, over time, may lead to the development of bronchiectasis and fixed airway obstruction. It is unclear why only small proportions of chronic asthmatics develop ABPA. Is this an inherent abnormality of the immune system responding to fungal antigens in an exaggerated way or a

predisposing condition that provides a favorable environment for fungal colonization(Wark and Gibson, 2001).

2.6. Diagnosis

In addition to radiology, other investigations utilized for the diagnosis and monitoring of ABPA include skin testing with *Aspergillus* antigens, peripheral eosinophil count, serum total IgE,Sputum examination often provides an important clue in patients with asthma and CF(Shah and panjali, 2016).

2.6.1. Skin testing

Since ABPA is basically an allergic response to *Aspergillus* antigens, skin sensitivity to *Aspergillus* is subsequently demonstrated in almost all patients with ABPA described to date. Although more than 150 species of *Aspergillus* exist, the main incriminating species are *A. fumigatus* followed by *A. flavus*, *A. niger*, *A. terreus*, and *A. nidulans*. Either the skin-prick test or the intradermal method can be employed for *Aspergillus* skin testing. There exists a marked variation in the *Aspergillus* antigen extract used for skin testing. This depends on the technique, potency and the geographical region. These factors, along with the type of test performed, expertise in interpretation of readings and patients' age variations, are reflected in the accuracy of the results. Although intradermal tests are more sensitive than the skin-prick tests, higher false positive results are noted (Shah and panjali, 2016).

2.6.2. Eosinophil count

Peripheral blood eosinophilia (>1000 cells·μL^{-1}), one of the major diagnostic criteria, is often the initial diagnostic indicator in a patient with asthma and fleeting pulmonary infiltrates. However, high eosinophil counts may be observed in numerous other diseases while normal levels may be found in patients who are already receiving oral corticosteroids. Given the poor specificity of this test, The ISHAM working group has relegated eosinophilia to "other" criteria. During exacerbations, when oral corticosteroids have not been initiated, most patients have an absolute eosinophil count ranging between 1000 and 3000 cells·mm^{-3}, which may return to near normal with oral corticosteroids (Shah and panjali, 2016).

2.6.3. Sputum examination:

In patients with productive cough, sputum eosinophilia is often present and fungal hyphae may be demonstrated on sputum smear examination. The presence of plugs in the sputum, coinciding with acute febrile illness, was noted in two of the three patients in the first report of ABPA by Hinson *et al*. In addition, sputum culture for *A. fumigatus* was positive in all three patients. Over the years, expectoration

of golden-brownish sputum plugs has been noted in up to 56% of patients with ABPA, whilst sputum culture yielded *Aspergillus* species in ~58% of cases. Detectable *A. fumigatus* DNA in the sputa of patients with ABPA, in whom routine cultures for *Aspergillus* were negative, was also observed. There is a role for sputum analysis in monitoring the severity of disease and in determining the course of the illness. As mentioned previously, higher levels of sputum eosinophilia and neutrophilia were found in patients with ABPA-central bronchiectasis when compared with serological ABPA. Sputum cultures and molecular testing may also help in assessing the response to antifungal therapy, as well as in identifying drug resistance to azoles (Shah and panjali, 2016).

2.6.4. Pulmonary function testing:

Pulmonary function testing in ABPA does not help the pulmonologist to assess the severity or the extent of the disease. During the remission stage, even in the presence of bronchiectasis, the lung volumes and flow rates could be normal if the asthma is well controlled. An obstructive airflow pattern is most commonly found during an acute episode or an exacerbation. Nevertheless, a restrictive pattern along with reduction in total lung capacity and impaired diffusion capacity of the lung for carbon monoxide may also be seen. Varying degrees of obstruction are noted in patients in the corticosteroid-dependent stage. When the disease progresses to the chronic fibrotic stage, the pulmonary function testing typically shows an irreversible mixed pattern characterized by airflow obstruction, reduced lung volumes and low diffusion capacity. Apart from obstructive airways disease, restriction, as well as a mixed pattern, has also been observed in either the acute or exacerbation stages (stages 1 and 3) of ABPA(Shah and Panjabi, 2016).

2.7. ABPA in Cystic Fibrosis

Cystic fibrosis is a fatal illness,the diagnosis of ABPA in patients with CF is difficult since it is common for both diseases to have several of the same clinical and laboratory features. Nevertheless, an early diagnosis of ABPA and the institution of treatment with corticosteroids are important to prevent additional destructive pulmonary changes, the primary criteria for ABPA include episodic bronchial obstruction (asthma), peripheral blood eosinophilia, immediate skin reactivity to Af antigen, precipitating antibodies against Af, elevated serum IgE level, history of pulmonary infiltrates (transient or fixed), and central bronchiectasis Other clinical features that may be helpful are: Af isolated from the sputum, based on similar criteria, Nelson *et a1* were able to demonstrate that 11% (5 of 46) of patients studied in a CF clinic had ABPA. Recently the demonstration of elevated Af-specific IgE or IgG serum

antibody levels was shown to be of considerable diagnostic importance in the detection of ABPA (Laufer *et al*., 1984).

2.8. ABPA in asthma:

Global burden of allergic bronchopulmonary aspergillosis with asthma and its complication chronic pulmonary aspergillosis in adults.Global prevalence of ABPA may be 0.7–3.5% of patients with asthma.ABPA is a TH2 hypersensitivity lung disease caused by bronchial colonization with *Aspergillus fumigatus* that affects asthmatic and/or CF patients. ABPA is characterized by exacerbations of asthma, worsening of pulmonary function, recurrent transient pulmonary infiltrates, peripheral blood and pulmonary eosinophilia, elevated total IgE level, and elevated *A. fumigatus* specific IgE, IgG, and IgA antibody levels. During episodes of ABPA exacerbations, thick brown mucoid sputum may contain *A. fumigatus* hyphaeCylindrical bronchiectasis of the central airways, particularly involving the upper lobes may be a consequence of pulmonary infiltrates due to eosinophilic inflammation(Knutsen, 2017).

2.9. Treatment of ABPA:

Treatment is designed first to control the acute episodes and then to limit the development of chronic lung disease. Most cases of ABPA require treatment with systemic Clinical and Developmental Immunology corticosteroids, and the treatment of choice is prednisone. Steroid therapy rapidly clears the eosinophilic infiltrates and the associated symptoms, although it is less effective at treating mucus impaction. In asthmatic ABPA patients, the usual starting dose is 0.5 mg/kg/day, taken each morning, and this does is maintained for 2 to 4 weeks while following the patient clinically and checking the chest radiograph for resolution of the acute process. After this induction treatment, the dose of prednisone should be reduced to 0.5 mg/kg given on alternate days. If mucus impaction persists and is associated with atelectasis, bronchoscopes should be performed to confirm the diagnosis and to attempt to remove the mucus plugs. Following resolution of the acute process, the dose of prednisone should be reduced over 1 to 3 months. Chronic treatment with corticosteroids is controversial, especially in adults, because only minorities of patients with ABPA are at risk of chronic lung disease. The relationship between acute episodes and lung damage is unclear, and the precise dose of prednisone is not certain, since acute exacerbations may continue while the patients are on low doses of steroid (Knutsen and Slavin, 2011).

2.10. Prognosis:

The prognosis of ABPA is good if the disease is detected early and treatment started promptly. It is important that the diagnosis is made and treatment commenced before there is permanent lung damage from bronchiectasis. In such patients, there should be no progression of the disease, although relapses

can occur many years later, and long-term follow up is recommended. In children with CF, the relapses seem to be more frequent than they are in patients with asthma, and careful surveillance is necessary to ensure resolution of the disease process. In some CF patients, it is difficult to wean the steroids without an increase in symptoms, such as dyspnea and wheezing; whether this is due to the underlying CF lung disease or due to patients going from stage II to stage III ABPA on withdrawal of steroids is unclear. Symptoms are not a reliable guide to therapy; therefore, it is important to reevaluate the chest radiograph and the serum IgE at regular intervals until a long-term remission is established (Knutsen and Slavin, 2011).

CHAPTER THREE

MATERIAL AND METHODS

CHAPTER THREE

MATERIALS AND METHODS

3. Materials and Methods

3.1. Study design

This study was descriptive, cross-sectional case study.

3.2. Study area and duration

This study was conducted in El-Shaab Teaching Hospital, from February to May 2018.

3.3. Study population

Patients with uncontrolled asthma and Cystic fibrosis .

3.4.Inclusion criteria

Patients with uncontrolled asthma and cystic fibrosis from all age groups with both sex.

3.5.Exclusion criteria

Patients with Tuberculosis.

3.6. Sample size

A total of one hundred subjects (n=100) were enrolled in this study.

3.7.Sampling Technique

This study was based on non-probability convenience sampling technique.

3.8. Data collection

Personal and clinical data were collected from patients by non self interviewing questionnaire from each subject (Appendix 1).

3.9. Ethical consideration

Permission to carry out the study was obtained from Research committee of college of Medical Laboratory Science Department of Microbiology, Sudan University of Science and Technology and from administration of El-shaab teaching hospital.

3.10. Specimens collection

Sputum specimens were collected, early morning Sputum samples were obtained using a non-invasive method and ideally should be collected before antifungal are started, the patients were instructed tocough deeply in a sterile universal container and processed as soon as possible.

3.11. Specimens processing

3.11.1. Macroscopically examination

The color (yellow, green or red) and consistency (mucoid, mucopurulent, purulent, and bloody) of sputum were observed.

3.11.2. Direct Microscopy

2.11.2.1. 20%KOH preparation

One drop of 20% KOH was placed in a clear glass slide and purulent part of sputum was selected and mixed, covered with coverslip incubated for a 2-3 minute in wet Petri dish then examined under the microscope (10x-40x)to see hyaline, septate, uniform in width (about 4µm) and branch dichotomously hyphae.

2.11.2.2. Direct Gram stain

One drop of normal saline was placed into clear glass slide and the purulent part of sputum mixed with normal saline air dry and red heat fixed, stained using gram stain technique put oil and examined in the microscope using oil immersion for gram-positive dichotomous hyphae.

3.11.3. Culture

In two Sabouraud Dextrose Agar, the purulent part of sputum sample inoculated, after incubated in two different temperatures: one in 25^{o}c and other in 37^{o}c for 3-5 day, examined for colonial morphology (they give different colors) and indirect microscopy (needle mount).

3.11.4. Needle mounts (Lacto Phenol Cotton Blue stain (LPCB))

Under aseptic condition placed one drop of lacto phenol cotton blue stain in clean glass slide, cut part of the colony with a mycological needle and put it in the slide, well mixed, covered with a cover slip and examined under the microscope using (40x) to observe conidia of *Aspergillus*.

3.11.5. Susceptibility testing

E test was performed in accordance with the manufacturer's instructions. Isolates were grown on potato dextrose agar slants at 35°C for 7 days to ensure adequate sporulation. Spore suspensions were prepared in sterile saline and adjusted to a concentration of 106spores/ml, corresponding to 80 to 82% transmittance at 530 nm. The agar formulation used for the E test was RPMI 1640 supplemented with 1.5% agar and 2% glucose and buffered to pH 7.0 with 0.165 M morpholinepropanesulfonic acid (MOPS) buffer. The 150-mm-diameter plates contained RPMI agar at a depth of 4.0 mm. The plates were inoculated by dipping a sterile swab into the spore suspension and streaking it across the surface of the agar in three directions.

The plates were dried at ambient temperature for 15 min before applying voriconazole and itraconazole E test strips. The plates were incubated at 37°C and read at 48 h. The E test MIC was read as the drug concentration at which the border of the elliptical inhibition zone intercepted the scale on the antifungal strips.

3.12. Data analysis

The collected data was analyzed using the computer program SPSS software version 11.5 for windows. The significance of difference was determined using Chi squire test Statistical significance was set at P> 0.05.

CHAPTER FOUR

RESULT

CHAPTER FOUR

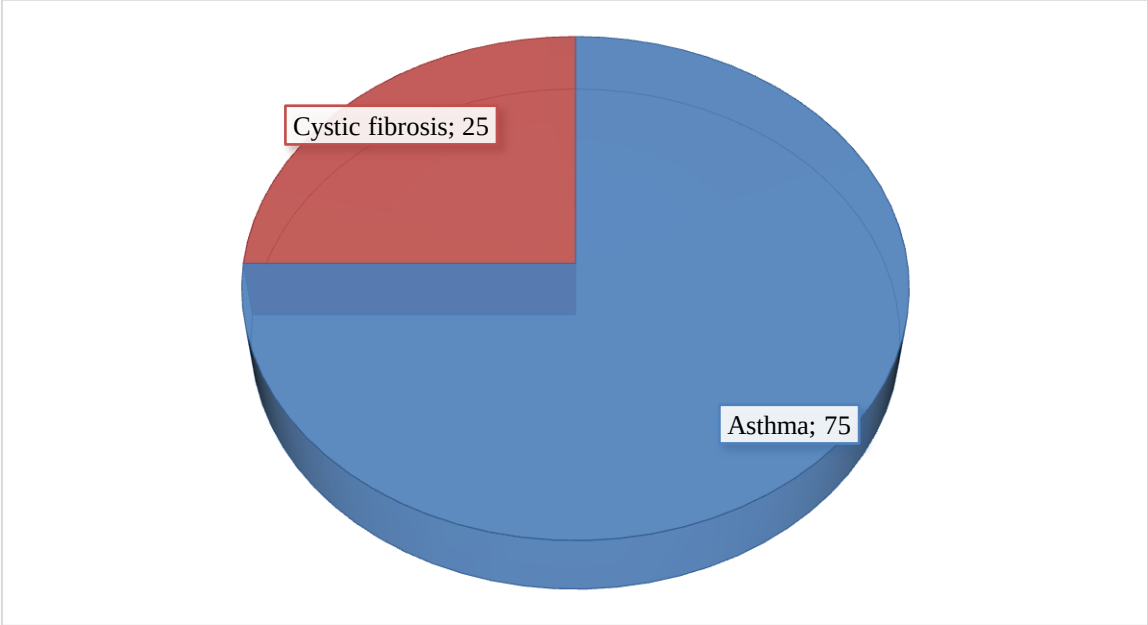
RESULTS

A total of 100 sputum specimens were included in this study, out of them cystic fibrosis patients were 25(25%) and asthmatic patients 75(75%) as shown in figure (4.1). Males were 43(43%) among them 10/43(23.2%) were from cystic fibrosis, and 33/43(76.7%)from asthmatic patients. While female 57(57%)in which 15/57(26.3%)from cystic fibrosis ,and 42/57(73.7%)from asthmatic patients as demonstrated in figure (4.2).

Aspergillus spp were isolated in 5/100(5%) from sputum specimens. *Aspergillus* spp were isolated in 2/25(8%) out of cystic fibrosis patients and 3/75(4%) in asthmatic patients as in figure (4.3). Out of 5 *Aspergillus* spp 3(60%) were *Aspergillus fumigatus* and 2(40%) were *Aspergillus terrus.*

|In this study there was no significant different between *Aspergillus* among asthmatic and cystic fibrosis patients *P*.value (0.42) table (4.1).

The five isolated samples undergo a sensitivity test (E. test) with two different drugs (itraconazole and voriconazole) in order to estimate the MIC. MIC (itraconazole mean=0.67,STD=0.5),(voriconazole mean=0.17,STD=0.125).



Figure(4 .1): Frequency of samples from asthma and cystic fibrosis

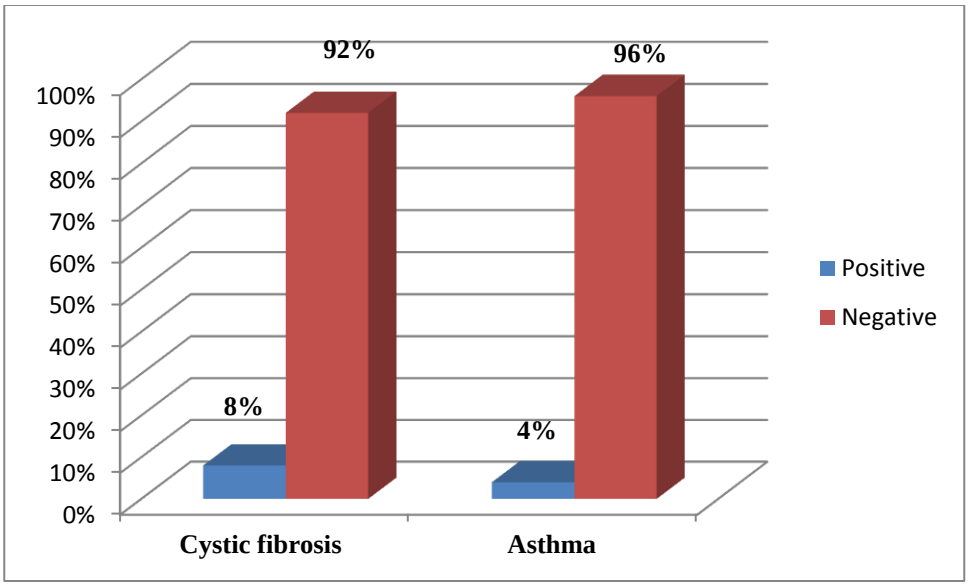


Figure (4.2): Frequency of gender among study population

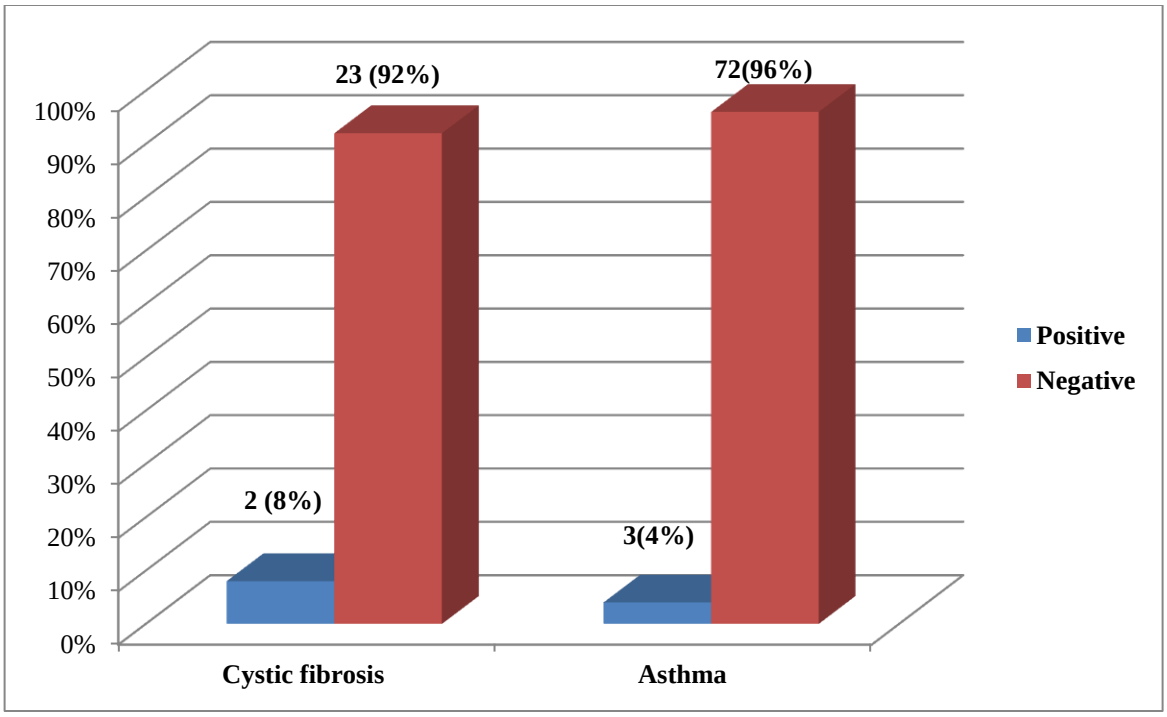


Figure (4.3): Distribution of *Aspergillus* on asthmatic and cystic fibrosis patients

Table (4.1): Correlation between *Aspergillus* and cystic fibrosis and asthma

	Culture results of *Aspergillus*		Total	*P* .value
	Positive%	Negative%		
Cystic fibrosis	2(4%)	23(96%)	25(25%)	0.42
Asthma	3(8%)	72(92%)	75(75%)	
Total	5(5%)	95(95%)	100(100%)	

CHAPTER FIVE

DISCUSSION

CHAPTER FIVE

DISCUSSION

5.1. Discussion:

In this study Aspergilli were isolated from 5(5%) out of 100 sputum sample of Asthmatic and Cystic fibrosis patient by commercial isolation method. The prevalence rate of aspergillus in Asthmatic patient were (4%), and (8%) in cystic fibrosis patient, this percentage is near the percentage in study of David *et al*(2013) in China the prevalence of aspergillus in asthmatic patient was (2.5%), The population prevalence of ABPA is not clearly known, but the prevalence in asthma clinics is reported to be around 13% (Agarwal, 2009)and prevalence of 2% to 15% in people with cystic fibrosis (Perisson *et al*., 2017).

ABPA affects approximately 1–2% of asthmatic patients and 7–9% of CF patients (knutsen, 2015).

This study reveal that the prevalence of allergic bronchopulmonary aspergillosis in asthmatic (4%) and cystic fibrosis patients is (8%) near to previous studies in developed countries due to difference in sample size, genetic makeup of population, socioeconomic status and personal hygiene, and voriconazole is more effective for treatment than itraconazole.

In the present study, voriconazole drug is determine to be more effective by its lower MIC than the itraconazole, (itraconazole mean=0.67,STD=0.5), (voriconazole mean =0.17, STD=0.125).

Allergic bronchopulmonary aspergillosis management includes corticosteroids to control the host immune response and antifungal agents to decrease the burden of the organism. Itraconazole is currently the first-line agent for symptomatic ABPA patients based on randomized, controlled clinical trials. Voriconazole is an alternative based on observational data (Jacobs, 2017).

Allergic bronchopulmonaryaspergillosis (ABPA) in asthma is a severe, life-affecting disease that potentially affects over 4.8 million people globally. In the UK, ABPA is predominantly caused by the fungus *Aspergillus fumigatus* (Overton, 2018).

There were no significant association between the prevalence rate of ABPA in Asthmatic and Cystic fibrosis disease (*P value*=0.42).

5.2. Conclusion

This study concludes that, the frequency of ABPA in cystic fibrosis and asthmatic patients was high and voriconazole is more effective than itraconazole.

5.3. Recommendations

Increase the sample size to validate the result of this study.

Use more advanced technique (PCR).

Instruct patients to care with health and immune status.

Fungal disease were neglected, we need more progression and care.

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APPENDCIES

Appendices

Appendix (1)

Questionnaire:

بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate study

College of Medical Laboratory Science

Department of Microbiology

Name: .....

Age: .....

Sex: Male: ☐ Female: ☐

Asthma: Yes ☐ No ☐

Cystic Fibrosis: Yes: ☐ No ☐

Appendix 2

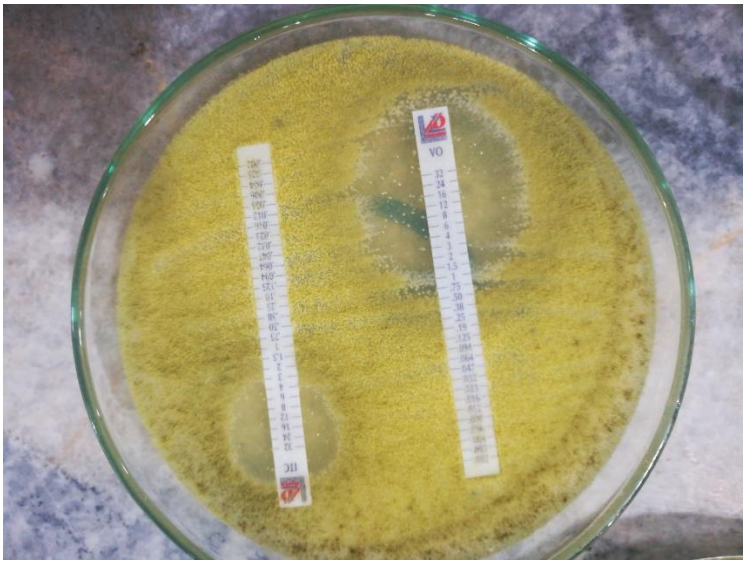

Figure 4:

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