بسم الله الرحمن الرحيم

Fungal Elments in Sputum Cytology Among Active and Post_Treated Plumonary Tuberculosis Patients in Al Managil Teaching Hospital, Gezira State, Sudan (2020)

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Examination Committee:

Name	Postion	Signature
	Chair person	.....
	External examiner	.....
	Internal examiner	.....

Date of examination …….

"وَمَنْ أَعْرَضَ عَنْ ذِكْرِي فَإِنَّ لَهُ مَعِيشَةً ضَنكًا وَنَحْشُرُهُ يَوْمَ الْقِيَامَةِ أَعْمَى ۝

قَالَ رَبِّ لِمَ حَشَرْتَنِي أَعْمَى وَقَدْ كُنتُ بَصِيرًا ۝ قَالَ كَذَلِكَ أَتَتْكَ آيَاتُنَا

فَنَسِيتَهَا وَكَذَلِكَ الْيَوْمَ تُنسَى" (طه:124)

Dedication

To my mother who scarify by her life to

Give me better life

To the soul of my father

Who left by his body but

still with us

To my sisters. The cause of my happiness

to my sister's daughters and sons

the candles which lighten our life

To all those who supported me

in this research.

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Abstract

Sputum cytology is still one of the most effective and producible sample for diagnosis and evaluation of lung diseases and disorders. For this, it can used for evaluation of apportunistic fungal pathogens on pulmonary tuberculosis patients, which is of leading cause of death worldwide. On this cross sectional laboratory base study, which used to evaluate the sputum cytology for presence of opportunistic fungal elements. A total 110 early morning expectorate sample collected from the period 1/3 to 30/6/2020. 69/110 (63%) from patients come for first diagnosis to the center of T.B and HIV. Al Managil teaching hospital, and 41/110 (37%) follow-up starting from second month until six month post- treated follow up. From each sample two slides was prepared. One of them fixed immediately before air-drying in 95% ethanol and later stain by PAS technique, the other fixed after air-drying on absolute methanol and stain by Giemsa stain. Regardless to other method of fungal identification such as serology or mycological culture, only depends on microscopic identification. The study found that 95/110 (86%) was negative for fungal elements, and 15(14%) was positive, 9(8%) positive in diagnosis group and 6(5%) in follow-up group. Moreover the most common infectious agents was Candida species 9/110 (8%), 6/110 (5%) present as yeast and 3/110 (2%) as Pseudohyhae. Followed by Aspergillus species 5/110 (4%) then actinomyces species 1/110(.9%). The incidence of infection is higher in rural (82%) males (55%) farmers (36%). In the majority of the pateints with negative results for fungi MDR-TB not detected 87(79%), followed by positive for fungi and also MDR-TB not detected 15 (13.6%), then (negative and positive) for fungi and MDR-TB with high rate with the same percentage 3(2.8%), and finely very low MDR-TB 1(.9%), medium MDR-TB 1(.9%) without any detection of fungal elements. The study recommend by including fungal testing and antifungal drugs on the pulmonary TB treatment plan as possible cause of complications.

العناصر الفطرية في الفحص الخلوي للقشع في مرضي السل الرئوي النشط وما بعد المعالجة في مستشفى المناقل التعليمي، ولاية الجزيرة، السودان (2020)

ملخص الدراسة

الفحص الخلوي للقشع يعتبر حتى الان واحد من الفحوصات الفعالة لتقييم وتشخيص امراض واضطرابات الرئتين. وبناءً على ذلك يمكن استخدامه لتقييم إصابات الفطريات الانتهازية المصاحبة لمرض الدرن الرئوي، والذي يعتبر واحد من الأسباب الرئيسة للوفاة على مستوي العالم. هذه الدراسة مختبرية مقطعة أجريت لتقييم الفحص الخلوي للقشع لوجود الفطريات الانتهازية عند مرضي الدرن الرئوي. اجمالي 110 عينه من التنخم الصباحي جمعت في الفترة بين 1\3 الي 30\6 2020. 69 (63%) لمرضي الحالات الجديدة، و41 (37%) لمرضي المتابعة بعد بداية العلاج بدأً من الشهر الثاني وحتى السادس. من أي عينه تم تحضير شريحتين، واحده تم تثبيتها قبل جفافها عن طريق الهواء بمحلول 95% ايثانول وصبغت لاحقاً بطريقة البيريوديك اسيد جيف. والثانية تم تجفيفها بالهواء ثم تثبيتها بميثانول مطلق وصبغت لاحقاً بطريقه صبغة جيمسا. بغض الظر عن الطرق الأخرى لتقييم الفطريات مثل فحص المصل او تزريع الفطريات وبالاعتماد على التقييم المجهري فقط وجدت الدراسة ان 110 \95(86%) من العينات سالبه لوجود محتوي فطري، و15\110(14%) موجبه للمحتوي الفطري.110\9(8%) لمرضي الحالات الجديدة و6\110(4%) لعينات المتابعة. أضف على ذلك أنواع المبيضات هي الغالبية بنسبة 9(8%), 6(5%) ظهرت كخميره و110\3(2%) كخيوط فطرية كاذبة، يليه خيوط فطر الرشاشيات 5 \110(4%)، ثم الشعيات بنسبة 1\110(,9%). حدوث الإصابة بالدرن الرئوي اعلي في مجموعة القرويين (82%)، الذكور (55%) المزارعين (36%). في الغالبيه العظمي للمرضي ذوي النتائج السالبة لفحص الفطريات الدرن المقاوم للأدوية المتعددة لم يحدد أيضا87(79%). يليهم مرضي العينات الموجبة لفحص الفطريات وسالبه للدرن المقاوم للأدوية المتعددة في نفس الوقت15 (13.6%). ثم العينات السالبة والموجبة للفطريات مع ايجابية الدرن المقاوم للأدوية المتعددة بنسبه عالية وعددهم3(2.8%) لكل مجموعة. واخيرا مرضي الدرن المقاوم للأدوية المتعددة بنسبه متوسطة ومنخفضه ونتيجة سالبة للفطريات1(.9%). الدراسة بأدراج الفحص والمضادات الخاصة بالفطريات ضمن الخطة العلاجية لمرضي الدرن الرئوي، باعتبارها من مسببات المضاعفات للمرضي

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A	Aaspregillus
AFB	Acid fast bacilli
AIDS	Aquired immuno deficiency syndrom
BAL	Broncho alveolar lavage
C	Candida
CNS	Central nervous system
C^{o}	Celsius
COPD	chronic obstructive pulomnary disease
DNA	Deoxyribonuclecic acid
HIV	Human immnuodeficiency virus
Ig G	Immunoglobulin G
M	Mycobactrium
MDR-TB	Multidrug resistant mycobacterium
MTB	Mycobacterium tuberculosis
P	penumocystis
PAS	Periodic acid Schiff"s
T B	Tuberculosis

Chapter one

Introduation

1:1 General Introduction

Tuberculosis remains one of the major causes of morbidity and mortality throughout the world, and is again occurring more frequently in Western countries (Gray,W and Kocjan, G.2010).

Sudan has a high burden of tuberculosis (TB) with an estimated 50,000 incident cases during 2009, when the estimated prevalence was 209 cases per 100,000 of the population. Few studies have been undertaken on TB in Sudan and the prevalence of drug resistant disease is not known(Sharaf Eldin *et al*.2011).

Pulmonary tuberculosis can be categorized as primary or postprimary (secondary). Primary pulmonary tuberculosis occurs soon after the initial infection with tubercle bacilli. Postprimary Disease Also called adult-type, reactivation, or secondary tuberculosis (Kasper, D.L and Fauci, A.S 2010).

Most cases present with pulmonary T.B disease, with classical symptoms which includes; Productive cough ,Haemoptysis, Breathlessness, Systemic symptoms—weight loss, night sweats, and malaise, Chest pain. Haemoptysis is more common with cavitatory disease, and up to two-thirds will be smear-positive (Chapman,S and Robinson,G.R .2014).

TB may cause persisting pulmonary damage in patients whose infection has been considered cured on clinical grounds.Chronic impairment of lung functions, bronchiectasis, aspergillomas, and chronic pulmonary aspergillosis have been associated with TB. Chronic pulmonary aspergillosis may manifest as simple aspergilloma (fungal ball) or chronic cavitary aspergillosis. (Jameson L.J, *et.al* .2018).

In developing countries, detecting infectious cases of tuberculosis by examining sputum for AFB, followed by adequate supervized treatment of smear positive individuals until they are completely cured, is the most effective way of reducing the transmission and infection rates of tuberculosis and spread of multi-drug resistant strains. In most developing countries, positive sputum smears due to mycobacteria other than the M. tuberculosis complex are rare.

Cultural techniques for detecting M. tuberculosis, although more sensitive, are slow and expensive.

Use screw-cap, leak-proof specimen containers (snap-closing containers are hazardous because they create aerosols). Sputum, not saliva is required to detect AFB. Examination of up to three specimens (at least one as an early morning specimen) may be required to detect the organisms (Cheesbrough, M. 2006)

If the patient is not able to expectorate adequately, expectoration can be induced by having the patient inhale nebulized water or saline solution. When prompt preparation of sputum is not possible, the patient can expectorate into a 70% ethanol solution, which prefixes the specimen (Cibas, E.S and Ducatman, B.S.2020).

Sputum can be processed in a variety of ways but all specimens must be regarded as potentially infective. The traditional ' pick and smear ' method, using alcohol fixation and Papanicolaou (PAP) staining is optimal for routine sputum examination. (Gray,W and Kocjan, G.2010).

Sputum specimens are judged adequate when plentiful pulmonary macrophages can be identified. The presence of columnar cells is ambiguous since they may be from the nasal passages or upper airways. (Gray,W and Kocjan, G.2010).

Although lung diseases caused by fungi have been known for many years in endemic areas, the movements of populations, treatment of patients with immunosuppressive agents, and mainly the onset of AIDS have significantly increased the prevalence of this group of diseases (Koss, L.G and Melamed, M.R. 2006).

Some of the fungi causing lung disease are purely saprophytic and grow along pre-existing cavities or necrotic lung tissue. Others, such as blastomycosis and coccidioidomycosis, are seen in well-defined geographical zones, where the fungal spores are found in the soil. They cause primary invasive infections in previously healthy people, in the absence of predisposing factors (Herrington, S.C .2014).

Many of the organisms can be identified in routinely Papanicolaou-stained cytologic material from the respiratory tract, although some require culture or special staining procedures for identification. Sputum or BAL specimens are commonly use for diagnosis (Koss, L.G and Melamed, M.R .2006).

Fungal infections produce granulomatous inflammation; the granulomas appear as collections of Epitheloid Histiocytes in the background of chronic inflammatory cells with or without necrosis. Confirmation of the diagnosis with microbiologic fungal cultures is always advised (Bibbo, M and Wilbur,D .2015).

Laboratory methods for the diagnosis of fungal infections remain based on three broad approaches: the microscopic detection of the etiologic agent in clinical material; its isolation and identification in culture; and the detection of either a serologic response to the pathogen or some marker of its presence, such as a fungal cell constituent or metabolic product. New diagnostic procedures based on the detection of fungal DNA in clinical material are presently being developed, but have not yet had a significant impact in most clinical laboratories (Kauffman, C.A , *et al* .2011).

1:1 Rational

Pulmonary tuberculosis represents one of the common causes of death, neglected tropical disease and socio-economic problem in Sudan.

Due to chronic nature of infectious causative mycobacteria, treatment that make the patients susceptible to opportunistic fungal infection. This makes fungal co-infection is the most common complications of T.B particularly on relapsed and untreated patients.

Sputum cytology consider as one of the most valuable, none invasive and producible respiratory system samples that can used to investigate fungal elements. Moreover, this investigation may reduce the disease burden and provide a solid ground for better clinical management of the patients.

1:2 General objectives

To evaluate sputum cytology in active and post_treated plumonary tuberculosis patients for fungal elements in Al Managil teaching hospital- Gezira state.

1:3 Specific objectives

1. To estimate the value of sputum sample for cytologic detection of fungal infections in respiratory system.
2. To determine the most common fungus species in the study area.
3. To test presence of fungal elements in sputum sample against multidrug resistance mycobacterium tuberclus bacili positive pateints.

Chapter tow

Litrature review

2:1 Anatomy of the Respiratory Tract

The respiratory tract can be categorized into upper and lower compartments. The upper airway extends from the sinonasal region to the larynx. The lower respiratory tract, which is the major focus of diagnostic respiratory cytopathology, extends from the trachea to the lungs. The tracheobronchial tree divides into progressively smaller units: bronchi, bronchioles, and respiratory acini (Cibas, E.S and Ducatman, B.S.2020).

2:2 Histology and Cytology of the Respiratory Tract

The trachea and bronchi are lined by a pseudostratified epithelium. The predominant cell is the ciliated columnar cell, which has a basally placed nucleus with finely textured chromatin. The luminal surface has a thick terminal bar with cilia. Goblet cells, present in a ratio of approximately one per six ciliated cells, also have a basally located nucleus but lack cilia, and their cytoplasm is distended by mucus. Goblet cells secrete mucus, whereas ciliated cells move the mucus and entrapped contaminants up the airway. Adjacent to the basement membrane are basal or reserve cells: small, undifferentiated cells that are the presumed forerunners of the ciliated and goblet cells. Neuroendocrine cells, or Kulchitsky cells, are also present in the respiratory epithelium, but they are identified only with special stains or ultrastructural examination: they are argyrophil-positive and possess dense-core granules (Cibas, E.S and Ducatman, B.S.2020).

The terminal bronchioles are lined by nonciliated cuboidal to columnar cells called club cells or respiratory exocrine cells (previously called Clara cells); they are not sufficiently distinctive with routine cytologic preparations and thus not specifically identified (Cibas, E.S and Ducatman, B.S.2020).

The alveolar lining consists of type I and type II pneumocytes. Type I pneumocytes, which are more numerous, are paper thin and cover the gas exchange portion of the alveolar surface.

The type II pneumocyte is more conspicuous: plump and cuboidal rather than flat. It secretes pulmonary surfactant, seen ultrastructurally as osmiophilic lamellar bodies. After lung injury, these cells function as reserve cells for the delicate type I pneumocyte. On cytologic preparations, type II pneumocytes are round and have vacuolated cytoplasm; they can be difficult to distinguish from macrophages (Cibas, E.S and Ducatman, B.S.2020).

Alveolar (pulmonary) macrophages vary in appearance depending on the amount and type of phagocytosed cytoplasmic material. In general, they have one or more round to oval nuclei and lacy or bubbly cytoplasm, often with small black particles from inhaled pollutants ("dust cells,"). After pulmonary hemorrhage, alveolar macrophages contain hemosiderin pigment, which is golden-brown rather than black (Cibas, E.S and Ducatman, B.S.2020).

2:3 General introdution about fungi

Fungi are eukaryotes with cell walls that grow as multicellular filaments (mold) or individual cells alone or in chains (yeast). Cell walls give fungi their shape. Yeasts are round to oval and mainly reproduce by budding. Some yeasts, such as Candida albicans, can produce buds that fail to detach and become elongated, producing a chain of elongated yeast cells called pseudohyphae (Kumar, V *et al* .2015).

Molds consist of threadlike filaments (hyphae) that grow and divide at their tips. They can produce round cells, called conidia, that easily become airborne, disseminating the fungus. Many medically important fungi are dimorphic, existing as yeast or molds, depending on environmental conditions (yeast form at human body temperature and a mold form at room temperature) (Kumar, V*et al* .2015).

Infection caused by fungus is known as mycosis (plural mycoses) (Kumar, S .2016)infections, are of four major types:

- Superficial and cutaneous mycoses are common and limited to the very superficial or keratinized layers of skin, hair, and nails.
- Subcutaneous mycoses involve the skin, subcutaneous tissues, and lymphatics and rarely disseminate systemically.
- Endemic mycoses are caused by dimorphic fungi that can produce serious systemic illness in healthy individuals (Kumar, V *et al* .2015).

Dimorphic fungi are filamentous (mold-like) in their natural habitat, but yeast-like in human tissue. In the laboratory, cultures grown at 25°C show the filamentous stage

and the yeast form is seen at 35°C. The laboratory diagnosis is mostly based on the isolation of the pathogen from a suitable clinical specimen, such as sputum, bronchial aspirate, or biopsied tissue (Mishra , S.K and Agrawall, D .2013).

- Opportunistic mycoses can cause life-threatening systemic diseases in individuals who are immunosuppressed or who carry implanted prosthetic devices or vascular catheters (Kumar, V *et al* .2015).

2:3:1 Systemic Mycosis

The terms "systemic mycoses" (singular mycosis) or "deep-seated mycoses" refer to fungal diseases involving internal organs, such as the lungs, brain, and kidneys. However, systemic mycoses may also develop into cutaneous, subcutaneous, or mucocutaneous mycoses, mostly as a secondary, but occasionally as the sole manifestation. The causal agents of systemic mycoses may be broadly divided into three groups: the dimorphic fungi, yeast-like fungi, and filamentous fungi or molds. Systemic mycoses are classic examples of airborne diseases. Respiratory tract is the usual portal of entry but infections due to traumatic implantation are also known. Lungs are often the primary site of infection from where the disease may spread to other parts of the body, including the CNS, bones, and skin. The three groups of the pathogens are discussed below (Mishra , S.K and Agrawall, D .2013).

2:3:2 Endemic mycosis or dimorphic fungi mycosis

The endemic mycoses are geographically restricted pathogens that exist as molds in specific environmental niches and that infect persons who encounter them (Jong E.C and Stevens D.L.2012).

Most patients infected with one of the endemic mycoses are a symptomatic or have such mild symptoms that it is thought that they have a self-limited viral illness. Therefore the discussion of symptoms and signs that follows concentrates on fewer than 5% of persons exposed to these organisms. When symptoms do occur, the predominant manifestations are pulmonary, which is not surprising given that the portal of entry is the lungs for these fungi (Jong E.C and Stevens D.L.2012).

The extent of disease manifested by a given patient depends on both the inoculum of the organism and the ability of the host to mount an effective immune response. The route of infection is almost always inhalation of the infectious conidia into the alveoli,

and therefore the major clinical manifestations are pulmonary. In addition, all of the endemic mycoses have the potential to disseminate hematogenously, and disease manifestations, especially in immunosuppressed patients, can reflect this widespread dissemination (Jong E.C and Stevens D.L.2012). Some of the well-known mycotic diseases caused by dimorphic fungi include Blastomycosis ,Coccidioidomycosis, Histoplasmosis , Paracoccidioidomycosis and Sporotrichosis (Mishra , S.K and Agrawall, D. 2013).

Endemic fungi can rarely present in non-endemic areas, and diagnosis is often delayed because of their non-specific and varied clinical features and the failure to obtain a detailed travel history. Fungal infection may mimic other diseases, such as TB and lung cancer, often leading to inappropriate investigations and treatment. Fungal infections can also cause granulomata on lung biopsy, which sometimes results in diagnostic confusion (e.g. with sarcoidosis). Infection in immunocompetent individuals is usually either asymptomatic or mild and self-limiting, although severe infection may rarely occur in apparently immunocompetent individuals. Outbreaks of disease may occur, as well as sporadic cases (Chapman,S and Robinson,G.R .2014).

2:3:3 Opportunistic mycosis

Patients with compromised host defenses, who are susceptible to ubiquitous fungi, are referred to as opportunistic fungi. Healthy people, if exposed to ubiquitous fungi are usually resistant. Causative Fungal Agents are Yeast and yeast-like fungi (Cryptococcus, Candida spp., Torulopsis). Filamentous fungi: (Aspergillus, Mucor, Absidia, Rhizopus, Cephalosporium, Fusarium, Penicillium, Geotrichum, Scopulariopsis) and Others: Pnuemocystis jiroveci (Kumar,S. 2016).

Candidiasis (candidiasis, moniliasis) is an infection of the skin, mucosa (Superficial) and rarely of the internal organs (Systemic candidiasis) caused by a yeast-like fungus Candida albicans, and occasionally by other Candida species. They are members of the normal flora of the skin, mucous membranes, and gastrointestinal tract. Important species of Candida found in man are: C. albicans; C. stellatoidea; C. tropicalis; C. krusei; C. guilliermondii; C. parapsilosis; C. glabrata C. viswanathii (Kumar, S. 2016).

Cryptococcosis (torulosis, European blastmycosis, Busse–Buschke disease) is subacute or chronic infection caused by the capsulate yeast Cryptococcus neoformans. It is most

frequently recognized as a disease of the central nervous system (CNS), although the primary site of infection is the lungs. The disease occurs sporadically throughout the world but it is now seen most often in patients with AIDS. Infection is usually acquired by inhalation but may sometimes be through skin or mucosa. The primary pulmonary infection may be asymptomatic or may mimic an influenza-like respiratory infection, often resolving spontaneously. Pulmonary cryptoccocosisis may lead to a mild pneumonitis. In patients who are compromised, the yeasts may multiply and disseminate to other parts of the body but preferentially to the central nervous system, causing cryptococcal meningoencephalitis. Other common sites of dissemination include the skin, eye, and prostate gland (Kumar, S. 2016).

Aspergillosis caused by Aspergillus species are ubiquitous saprophytes in nature, and aspergillosis occurs worldwide. The most important species are A. fumigatus, A. niger, A. flavus, A. terreus and A. Nidulans .Following inhalation of conidia, atopic individuals often develop severe allergic reactions to the conidial antigens. In immunocompromized patients, the conidia may germinate to produce hyphae that invade the lungs and other tissues (Kumar, S.2016).

Aspergillosis either cause localized infections such as sinusitis (A. flavus and A. Fumigatus), mycotic keratits (A. flavus and A. Fumigatus), or otomycosis; (A niger). Or may be systemic aspergillosis which inculde the following forms:

- Pulmonary aspergillosis present as allergic asthma, bronchopulmonary aspergillosis; the conidia germinate and hyphae colonize the bronchial tree without invading the lung parenchyma. This phenomenon is characteristic of allergic bronchopulmonary aspergillosis or present as colonizing aspergillosis (aspergilloma) (Kumar, S. 2016).
- Colonizing aspergillosis usually develops in preexisting pulmonary cavities, such as in tuberculosis or cystic disease. It is also referred to as fungus ball. The fungus grows into large ‘balls’ (as pergilloma). Cases of as pergilloma rarely become invasive (Kumar, S.2016).
- Invasive aspergillosis this form occurs in severely immunocompromised individuals. Disseminated aspergillosis involving the brain, kidney and other organs is a fatal complication (Kumar, S.2016).
- Other forms of systemic aspergilosis inculde endocarditis and paranasal granuloma (Kumar, S. 2016).

Other fungal agents inculdes Pneumocystis jiroveci Until recently, P. jeroveci was thought to be a protozoan. Molecular studies indicate that Pneumocystis carinii is a fungus with a close relationship to ascomycetes. P. jiroveci is normally a commensal in the lung, spread by respiratory droplets. In immunocompetent individuals, infection is asymptomatic. However,in imunocompromised patients, serious lifethreatening pneumonia can develop (Kumar, S. 2016).

Since the early 1980s, it has remained one of the primary opportunistic infections found in patients with AIDS.Almost any fungus may invade a severely immunocompromized host and infections with many common fungi, including Fusarium species, Trichosporon beigelii and Pseudallescheria boydii have been reported (Kumar, S. 2016).

2:4 Diseases of the respiratory system

The majority of diseases of the respiratory system present with cough and/or dyspnea and fall into one of three major categories:

(1) Obstructive lung diseases

(2) Restrictive disorders

(3) Abnormalities of the vasculature. (Jameson L.J, *et.al* .2018).

Obstructive lung diseases are most common and primarily disorders of the airways, such as asthma, chronic obstructive pulmonary disease (COPD), bronchiectasis, and bronchiolitis. Diseases resulting in restrictive pathophysiology include parenchymal lung diseases, abnormalities of the chest wall and pleura, and neuromuscular disease. Pulmonary embolism, pulmonary hypertension, and pulmonary venoocclusive disease are all disorders of the pulmonary vasculature. (Jameson L.J, *et.al* .2018).

Although many specific diseases fall into these major categories, both infective and neoplastic processes can affect the respiratory system and result in myriad pathologic findings. Disorders can also be grouped according to gas exchange abnormalities, including hypoxemic, hypercarbic, or combined impairment. (Jameson L.J, *et.al* .2018).

Many patients will subsequently undergo pulmonary function testing, chest imaging, blood and sputum analysis, a variety of serologic or microbiologic studies, and diagnostic procedures, such as bronchoscopy. (Jameson L.J، *et.al* .2018).

2:4:1 Pneumonia

is pulmonary infection, caused by viruses, bacteria, fungi, or parasites. A predisposing factor, such as occult lung cancer, with obstruction behind it, cystic fibrosis, immunosuppression, aspiration, and so on, should always be sought. Lung infections are classified based on the anatomy or aetiology. Pneumonias may be localized, affecting a lobe (lobar pneumonia) or diffuse, affecting lung lobules, bronchi, and bronchioles (bronchopneumonia) (Herrington, S.C .2014).

2:4:2 Pulmonary Tuberculosis

Tuberculosis remains one of the major causes of morbidity and mortality throughout the world, and is again occurring more frequently in Western countries. This is partly attributable to the increasing numbers of disadvantaged groups within affluent societies but also due to the emergence of resistant strains of the organism and because conditions associated with immunosuppression are becoming more common. The latter group of patients are also susceptible to infection with atypical mycobacteria such as M. avium-intracellulare, M. kansasii and M. Fortuitum. The natural history and pathogenesis of pulmonary tuberculosis were expounded by Rich, in 1951. The causative organism was isolated in 1852 by Koch, and antibiotic treatment has been available from the 1940s. Awareness of the pathology and cytological findings is important to ensure early diagnosis and treatment. (Gray,W and Kocjan, G. 2010). Tuberculosis is classified as pulmonary, extrapulmonary, or both. (Kasper, D.L and Fauci, A.S. 2010).

2:4:3 Classification of Pulmonary Tuberculosis

Pulmonary tuberculosis can be categorized as primary or postprimary (secondary). Primary Disease Primary pulmonary tuberculosis occurs soon after the initial infection with tubercle bacilli. In areas of high tuberculosis transmission, this form of disease is often seen in children. Because most inspired air is distributed to the middle and lower lung zones, these areas of the lungs are most commonly involved in primary tuberculosis. The lesion forming after infection is usually peripheral and accompanied in more than half of cases by hilar or paratracheal lymphadenopathy, which may not be detectable on chest radiography. In the majority of cases, the lesion heals spontaneously and may later be evident as a small calcified nodule (Ghon lesion) (Kasper, D.L and Fauci, A.S .2010).

Postprimary Disease Also called adult-type, reactivation, or secondary tuberculosis, postprimary disease results from endogenous reactivation of latent infection and is usually localized to the apical and posterior segments of the upper lobes, where the substantially higher mean oxygen tension (compared with that in the lower zones) favors mycobacterial growth. In addition, the superior segments of the lower lobes are frequently involved. The extent of lung parenchymal involvement varies greatly, from small infiltrates to extensive cavitary disease.With cavity formation, liquefied necrotic contents are ultimately discharged into the airways, resulting in satellite lesions within the lungs that may in turn undergo cavitation. Massive involvement of pulmonary segments or lobes, with coalescence of lesions, produces tuberculous pneumonia (Kasper, D.L and Fauci, A.S .2010).

2:4:4 Symptoms of pulmonary tuberculosis

Most cases present with pulmonary T.B disease, classically:

- Productive cough
- Haemoptysis
- Breathlessness
- Systemic symptoms—weight loss, night sweats, and malaise
- Chest pain (Chapman,S and Robinson,G.R .2014).

Haemoptysis is more common with cavitatory disease, and up to two-thirds will be smear-positive. Most haemoptysis is small volume. Massive haemoptysis is rare and is most common as a consequence of destruction of a lobe, with consequent bronchiectasis formation. Most haemoptysis will resolve with antituberculous chemotherapy (Chapman,S and Robinson,G.R .2014).

2:4:5:T.B treatment, Follow up, And Drug resistance

All treatment programs should be recommended and preferably under-taken by physicians and health care workers experienced in the management of mycobacterial diseases. The most important impediment to lack of adequate therapy world-wide is the lack of adherence to the treatment. Cavitary tuberculosis is often treated for 9 months. Extrapulmonart uberculosis can be treated effectively with either a 6- or 9- month regimen. However, military tuberculosis,

bone and joint tuberculosis, and tuberculous meningitis in infants and children may require treatment for 12 months or more (Wittich, C.M and Beckman, T.J .2016).

Drug- resistant tuberculosis is an increasingly recognized problem. Drug resistance can develop against a single first- line drug (Chapman,S and Robinson,G.R. 2014).

Multidrug-resistant TB (MDR-TB) defined as MTB resistant to two or more first-line agents, usually isoniazid and rifampicin. It's treatment is complex and time-consuming. MDR-TB is not more infectious than other forms of TB, but the consequences of acquiring it are more serious. 3.6% of new TB cases in the world have MDR-TB. The frequency varies between countries (Chapman,S and Robinson,G.R .2014).

Risk factors for resistant disease are Previous anti-TB treatment, prior treatment failure, Lack of response to intensive phase of standard short-course therapy/treatment failure, HIV infection, Contact with patients with drug-resistant disease, History of poor adherence, aggravated by social deprivation or substance abuse, residence in regions with high prevalence of drug-resistant disease (Chapman,S and Robinson,G.R .2014).

Treatment failure/disease results in relapse which is usually due to poor compliance. Drug resistance may have developed repeat cultures and sensitivity testing in this situation. Consider specific molecular tests for rifampicin/isoniazid resistance. If found, then treat as for MDR-TB (Chapman,S and Robinson,G.R .2014).

Follow-up at 12 months after treatment completion is recommended for patients treated for drug-resistant TB. relapse after good compliance is usually due to fully sensitive organism; therefore, treatment can be with the same regime again. relapse due to poor compliance needs a fully supervised regime (Chapman,S and Robinson,G.R .2014).

2:4:6 Post-TB Complications and fungal infections

TB may cause persisting pulmonary damage in patients whose infection has been considered cured on clinical grounds. Chronic impairment of lung functions, bronchiectasis, aspergillomas, and chronic pulmonary aspergillosis have been associated with TB. Chronic pulmonary aspergillosis may manifest as simple aspergilloma (fungal ball) or chronic cavitary aspergillosis. Early studies revealed that, especially in the presence of large residual cavities, Aspergillus fumigatus may colonize the lesion and produce symptoms such as respiratory impairment, hemoptysis, persistent fatigue, and weight loss, often resulting in the erroneous diagnosis of TB recurrence.(Jameson L.J, *et.al* .2018).

The detection of Aspergillus precipitins (IgG) in the blood suggests chronic pulmonary aspergillosis, as do radiographic abnormalities such as thickening of the pleura and cavitary walls or the presence of a fungal ball inside the cavity. Treatment is difficult (Jameson L.J, *et.al* .2018).

Bronchiectasis, bronchial obstruction, and airway stenosis (uncommon) may result from endobronchial disease, though this is much less common in the post-chemotherapy era. It is more common in the presence of extensive parenchymal disease and is associated with lymph node enlargement, with compromise of airway size (Chapman,S and Robinson,G.R .2014).

Pleural disease is due to either progressive disease or reactivation of latent infection. It probably represents an increased immune response a delayed-type hypersensitivity reaction to mycobacterial antigens, rather than a diminished one, which is the case in other forms of TB infection (Chapman,S and Robinson,G.R .2014).

Pneumothorax is rare and results from the rupture of a peripheral cavity. Can lead to the formation of a bronchopleural fistula. Other complications such as draining abscess and right middle lobe syndrome compression of the right middle lobe bronchus by hilar lymph nodes leads to lobar collapse (Chapman,S and Robinson,G.R .2014).

2:5 Laboratory Procedures for the Diagnosis of Fungal Infection as general

Laboratory methods for the diagnosis of fungal infections remain based on three broad approaches: the microscopic detection of the etiologic agent in clinical material; its isolation and identification in culture; and the detection of either a serologic response to the pathogen or some marker of its presence, such as a fungal cell constituent or metabolic product. New diagnostic procedures based on the detection of fungal DNA in clinical material are presently being developed, but have not yet had a significant impact in most clinical laboratories (Kauffman, C.A , *et al* .2011).

The question of when and how far to go with the identification of fungi recovered from clinical specimens presents an interesting challenge. The current emphasis on cost containment and the ever-increasing number of opportunistic fungi causing infection in compromised patients prompts consideration of whether all fungi recovered from clinical specimens should be thoroughly identified and reported (Tille, P.M .2017).

A study by Murray et al focused on the time and expense involved in identifying yeasts from respiratory tract specimens. Because these are the specimens most commonly submitted for fungal culture, the researchers questioned whether identifying every organism recovered was important. After evaluating the clinical usefulness of information provided through the identification of yeast recovered from respiratory tract specimens, they suggested the following: (Tille, P.M .2014).

- Routine identification of yeasts recovered in culture from respiratory secretions is not warranted, but all yeasts should be screened for Cryptococcus neoformans complex.
- All respiratory secretions submitted for fungal culture, regardless of the presence or absence of oropharyngeal contamination, should be cultured, because common pathogens, such as H. capsulatum, B. dermatitidis, C. immitis, and S. schenckii, may be recovered.
- Routine identification of yeast in respiratory secretions has little or no value for the clinician and probably represents "normal flora," except for C. Neoformans (Tille, P.M. 2014).

2:5:1 Histopathologic Examination

Histopathologic examination of tissue sections is one of the most reliable methods of establishing the diagnosis of subcutaneous and systemic fungal infections. However, the ease with which a fungal pathogen can be recognized in tissue is dependent not only on its abundance, but also on the distinctiveness of its appearance. Many fungi stain poorly with hematoxylin and eosin, and this method alone may be insufficient to reveal fungal elements in tissue. There are a number of special stains for detecting and highlighting fungal organisms, and the clinician should request these if a mycotic disease is suspected. Methenamine-silver (Grocott or Gomori) and periodic acid-Schiff (PAS) staining are among the most widely used procedures for specific staining of the fungal cell wall. Mucicarmine can be used to stain the capsule of C. Neoformans (Kauffman, C.A , *et al* .2011).

2:5:2 Cytological Examination

Many of the respiratory fungal infections are readily detectable by cytologic methods. In these diseases, the etiologic agent is visible and in some cases has a morphology on which a specific diagnosis may be based. The detection of these fungi in a stained cytologic specimen may be the first clue to the nature of a patient's problem. The

accuracy of observation is dependent on the ability of the cytologist to appreciate the various forms that the fungi may assume (Bibbo, M and Wilbur,D .2015).

Fungal infections produce granulomatous inflammation; the granulomas appear as collections of epithelioid histiocytes in the background of chronic inflammatory cells with or without necrosis. Confirmation of the diagnosis with microbiologic fungal cultures is always advised (Bibbo, M and Wilbur,D .2015).

2:5:3 Sputum collection, preparation and examination

Sputum Sputum consists of a mixture of cellular and noncellular elements that are cleared by the mucociliary apparatus. It was once the most common respiratory tract specimen because it is relatively easy to obtain, with little discomfort to the patient. Sputum cytology is generally reserved for symptomatic individuals; as a screening test (e.g., in symptomfree smokers), sputum cytology is not effective in decreasing rates of death from lung cancer. With the advent of bronchoscopy and FNA, its use as the mainstay in respiratory cytology has declined significantly (Cibas, E.S and Ducatman, B.S.2020).

Collecting multiple sputum samples over several days optimizes sensitivity. Early morning, deep cough specimens are preferred. If the patient is not able to expectorate adequately, expectoration can be induced by having the patient inhale nebulized water or saline solution. When prompt preparation of sputum is not possible, the patient can expectorate into a 70% ethanol solution, which prefixes the specimen. A simple method of sputum preparation is known as the “pick and smear” technique, whereby fresh sputum is examined for tissue fragments, blood, or both.(Cibas, E.S and Ducatman, B.S.2020).

Smears are prepared from areas that contain these elements and immediately fixed in 95% ethanol. A modification of this is the Saccomanno method, which calls for sputum to be collected in 50% ethanol and 2% carbowax; it must be performed in a biologic safety hood because of the risks of infection from aerosolization. The specimen is then homogenized in a blender and concentrated by centrifugation. Improved sensitivity has also been demonstrated by the use of dithiothreitol, N-acetyl-L-Cysteine, or CytoRich Red for mucolysis and homogenization. Smears are made from the concentrated cellular material. Sputum can also be processed using thin-layer methods or embedded in paraffin for cell block sections Cibas, E.S and Ducatman, B.S.2020).

Criteria for assessing adequacy of samples when a sample providing enough cells for confident accurate diagnosis can be regarded as adequate. However, misleading reports are sometimes given if the specimen does not include appropriate material confirming the origin of the sample, or if there is insufficient abnormal material to ensure correct interpretation. Hence, it is one of the prime tasks of the cytologist to assess whether a specimen is suitable for diagnosis or whether the test should be repeated (Gray,W and Kocjan, G. 2010).

Sputum specimens are judged adequate when plentiful pulmonary macrophages can be identified. The presence of columnar cells is ambiguous since they may be from the nasal passages or upper airways. Macrophage counts have been used to quantify the adequacy of sputum specimens, and to relate these findings to smoking status, but the procedures are too time-consuming for routine laboratory work. All samples irrespective of their apparent quality should always be screened fully as malignant cells are occasionally found (Gray,W and Kocjan, G. 2010).

Specimens consisting merely of squamous cells, bacteria, and Candida organisms are unsatisfactory because they represent only oral contents. Even ciliated cells, which also line the sinonasal passages, do not guarantee that a sample is from the lower respiratory tract (Cibas, E.S and Ducatman, B.S. 2020).

2:5:4 Culture

Isolation in culture will permit most pathogenic fungi to be identified. Most of these organisms are not fastidious in their nutritional requirements and will grow on the media used for bacterial isolation from clinical material. However, growth on these media can be slow, and development of the structures used in fungal identification can be poor. For these reasons, most laboratories use several different culture media and incubation conditions for recovery of fungal agents. However, a variety of additional incubation conditions and media may be required for growth of particular organisms in culture. The laboratory should be made aware of the particular fungal agent(s) that are suspected in a given sample so that the most appropriate media can be included (Kauffman, C.A , *et al* .2011).

2:6 Previous studies

Study conducted by Elizabeth Nyambura, Vivian Matiru1 and Christine Bii between July 2009 and August 2009, On which 172 sputum samples were collected from Mbagathi

District Hospital TB clinic and trasfered to Mycology Laboratory, Centre for Microbiology Research (CMR), Kenya Medical Research Institute (KEMRI). Among the 172 sputum samples analyzed 14/172(8.1%) were positive for fungal elements, 50/172(29.1%) were positive for yeast cells, 1/172(0.6%) of the speciments were positive for both yeast and gram positive rods. One (0.6%) was positive for yeast cells together with fungal elements, and 3/172(1.7%) of the samples were positive for Gram negative rods and 10/172 (5.8%) were positive for gram positive cocci, 8/172(4.7%) were Gram positive rods, while 85/172 49.4% of samples were scored negative for potential pathogens on Gram reaction (Mwaura E.N,*et al* .2013).

Pulmonary fungal pathogens isolation in Mycobacterium tuberculosis patients were: 46/172 (26.7%). Yeasts were isolated in 46/172 (26.7%) samples, in which 33/172 (19.2%) were Candida albicans, 3/172 (1.7%) were Candida dubliensis, 1/172 (0.6%) was Candida guilliermondii, 3/172 (1.7%) were Candida tropicalis. Cryptococcus lauretii was isolated in 2/172 (1.2%) of the samples. Filamentous fungal colonization with Mycobacterium tuberculosis was as follows: Aspergillus flavus 2/172 (1.2%), A. fumigatus 3/172 (1.7%), A. niger 4/172 (2.3%), Scytalidium hyalinum 2/172(1.2%) and 4/172(2.3%) Trichosporon asahii (Mwaura E.N,*et al* .2013).

Among the pathogens isolated; 46/172 (26.74%) were yeasts and 33/172 (19.2%) were Candida albicans, 3/172 (1.7%), Candida dublieniensis and C. tropicalis each, 1/172 (0.6%) were Candida guilliermondii, 2/172 (1.2%) were Cryptococcus lauretii. The samples negative for yeast were 126/172 (73.3%). Pneumocystis jirovecii Toluidine O Blue staining for Pneumocystis jirovecii detected 19/172 (11.0%) samples positive for Pneumocystis jirovecii oocysts (Mwaura E.N,*et al.* 2013).

A Cross-sectional study of one year duration (January 2015-December 2015) was conducted on patients who were clinically diagnosed cases of Pulmonary Tuberculosis (Group 1) attending Out-Patient Department and In-Patient Department of TB and Chest Diseases and RNTCP lab at KLE′S Dr. Prabhakar Kore charitable Hospital and MRC and on post treated cases of pulmonary tuberculosis having respiratory symptoms (Group 2) at District Hospital, Belagavi. Fifty sputum samples were taken from each group and samples were processed in Bio-safety cabinet II, adhering to Universal safety precautions in the Department of Microbiology, J.N. Medical College, Belagavi. A total of 100 patients suffering from Pulmonary Tuberculosis and Post-treated cases of Pulmonary-TB with

respiratory symptoms were undertaken for the present study. The mean age group in the present study was 40 ± 10 years. Out of 50 participants in each group, in Group 1, 74%were males and 26% were females while in group 2, 72%were males and 28% were females. Of the 100 patients, 55% were positive for opportunistic fungal infection. Amongst them, 58% were in group 1 and 52% were in group 2 (Bin Najeeb, M.A and Nagmoti, M.B.2019).

In group 1, 44% of males and 14% of females had opportunistic fungal infections. And in group 2, 38% males and 14% females showed opportunistic fungal infection. 31% isolates were yeasts (Candida, Cryptococcus and Rhodotorula glutinis) and 24% isolates were molds (Aspergillus spp.) (Bin Najeeb, M.A and Nagmoti, M.B.2019).

Chapter three

Materials and methods

3:1 Study design

Cross sectional, laboratory based study to evaluate sputum cytology in active and post_treated plumonary tuberculosis patients for fungal elements in Al Managil teaching hospital- Gezira state.

3:2 Study area and duration

Al Managil teaching hospital. Gezira state. Sudan from 1\3 to 30\6\2020.

3:3 Sample size

110 patients.

3:4 Inclusion criteria

Patients attended to tuberculosis center. Al managil teaching hospital, either for diagnosis or post_treatment follow up.

3:5 Methedology

3:5:1 Specimen collection and preparation:

Early morning Sputum sample collected in sterile container, after instructing the patient for proper method of expectoration and deep coughing to get satisfactory material for cytologic evaluation, smear prepared as two slides one slide immediately fixed in 95% ethanol, and the other allowed to air dry then stain. One by PAS staining technique and the other with Giemsa stain

3:5:2 Staning procedure:

McManus' PAS method for glycogen and fungal cell walls

1. Fix in 95%ethanol for 15 minutes and take to water
2. Oxidize in periodic acid solution for 5 minutes.
3. Rinse in distilled water.
4. Place in Schiff's reagent for 15 minutes.
5. Wash in running tap water for 10 minutes to allow pink color to develop.
6. Counterstain for a few seconds in working light green solution.
7. Dehydrate in 95% alcohol, absolute alcohol and clear in xylene.

Mount in resin-based mountant (Suvarana. S.K *et al* .2019).

8. **Results**

Fungal cell walls and glycogen magenta to red

Background pale green (Suvarana. S.K *et al* .2019).

Giemsa staining procedure

1. 95% Ethyl alcohol 15 dips
2. 80% Ethyl alcohol 15 dips
3. 70% Ethyl alcohol 15 dips
4. Wash in distiled water 15 dips
5. Giemsa working stain 2 hours
6. 1% Acetic acid 1 quick dip
7. 100% ethyl alcohol until there is only a slight bluish tint to the alcohol that runs off the slide
8. Xylene 10 dips
9. Mount with permanent mounting medium (Koss, L.G and Melamed, M.R. 2006).

3:6 Data type:

Primary data, informations will be collected using questioners

3:7 Data analysis

Data was analyze using computer program (SPSs version 22), Microsoft Excel program.

3:8 Results

Will be display as tables and figures

3:9 Ethical consideration:

Approved from ministry of health of the Gezira state.

All patient fill verbal approval either to share in the study or refuse.

Chapter four

Results and discussion

4:1 Results

The participants were 110 patients with 60 (55%) males and 50 (45%) females (figure-1:4). The age range from 10-86 with mean age 40 years (Figure-2:4). Among these group 69 (63%) patients present for the first time for diagnosis and 41 (37%) for follow up some for the second month while other for third, fourth, fifth and sixth monthes respectively (figure-3:4).

The majorty of patients were farmers (36%) Figure-4:4, from rural areas (82%) (figure-5:4).

Cytologic evaluation of sputum sample for fungal elemnts include 95 (86%) negative, 9(8%) candida species 6(5%) yeast and 3(3%) pseudohphae. 5 (4%) Aspregillus species hyphae, and 1(0.9%) actinomyces speies (Figures 6.4:1 and 6.4:2).

The correlation of the postive samples for fungi to the status either diagnosis or post treated among 69 (63%) of diagnosis 9 (8%) was postive and among 41 (37%) of post treated follow up 5 (4%) was postive as explain in tables 1:4and 2:4.

Testing of fungal elements against MDT-TB results in 87(79%) MDR-TB not detected and negative for fungi, followed by positive for fungi and also MDR-TB not detected 15(13.6%), then (negative and positive) for fungi and MDR-TB with high rate with the same percentage 3(2.8%), and finely very low MDR-TB 1(.9%), medium MDR-TB 1(.9%) without any detection of fungal elements this explain in figure 7:4.

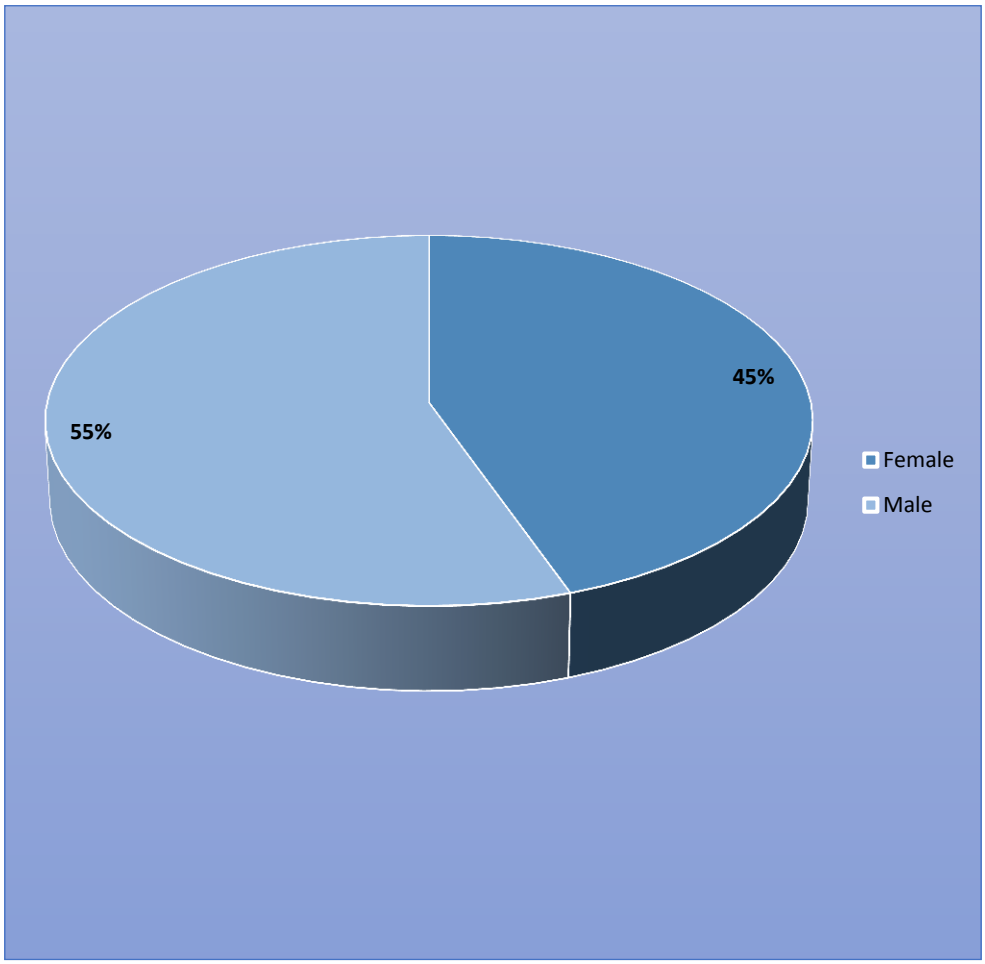

Figure 1:4 Distribution of the group according to the gender

The higher percentages of pateints were male 60 (55%), with percent 45 %(50 patients) females

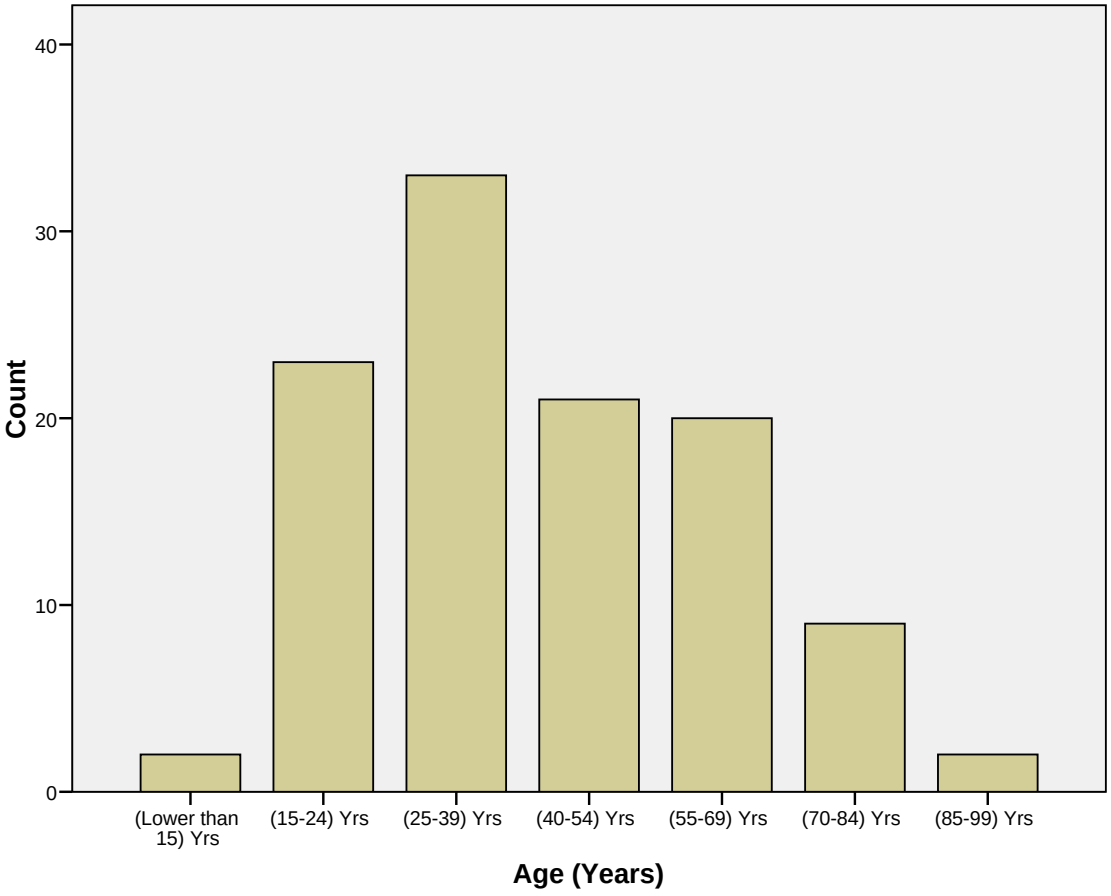

Figure 2:4 Distribution of participants according to age groups

Most of patients in age range (25-39) years, followed by (15-24) years, then (40-54) years that mean most affected groups are adults rather than children or old people

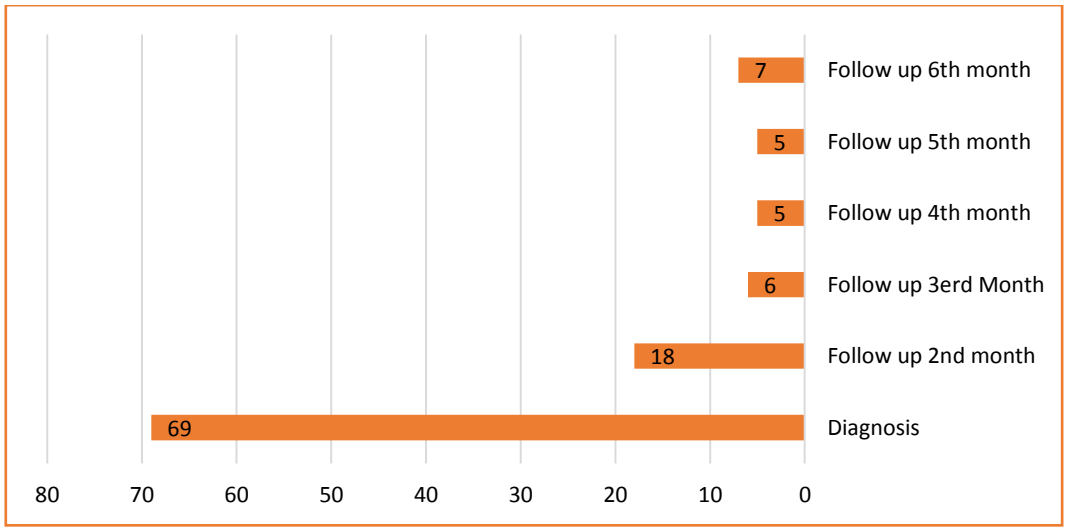

Figure 3:4 Distrbution of participants according the test presnt for

The majority of patients whose attend to the center for first time diagnosis, followed by second month follow up and sixth month follow up (last month of treatment course).

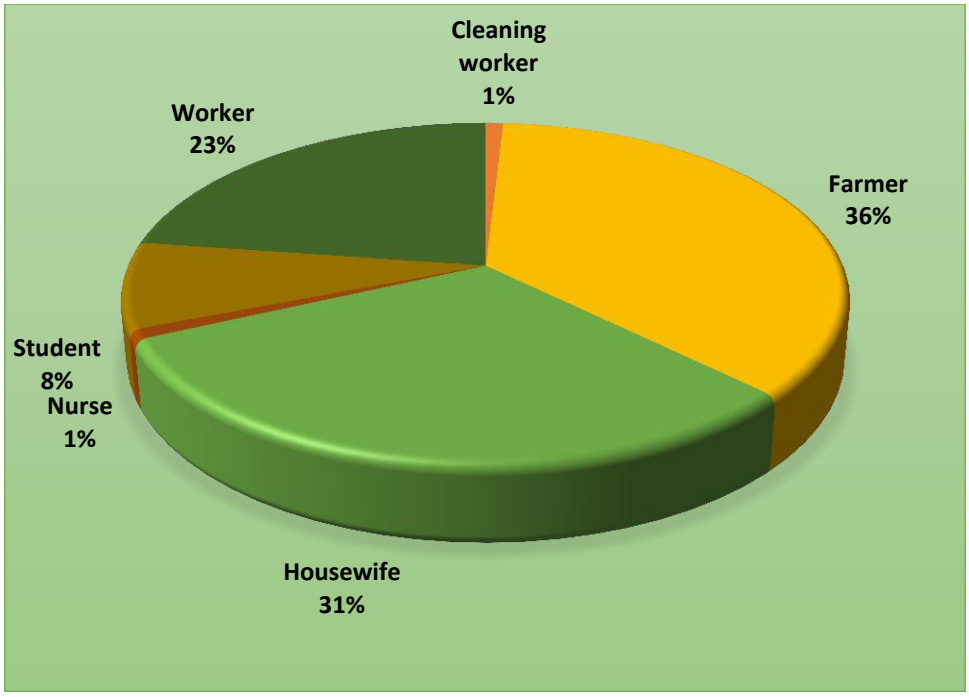

Figure 4:4 Distribution of participantsaccording to occupation

Farmers are most commonly affected working group followed by housewifes the the workers and this may retains the nature of the study area.

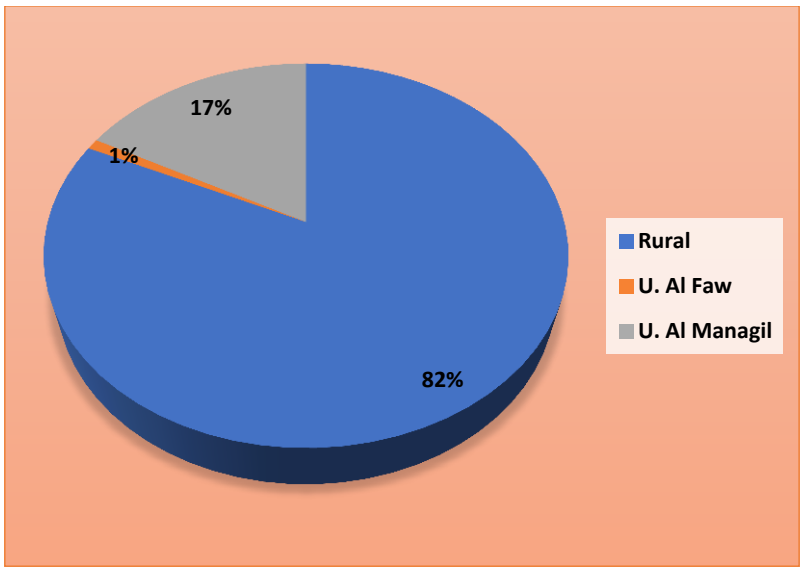

Figure 5:4 Residencce of the participants

Most of the pateins were rurals followed by urbans and this may related the the geographical location of the study area.

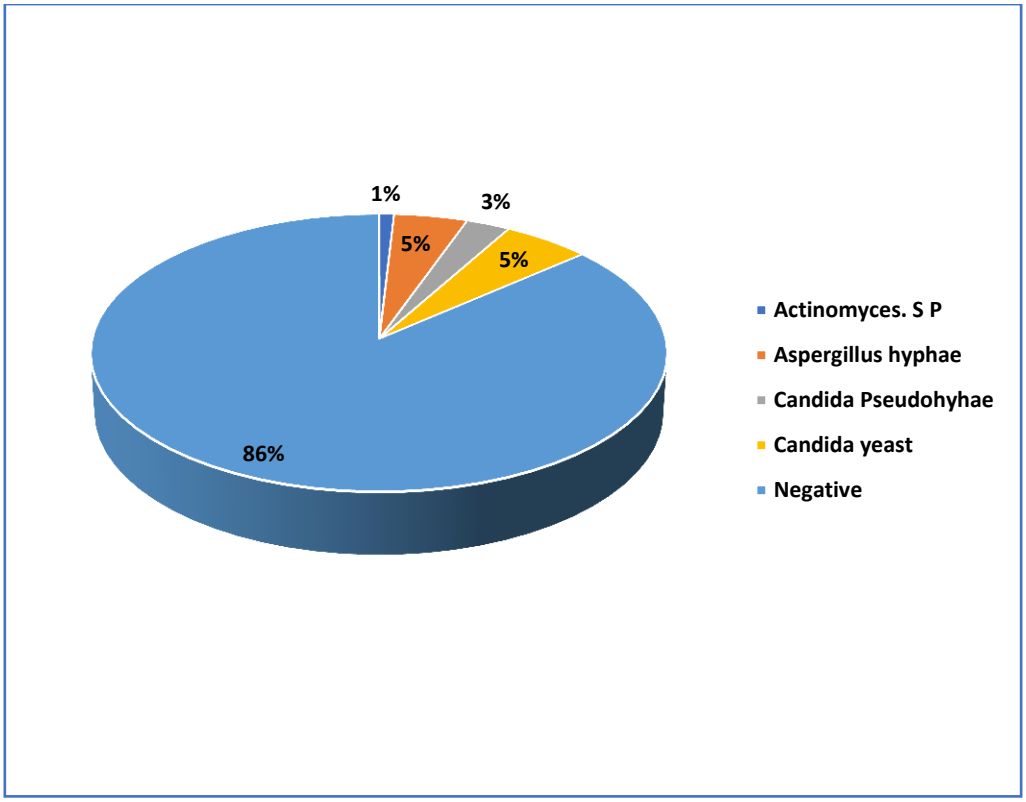

Figure 6:4 Results of microscopic examination for fungal elements

The positive cases were15(14%) and 95(86%) were negative, most common species in positive cases is candida as (yeast and pseudohyphe) 9 (8%), then aspergillus6(5%) Species and actinomyces 1(.9%)

Table 1:4 Cases procesing summary (results for fungal elemnts)

	Cases					
	Valid		Missing		Total	
Test for TB results for fungal elemnts	N	percent	N	percent	N	percent
	110	100%	0	.0%	110	100%

Table 2:4 TB diagnosis results for fungal elemnts Crosstabulation

		Results for funal elemnts		Total
		Negative	Positive	
Test for TB	first time diagnosis	60 63.2%	9 60%	69 62.7%
	Followup(2-3 months)	23 24.2%	2 13.3%	25 22.7%
	Followup (4-6 months)	12 12.6%	4 26.7%	16 14.6%

Total	95 100%	15 100%	110

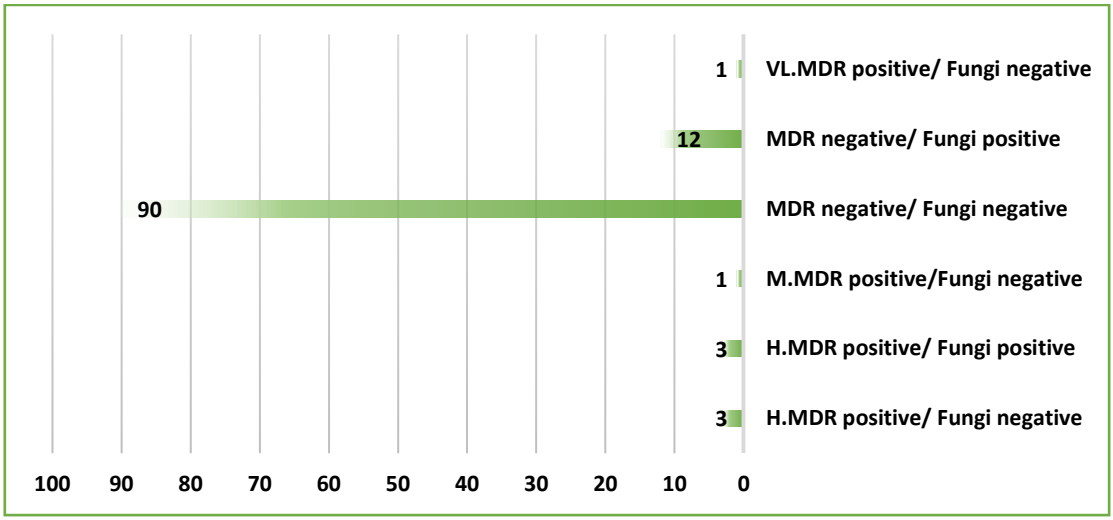

Figure 7:4 Fungal test resluts related to MDR-TB

In the majority of the pateints with negative results for fungi MDR-TB not detected, followed by positive for fungi and also MDR-TB not detected, then (negative and positive) for fungi and MDR-TB with high rate with the same percentage , and finely very low MDR-TB, medium MDR-TB without any detection of fungal elements.

4:2 Disscusion of results:

Pulmonary tuberculosis is one of tropical infectious disease associate with the disadvantaged groups within affluent societies. The disease is destructive that cause cavitation on the lung, with nature of chronic course of the disease and prolong treatment of the tuberculin bacilli superadded by emergence of resistant strains of the bacilli these factors results in immunosuppression, which is favorite environment for opportunistic fungi to flourish and cause complications both in primary, and post-treated patients.

Add to all factors mention previously the geographical location of the study area,leads to that most affected group are farmer from rural area which are clearly appear on the result of the study with 36%,82% respectively.

Cytologic examination for sputum samples in 110 patients 60(55%) males and 50(45%) females. The age range from 10-86 with mean age 40 years, most of the pateints in age group

range 25-39 years, followed by group 15-24 years, then 40-54 years, that clearly indicates adults are more affected group rather than children and old people.

The results of the study was similar to results obtained by Sharaf Eldin *et al*,on May to December 2005. Omdurman hospital, Sudan. on which full drug resistance data was obtained for strains from 235 patients.The median age of participants was 35 years and the interquartile range was 26-45 years.

As in this study the male pateints are majority which represents one hundred and seventy five (74%) of patients were male.

Sharaf Eldin *et al* (2005). reported occupations included unskilled worker or laborer 31% (n = 72), housework 21% (n = 50), business 11% (n = 26), student 10% (n = 24), unemployed 8% (n = 19), farmer 7% (n = 17), driver 5% (n = 11) or soldier 2% (n = 4). Which are differ from results of this study that found farmers are most commonly affected group 36%(n=39).

Among these age groups 69 (63%) present for the first time for diagnosis and 41 (37%) for follow up. The follow up groups classify as two subgroups first three months starting from the second month, and the other subgroup include the third to six month which is the last month of the treatment course according to WHO protocol apply on the center. According to this classification 25 (22.7%) follow up for the first three months of treatment, and 16(14.6%) form forth to six months from starting of treatment.

Positive for total 110 samples is 15(14%). 9(8.%) on first diagnosis samples and 6(5%) on follow up patients, 3(2%) on first three months follow up and 4(3%) on fourth, fifth and sixth months of follow up.

Regardless to the specific identification and other microbiologic or serologic identification of the species, only depends on the cytologic identification with using of special stains for fungi, PAS and Giemsa stains. The species which are demonstrated on positive sample are Candida species 9(8%) both as yeast 6(5%) and Pseudohyhae 3(3%), and dichotomous hyphae of Aspergillus species 5(4%) and actinomyces species 1(1%), these results were similar on the fungal species which found in positive samples ; to the results of the observational analytic study with a cross-sectional design of all pulmonary TB patients who were hospitalized in Dr. Soetomo Hospital Surabaya,Indonesia conducted by Soedarsono Soedarsono *et al* from March 2018 to February 2019 on which fungal isolates were found in 148/193 (77%) pulmonary TB patients. Candida species was found 99% among 148 fungal positive

culture. Candida albicans was the most common found fungal species (54.05%), followed by Candida sp (26.35%), Candida glabrata (10.13%), Candida krusei (5.4%), and Candida tropicalis (1.35%).

But In contrast to results of the study of Elizabeth Nyambura Mwaura et.al which is coducted in July to August 2009 this study, not demonstrate presence of Pneumocystis jirovecii that is always, associate with HIV infection, and there is no patient get positive for TB. Their study results Pneumocystis jirovecii Toluidine O Blue staining for Pneumocystis jirovecii detected 19/172 (11.0%) samples positive for Pneumocystis jirovecii oocysts

The results of the study are similar to the results of the study of Mohammad Aadam Bin Najeeb and Mahantesh B Nagmot in January 2015-December 2015 in common opprtunstic fungal specices.

In the majority of the pateints with negative results for fungi MDR-TB not detected 87(79%), followed by positive for fungi and also MDR-TB not detected 15 (13.6%), then (negative and positive) for fungi and MDR-TB with high rate with the same percentage 3(2.8%), and finely very low MDR-MTB 1(.9%), medium MDR-MTB 1(.9%) without any detection of fungal elements.

These results are in contrast to cross sectional observational study carried out in the department of Microbiology and Out Patient Department(OPD) of Pulmonary Medicine, Jorhat Medical College and Hospital, Jorhat, India. from 20th July-20th September 2017 by Deka Bhakhita *et al* on which the prevalence of mycotic co infection was highest in Multi-drug Resistant TB (MDR-TB) (60%) followed by Category II (35.71%) than Category I (19.40%) DOTS recipients.

Chapter Five

Conclusion and recommendations

5:1 Conclusion:

According to the study, opportunistic fungal infection with pulmonary T.B represents a noticeable percent both in active and post treated patients. Candida and Aspergillus species are the most common causative agents. This co- infection results in persistence of the respiratory symptoms in spite of T.B treatment, or even became worth as disseminated infection, which finally ends on death.

Sputum samples can be used for cytologic diagnosis of fungal infection in pumonary tuberculosis paintes, with aid of using special stains can produce a quite good results.

5:2 Recommendations:

- Awareness for physicians, laboratory personnel and even patients of pulmonary T.B about possibility of fungal Co infection, which may results in serious complications.
- Further investigations such as PCR, DNA sequencing and molecular methods should be introduce on T.B centers for better diagnosis not only for fungal pathogens but also for all diseases associated with T.B disease.
- Laboratory personnel should be train for mycological isolation and identification.
- A clear plan of treatment for fungal pathogens should be apply with regular plan of T.B treatment and administration of antifungal drugs when the test for fungi appear positive as quick as possible.

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Appendix

Reagents:

Ethanol (absoulte 100%, 95%, 70%. 50%) concentrations.

Methanol 100%

Xylene

Schiff's reagent

.5% Periodic acid

.2% Light green

Giemsa stain

1% Acetic acid

Other requirements:

Frosted end slides

Cover glass

Coplin jars

Filter papers

Wooden sticks

Sterile containers

بسم الله الرحمن الرحيم

University of Gazira

Faculty of postgraduate studies/ medical laboratories sciences

Histopathology and cytology department

Questioner

(1) Name……

(2) Age……

(3) Sex……

(4) Residence……

(5) Occupation……

(6) Marital status **Married** **Single** **separated**

Divorced **Widow**

(7) Clinical symptoms

……

……

……

……

(8) When the symptoms start

……

……

(9) History of previous medication

a. Date

b. Type of medicine

(10) Other respiratory disease

(11) If there is other disease

Chronic **acute**

Notes

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Candida Spp Pseudhyphae on positive sputum sample