Sudan University of Science and Technology

College of Graduate Studies

Detection of Multi Drug Resistant *Mycobacterium tuberculosis* among Pulmonary Tuberculosis patients in Shendi city River Nile state–Sudan 2019

الكشف عن المتفطرة السلية المقاومة للأدوية المتعددة بين مرضى السل الرئوي في مدينة شندي ، نهر النيل – السودان 2019

A Thesis Submitted in Partial Fulfillment for Requirement of M.Sc. Degree in Medical Laboratory Sciences (Microbiology)

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December 2020

بسم الله الرحمن الرحيم

الآيـــة

قال تعالى

﴿ فَتَعَالَى اللَّهُ الْمَلِكُ الْحَقُّ وَلَا تَعْجَلْ بِالْقُرْآنِ مِن قَبْلِ أَن يُقْضَى إِلَيْكَ وَحْيُهُ وَقُل رَّبِّ زِدْنِي عِلْماً ﴾

صدق الله العظيم

سورة طه - الآية (114)

Dedication

My humble effort is dedicated to

My loving Mother, Fathers and Aunts

My sweet brothers and sister

To teachers everywhere in the world those teach so other minds can grow

Acknowledgements

First and for most thanks to **ALMIGHTY ALLAH** for giving me strength, health and determination to accomplish this research work.

I would like to thank my supervisor **Dr. Kawther Abdalgaleil Mohammed Salih Ibrahim** for continuous guidance throughout the path of this study.

Moreover, l would like to acknowledge the crucial role of the staff of Department of Microbiology and staff of Shendi Teaching Hospital.

Furthermore, my thanks are extended to Dr. Hamza Hassan, Dr. Abdelmonem Abureeda

And Mr. Mohamed El Toum and all my colleagues who supported and encouraged me throughout my research.

Abstract

Multi Drug resistant tuberculosis caused by *Mycobacterium tuberculosis* resisting to at least isoniazid and rifampicin and considered as a man-made phenomenon and detecting of such resistance at the earliest is essential to limit the spread of the evolving.

This is a cross sectional and laboratory based study was conducted in Shendi , River Nile State ,Sudan during the period from July 2019 to December 2019, aimed to determining the frequency of Multi Drug resistant among TB patients in Shendi to find the most risk factors associate with generating TB-resistance since using of special laboratory tests a fifty two samples from TB positive patients applied on Xpert MTB/RIF automated sample-processing and real-time PCR platform to detect *M.tuberculosis* and rifampicin resistance in a single-use-cartridge hands-free step ,The result showed from a fifty two patients referred to central laboratory and detected for RIF/ISO volunteered a distribution into (43 /52) (82.7 %) were active TB patients negative MDR compare with (9/52) (17.3%) were MDR TB patients.17.3% (9/ 52) positive for MDR-TB, And when it comes to risk factors the people who live in rural was most frequent (100%) MDR positive than who live in urban area (0%) And 6 patients with HIV co infection was positive MDR-TB (67%) patient and 2 patient (22%) was negative for MDR-TB and patient with previous treatment found positive for MDR-TB 8 (89%) from total 9 MDR-TB.

There was a significant association between *MDR-TB* and previous treatment also significant association between *MDR-TB* and Geographic area also negative relation with HIV co infection while there was no significant association between *MDR-TB* and gender of patients, occupation, level of education and *Mycobacterium tuberculosis* quantity and age.

In conclusion this frequency is considered obstacle for TB control program so detection of resistance and adequate treatment is crucial. And MDR-TB mostly prevalent in rural area due to lack of health services and/or difficulties in accessing to health services

المستخلص

السل المقاوم للأدوية المتعددة هو مرض عدوى قاتل تسببه المتفطرة السلية التي تقاوم على الأقل أيزونيازيد وريفامبيسين، وتعتبر ظاهرة من صنع الإنسان. واكتشاف هذه المقاومة في أقرب وقت ممكن أمرضروري للحد من انتشار التطور.

كانت هذه دراسة مقطعية ومخبرية أجريت في ولاية نهرالنيل شندي – السودان خلال الفترة من يوليو 2019 إلى ديسمبر 2019 ، وتهدف إلى تحديد وتيرة الإصابة بالسل المقاوم للأدوية المتعددة بين مرضى السل في شندي والعثور على أكثر عوامل الخطر المرتبطة بتوليد السل – المتعدد المقاومة للأدوية ،و منذ أصبح استخدام الاختبارات المعملية الخاصة القادرة على اكتشاف أنماط مقاومة السل المقاوم للأدوية المتعددة أمرا ممكن ، أخذت 52 عينة من مرضى السل وأجريت عليهم فحص بي سي آر باستخدام جهاز(جين اكسبيرت) لفحص بكتيريا السل المقاومة للريفامبيسين وأظهرت النتيجة من 52 مريضًا مصابين بالسل أن (52/43) (82.7٪) هم مرضى سل سلبيين الاصابة بالسل المتعدد المقاومة للأدوية وأن (52/9) (17.3٪) من مرضى السل لديهم سل مقاوم للأدوية المتعددة وهؤلاء المرضى هم متطوعين في هذا البحث .

وعندما يتعلق الأمر بعوامل الخطر ، كان الأشخاص الذين يعيشون في المناطق الريفية أكثر شيوعًا (100٪) إيجابيي من الذين يعيشون في المناطق الحضرية (0٪) سلبييين وكان 6 مرضى مصابين بفيروس العوز المناعي البشري إيجابيين (67٪) وكان مريضان من مرضى السل ونقص المناعة (22٪) سلبين و بالنسبة لمرضى السل الذين تم علاجهم سابقا وعاودهم السل وجدنا أن 8 (89٪) من الذين تم علاجهم سابقا من إجمالي 9 من مرضى السل لديهم السل المقاوم للأدوية المتعددة .

في الختام يعتبر هذا التواتر عقبة أمام برنامج مكافحة السل ، لذا فإن اكتشاف السل المقاوم والعلاج المناسب مهم. ينتشر السل المقاوم للأدوية المتعددة في المناطق الريفية بسبب نقص الخدمات الصحية في المناطق الريفية و/أو الصعوبات في الوصول إلى الخدمات الصحية كما أن هناك ارتباط كبير بين السل المقاوم للأدوية المتعددة والعلاج السابق أيضًا علاقة سلبية مع عدوى فيروس العوز المناعي البشري بينما لم يكن هناك ارتباط كبير بين السل المقاوم للأدوية المتعددة وجنس المرضى والمهنة ومستوى التعليم وكمية بكتيريا السل المتفطرة وعمر المرضى.

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List of abbreviation

AFB	**Acid Fast Bacilli**
CDC	**Centers for disease control**
CFU	**Colony Forming Unit**
DC	**Dendritic Cell**
DNA	**Deoxyribonucleic acid**
DST	**Drug-susceptibility testing**
HIV	***Human immunodeficiency virus***
IGRA	**Interferon-Gamma Release Array**
INH	***Isoniazid***
LPA	**Line Probe Assay**
MDR	***Multidrug-resistant***
MDR-TB	**Multi Drug Resistant Tuberculosis**
MOTTB	**Mycobacterium Other Than Tuberculosis**
MTB	**Mycobacterium Tuberculosis**
NK	**Natural Killer cell**
NTP	**National Tuberculosis Control Program**
PTB	**Patients with Pulmonary Tuberculosis**
PZA	***Pyrazinamide***
RIF	***Rifampin***
RNA	**Ribonucleic acid**
RR-TB	**Rifampicin Resistant Tuberculosis**
SLD	***Second line drugs***
TB	***Tuberculosis***
TDR	***Total drug resistant***
TDR-TB	**Total Drug Resistant Tuberculosis**
TDR-TB	**Total Drug Resistant Tuberculosis**
TLR	**Toll like Receptor**
WHO	***World Health Organization (WHO)***
XDR	***Extensively drug-resistant***
XDR-TB	**Extensively drug-resistant Tuberculosis**
IL 2	**Interleukin 2**
IL 17	**Interleukin 17**
NK cell	**Natural killer cell**

CHAPTER I

INTRODUCTION

1.1. Background

Tuberculosis is a deadly infectious disease caused by the bacteria *Mycobacterium tuberculosis* (*Nasiruddin et al*., 2017)

Each year 8–9 million persons develop the disease and approximately two million people die of (Tuberculosis) TB or its complications (Niţu *et al.*, 2017).

During the 1990s, multidrug-resistant tuberculosis (MDR-TB), defined as resistant to at least isoniazid and rifampin, emerged as a threat to TB control (*Hu* et *al.*, 2017) .

Now (MDR) is a serious global threat with prevalence about 1-2% of TB cases around the world which mainly occur in many developing countries due to poor treatment of TB the Problems related to MDR-TB is the requirement of a very toxic and expensive medication for therapy leads to less influential and more toxic second line drugs (SLD) Fluroquinolones with higher mortality level than susceptible TB (Muhammad *et al*,. 2019) which can then be transmitted, and patients experience many adverse effects from the drugs. In some cases even more severe drug-resistant TB may develop Extensively Drug-Resistant TB, (XDR-TB), which is a form of multidrug-resistant TB It has been reported in 117 countries worldwide. (WHO, 2018).

In 2017 an estimated 10 million people developed tuberculosis and 4 million people with tuberculosis remained undiagnosed and untreated. There were 558,000 new cases of drug-resistant TB 82% of which were (MDR-TB-resistance to isoniazid and rifampicin), and 8.5% has extensively drug resistant TB (XDR-TB -MDR-TB (Petersen *et al.*, 2019) Recently another dangerous form of TB bacillus was identified which was named Totally Drug Resistant (TDR-TB) or extremely drug resistant TB. These strains were resistant to all first- and second-line anti-TB drugs. Collectively, it is accepted that 2% of MDR-TB strains turn to be TDR-TB (Velayati and Farbahod .,2016) and those resistant types considered obstacles for to control tuberculosis according to the goals of the WHO Strategy to end global tuberculosis epidemic by 2030 (Eskild *et al* .,2019).

Drug resistance can be detected using special laboratory tests which tested the bacteria for sensitivity to the drugs or detect resistance patterns. These tests can be molecular in type (such as Xpert MTB/RIF) provide results within hours and have

been successfully implemented even in low resource settings or else culture-based (WHO,2018) more over a better knowledge of the mechanisms of action of anti-TB drugs and the development of drug resistance allow identifying new drug targets and better ways to detect drug resistance (WHO ,2018).

1.2. Rationale

Tuberculosis (TB) one of the top 10 causes of death globally, despite the availability of curative treatment (MDR-TB) accounts for a sizable proportion of TB which are more disabling patient With outcomes compared to non-resistant strains (Dheda, ,2019) and consider a public health threat especially in Sudan which has a socioeconomic impact and threatens global public health. Because of poor treatment outcomes Sudan may suffer from high prevalence rates (Ali *et al*, .2019).

Rapid detection and timely initiation of effective treatment is critical to rendering MDR -TB cases non-infectious (Fox *et al*., 2017), Since Sudan is a large country with a diverse population and history of civil conflict and poverty levels are high, Sudan has a high burden of TB Few studies have been undertaken on TB in Sudan and the prevalence of drug resistant TB unknown *(*Eldin *et al.,* 2011).

Treatment short course and continuously survey the prevalence of drug resistance cases is important to prevent the development of new cases and treat existing patients (Tembo and Malangu., 2019).

So applying this study to have more data about the prevalence of MDR TB in Shendi city using GeneXpert Technique among tuberculosis patients those who starting treatment and those who relapsed due to cutting or fail of treatment to control MDR TB dissemination since surveillance is one of the controlling strategy besides observing treatment process.

1.3. Objectives

1.3.1. General objective

To determine the frequency of MDR-TB among pulmonary tuberculosis patients in Shendi city, River Nile, Sudan

13.2. Specific objective

1-To detect MDR-TB using Gene Xpert Technique among infected TB patients.

2-To determine the frequency of MDR among TB patients to factors (HIV, Age, Previous treatment, status address age, gender and occupation state, quantity of mycobacterium education) associated with developing resistance among TB patients.

3-To find possible association between possible risk factors (HIV, previous treatment , status address, age, gender and occupation state, quantity of mycobacterium education) and resistance development among TB patients.

CHAPTER II

LITERATURE REVIEW

2.1 *Mycobacterium tuberculosis*

2.1.1 Definition of TB and TB infection

TB is a contagious infectious disease caused by *Mycobacterium tuberculosis* with chronic evolution widespread in the population, which if untreated has a significant fatality (Niţu, *et al*., 2017) Development of TB in an exposed individual is a two-stage process following infection. In most infected persons, infection is contained by the immune system and bacteria become walled off in caseous granulomas or tubercles. In about 5% of infected cases, rapid progression to tuberculosis will occur within the first two years after infection (Narasimhan *et al*., 2013), The risk of TB development varies between people, depending on factors such as infectious dose and time since infection, *M. tuberculosis* strain, smoking and host immunity, which is influenced by co-morbidity, malnutrition, HIV infection, and other factors,.(Hoa ,2013)

2.1.2 Table (1-2) Tuberculosis classification system

The tuberculosis classification system contain 6 types according to CDC starting with class 0 and end with class 5 (CDC, 2011)

Classification System for TB		
Class	**Type**	**Description**
0	No TB exposure Not infected	No history of exposure Negative reaction to tuberculin skin test
1	TB exposure No evidence of infection	History of exposure Negative reaction to tuberculin skin test
2	TB infection No disease	Positive reaction to tuberculin skin test Negative bacteriologic studies (if done) No clinical, bacteriologic, or radiographic evidence of TB
3	TB, clinically active	*M. tuberculosis* cultured (if done) Clinical, bacteriologic, or radiographic evidence of current disease
4	TB Not clinically active	History of episode(s) of TB **or** Abnormal but stable radiographic findings Positive reaction to the tuberculin skin test Negative bacteriologic studies (if done) **and** No clinical or radiographic evidence of current disease
5	TB suspect	Diagnosis pending TB disease should be ruled in or out within 3 months

(CDC, 2011)

2.1.3TB treatment

Table (1-1) Drug Susceptible TB Disease Treatment Regimens (CDC, 2016)

Regimens for treating TB disease have an intensive phase of 2 months, followed by a continuation phase of either 4 or 7 months (total of 6 to 9 months for treatment).

	INTENSIVE PHASE		**CONTINUATION PHASE**				
Regimen	**Drugs[a]**	**Interval and Dose[b] (minimum duration)**	**Drugs**	**Interval and Dose[b,c] (minimum duration)**	**Range of Total Doses**	**Comments[c, d]**	**Regimen Effectiveness**
1	INH RIF PZA EMB	7 days/week for 56 doses (8 weeks) *or* 5 days/week for 40 doses (8 weeks)	INH RIF	7 days/week for 126 doses (18 weeks) *or* 5 days/week for 90 doses (18 weeks)	182 to 130	This is the preferred regimen for patients with newly diagnosed pulmonary TB.	
2	INH RIF PZA EMB	7 days/week for 56 doses (8 weeks) *or* 5 days/week for 40 doses (8 weeks)	INH RIF	3 times weekly for 54 doses (18 weeks)	110 to 94	Preferred alternative regimen in situations in which more frequent DOT during continuation phase is difficult to achieve.	
3	INH RIF PZA EMB	3 times weekly for 24 doses (8 weeks)	INH RIF	3 times weekly for 54 doses (18 weeks)	78	Use regimen with caution in patients with HIV and/or cavitary disease. Missed doses can lead to treatment failure, relapse, and acquired drug resistance.	
4	INH RIF PZA EMB	7 days/week for 14 doses then twice weekly for 12 doses[e]	INH RIF	Twice weekly for 36 doses (18 weeks)	62	Do not use twice-weekly regimens in HIV-infected patients or patients with smear positive and/or cavitary disease. If doses are missed then therapy is equivalent to once weekly, which is inferior.	

2.2.1. Multi Drug Resistant –Tuberculosis

2.2.1.1. Definition of MDR –TB

Multidrug-resistant tuberculosis (MDR-TB) a man-made phenomenon and arises due to inadequate treatment of drug-sensitive TB (Sharma *et al.*, 2011*)* resisting to at least isoniazid and rifampicin and it influence the future of global TB control (Dennis *et al*., 2015)

2.2.1.2. Risk factors to develop MDR –TB

The prevalence of MDR-TB due to lack or weak tuberculosis control programs in some epidemic country ,and previous treatment for TB is the strongest risk factor for development of MDR-TB *(*Sharma *et al .,* 2011) Which came with a recent problem that multidrug-resistant tuberculosis is epidemic now *(*Galli *et al* .,2016) However being a previously treated tuberculosis patient and having a positive smear found to be factors associated with the prevalence of multidrug/rifampicin-resistant tuberculosis in which age, sex, living in urban area and HIV status were not associated with this *(*Tembo and Malangu ,2019) Studies carried in Sudan showed the geographic region of origin of the patient, being most frequently observed as a risk factor (Eldin *et al* .,2011) The ability of *M. tuberculosis* for the development of drug resistant gene were found to be associated with many factors, however hypertension and diabetes mellitus were of no significant in the contribution of developing drug resistance (Enan ,2018)

2.3. Classification of drug resistant TB

2.3.1. Classification of MDR-TB Resistance according to acquisition

Patients with MDR drug-resistant tuberculosis are classified as:

2.3.1.1. having acquired drug-resistant Acquired MDR-TB refers to resistance developed during or following chemotherapy in patients who had previously been regarded as DS TB (Li *et al* .,2017)

2.3.1.2. Primary drug-resistant disease. Primary MDR-TB characterizes patients who have no prior TB treatment history or treatment of less than one month (Li *et al* .,2017) Only cases of primary drug resistance are assumed to be due to inability to accurately determine resistance patterns in cases of mixed infection (Multiple infections with different strains of *Mycobacterium tuberculosis)*may exacerbate delays in the diagnosis of drug-resistant tuberculosis, which could have implications for the individual patient and the spread of drug-resistant strains (Van Rie *et al* .,2005).

2.3.2. Classification based on type of drug resistance

2.3.2.1. Monoresistance:

Resistance to only one first-line anti-TB drug for example resistance to isoniazid (INH) alone and it is can be risky because INH is the most potent anti-TB drug and is the main part of any first-line treatment regimen for TB (Varahram *et al* .,2014)

2.3.2.2. Polydrug resistance:

Resistance to more than one first-line anti-TB drug (other than both isoniazid and rifampicin) (WHO .,2014) or can be resistance to at least two or more drugs, but excluding the INH and RIF combination (Yang *et al* .,2014).

2.3.2.3. Multidrug resistance (known as MDR-TB):.

Multidrug-resistant TB (MDRTB), caused by TB bacilli resistant to both isoniazid (INH) and rifampin (RIF), poses difficulties in diagnosis and treatment, with lower survival rates (especially in HIV-infected persons) associated high costs of TB control programs. The World Health Organization (WHO) estimates current MDRTB rates in new and previously treated cases globally to be 2.9 and 15.3%, respectively (Kontsevaya *et al* ., 2011)

2.3.2.4. Extensive drug resistance (known as XDR-TB):

Multidrug resistance plus resistance to any fluoroquinolone and at least one of three second-line injectable drugs (amikacin, capreomycin or kanamycin)(World Health Organization ,2014). (XDRTB) which is caused by MDRTB bacilli with additional resistance to a fluoroquinolone [FQ] and one or more injectable drugs) has been increasing in Russia due to multiple incomplete treatment regimens and poor infection control practice. Cases of XDRTB show a high rate of treatment failure and mortality especially in XDRTB patients with concomitant HIV infection (Kontsevaya *et al* ., 2011)

2.3.2.5. Rifampicin resistance (known as RR-TB):

Resistance to RIF only and it is largely attributed to nucleotide substitutions in an 81-bp core region of the rpoB gene In contrast, resistance to INH occurs by mutations in several genes, in particular inhA and katG, and to a lesser extent in ahpC, oxyR, kasA, furA and ndh(Coovadia *et al* .,2013)

2.3.2.6. Totally drug resistant (TDR) - TB:

Totally drug-resistant tuberculosis (TDR-TB) refers to *M.tuberculusis* clinical strains that show in vitro resistance to all first to all first- and second-line drugs tested (INH, RIF, SM, ethambutol, pyrazinamide, ethionamide, PAS, cycloserine, ofloxacin, amikacin, ciprofloxacin, capreomycin and kanamycin) These *M.tb* strains were also

resistant to rifabutin, clofazimine, dapsone, clarithromycin and thiacetazone. The authors suggested the new term “XDR-TB" to define this (TDR-TB) strain (Parida *et al*., 2015)

2.4. Genetic structure of MDR TB

Mutations in the genes *rpo B* (rifampicin), *katG* (isoniazid), *inhA*-promoter (isoniazid), *rpsL* (streptomycin) and *embB* (ethambutol) were responsible for the majority of resistance observed in Mycobacterium (Phelan *et al.,* 2016) and when come to rifampicin monoresistance and the high resistance to RIF they found association with various point mutations inside and outside of the Rifampicin-resistance-determining region (RRDR) of the *rpoB* gene (Khalid *et al* .,2015).

2.4.1. Genes *rpo B* (rifampicin)

The molecular mechanism of rifampicin-resistance demonstrating that resistance is mostly due to chromosomal mutations in *rpoB* gene encoding the RNA polymerase β chain (Alifano *et al* .,2015).

2.4.2. katG and *inhA*-promoter (isoniazid)

(INH) resistance of *Mycobacterium tuberculosis* is caused by mutations in the *katG* and *inhA* genes encoding for catalase-peroxidase and inhA, (*Mycobacterium tuberculosis* is isoniazid (INH)) respectively (Tseng *et al.,* 2015)

2.4.3 rpsL (streptomycin)

Streptomycin resistance is mainly related to mutation in gene rpsL and in lesser amount to gene rrs, other factors such as changes in permeability of the wall and membrane and modifying enzymes of aminoglycosides are slightly effective(İk , 2015). Mutation in gene rpsL depends on changes in codons 43 in 70% cases and codon 88 in much less cases. Streptomycin resistance occurs because of occurrence of mutation in genes such as rpsL and rrs that are respectively coding of 16SrRNA and ribosomal protein S12. They are responsible for high levels of resistance to Streptomycin (İk. 2015).

2.4.4. embB (ethambutol)

Ethambutol inhibits arabinogalactan and lipoarabinomannan biosynthesis in mycobacteria. , its associated with simultaneous resistance to ethambutol, isoniazid, and rifampin, with a specificity of 97% for diagnosing multidrug resistance associated with ethambutol, indicating its potential as a molecular marker for several drugs (Cuevas-Córdoba *et al* , 2015).

2.5. Pathophysiology

2.5.1. TB Pathophysiology

Tuberculosis is an infection caused by the rod-shaped, non-spore-forming, aerobic bacterium *Mycobacterium tuberculosis*. Mycobacterium commonly measure 0.5 μm by 3 μm, and classified as Acid-fast bacilli, and have a unique cell wall structure crucial to their survival (Kinsi Jama, 2019).

The well-developed Cell wall contains a considerable amount of a fatty acid, mycolic acid, covalently Attached to the underlying peptidoglycan-bound polysaccharide arabinogalactan (Biopolymer Consisting of arabinose and galactose monosaccharide), providing an extraordinary lipid barrier and this barrier is responsible for many of the medically challenging physiological characteristics of Tuberculosis, including resistance to antibiotics and host defense mechanisms. *(*Kinsi Jama , 2019).

The composition and quantity of the cell wall components affect the bacteria's virulence and growth rate. The Peptidoglycan polymer confers cell wall rigidity and is just external to the bacterial cell Membrane, another contributor to the permeability barrier of mycobacterium. Another important Component of the cell wall is lipoarabinomannan (Glycolipid and major virulence factor in the Bacteria genus Mycobacterium), a carbohydrate structural antigen on the outside of the organism that is immunogenic and facilitates the survival of mycobacteria within macrophages. (Kinsi Jama , 2019)

2.5.2. MDR TB Pathophysiology

Immune studies suggest that MDR bacilli can act like drug sensitive Mtb manipulating the host responses to support the survival and spread of the bacilli. MDR-Mtb infection clearly stimulates broad immune responses, but these vigorous MDR-Mtb-induced responses fail to control the infection (Yang *et al.*,2018). Instead, these potentially overreactive or inflammatory responses may play a role in the development of severe TB lesions that enhance airborne Mtb transmission.. (Yang *et al.*,2018).

2.6. TB Immunology to tuberculosis infection

Infectious droplet nuclei are deposited in the alveolar spaces where *M. tuberculosis* can be phagocytosed by alveolar macrophages epithelial cells, dendritic cells (DC) and neutrophils. Alveolar macrophages and DC are then believed to transport *M. tuberculosis* to local lymph nodes where T cells are primed and clonally expanded *(*Dheda, *et al*., 2010)

2.6.1. Innate immunity to *M. tuberculosis*

Upon entry into the host lungs by aerosol inhalation, *M. tuberculosis* interacts with toll-like receptors (TLR), complement receptor 3, mannose receptor, scavenger receptor, receptor, DC-specific intercellular-adhesion-molecule-3-grabbing non-integrin, on the surface of macrophages and DC). These receptors recognize components of *M. tuberculosis* such as lipoprotein, CpG-containing DNA, mannose-capped lipoarabinomannan and phosphatidylinositol mannoside, respectively, Interferon-γ, secreted from activated T cells and NK cells have the capability to activate macrophages and promote bacterial killing by permitting phagosomal maturation and production of antimicrobial reactive nitrogen intermediates and reactive oxygen intermediates (Dheda *et al* .,2010)

2.6. 2. Adaptive immunity to *M. tuberculosis*

Mycobacterium-infected macrophages and DC of the innate immunity present antigens to T cells and B cells that belong to adaptive immunity. Cytokine IL12p40 plays a fundamental role in the pathogen-induced activation of pulmonary DC. Macrophage apoptosis that releases apoptotic vesicles to carry *mycobacterium* antigens to uninfected DC can lead to more effective antigen presentation and that lead to activation CD4 T cell and CD8 T cell ,The CD8 cytolytic T lymphocyte secrete granulisin enzymes and perforins to kill mycobacteria infected – cell and capable of protection against secondary mycobacterial infection *(*Dheda, *et al* ., 2010)

2.6.3. MDR-TB Immunology

In vitro comparative study done in vitro they found after MDR-Mtb infection upregulated the expression of caspase 3, an apoptotic/cell death surrogate marker, in macrophages/monocytes and induced substantial innate-like IFN-γ and TNF-α responses in the CD3-lymphocyte population and the Mtb phospho antigen-specific γ δ T-cell subset, respectively. Concurrently, broad adaptive responses of T-cell subpopulations producing Th1, Th17, Th22, and CTL cytokines were detected after primary MDR-Mtb infection (Yang *et al*., 2018) .

2.7. Epidemiology of MDR –TB

An estimated 3.9% of new TB cases and 21% of previously treated cases had rifampicin-resistant (RR) or multidrug-resistant (MDR) TB in 2015 *(*Dean *et al.*, 2017)

However the geographical distribution of MDR-TB is highly variable from Eastern Europe to Asia. In 2016, the largest number of MDR/RR-TB was reported from the European Region followed by South East Asia in the Western Pacific region, 21 252

cases of MDR/RR-TB were notified) (Lange *et al.,* 2018) And from data collected globally in 2012, there were an estimated 450,000 cases of multidrug resistant (MDR)-TB and 170,000 deaths were due to it. MDR-TB is caused by strains of Mycobacterium tuberculosis that are resistant to at least rifampicin and isoniazid *(*WHO,. 2018) .

Despite the fall in incidence and mortality of multidrug-resistant (MDR) the WHO estimated there were 450,000 incident cases of drug-resistant TB ,170,000 drug-resistant TB-related deaths in 2012 worldwide (Günther ,2014) from Belarus, where 38% of new cases and 72% of retreatment cases are estimated to have MDR/RR‑TB (Lange *et al* ., 2018).

The estimation of MDR-TB in Sudan which is a large country with a diverse population and history of civil conflict and poverty due to that 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 TB (Eldin *et al* ., 2011) In study done in Sudan to estimate the MDR-TB amoung tuberculosis patients 54 of total 239 found multi-drug resistant (Adam *et al* .,2017)

2.8. Susceptibility to TB and MDR-TB

Drug susceptibility testing (DST) is performed to test for resistance to any of the first-line anti-tuberculosis drugs. Resistance to isoniazid and rifampicin in the first line drugs is diagnosed as multidrug resistant TB (MDR-TB) (Agyeman and Ofori-Asenso ., 2017).

DST involving second-line drugs are conducted under special cases such as previous TB treatment, contact with patient diagnosed of drug resistant TB, confirmed resistance to first-line anti-TB drugs or positive cultures following more than 3 months of treatment (Following second-line drug susceptibility test, diagnosis can be made for extensively drug resistant TB (XDR-TB) if in addition to isoniazid and rifampicin resistance, the TB isolate shows additional resistance to at least one of the three injectable second line drugs (i.e., amikacin, kanamycin or capreomycin) and any of the fluoroquinolones (Agyeman and Ofori-Asenso *,2017).*

2.9. Diagnosis of MDR –TB

Testing for active TB is achieved either through skin or blood tests. The skin test is known as Mantoux tuberculin test) ,The blood tests are also known interoferon

gamma release array (IGRA) (Agyeman and Ofori-Asenso .,2017) ,And there are a number of currently available WHO-recommended diagnostic techniques for detection of resistance of *M. tuberculosis* isolates *(*Gilpin *et al .,* 2016*)* Those techniques can be categorized to:

2.9.1. Mantoux tuberculin test

The reaction to intracutaneously injected tuberculin is the T-cells sensitized by prior infection are recruited to the skin site where they release lymphokines these lymphokines induce induration through local vasodilatation, edema, fibrin deposition, and recruitment of other inflammatory cells to the area, (Nayak and Acharjya .,2012) The Mantoux test does not measure the degree of hypersensitivity to tuberculin and the results of this test must be interpreted carefully after48-72 hrs of testing diametr of 0-4 represent negative and at medical risk factors if the size of indurations the result is positive (5 mm, 10 mm, or 15 mm) (Nayak and Acharjya .,2012).

2.9.2. Phenotypic methods to detect MDR-TB

M. tuberculosis isolates that cannot grow at 'critical' concentrations (the drug concentration (in mg/L or μg/ml) included in the culture medium)were then defined as susceptible, whereas those that can grow were considered resistant (Schön *et al .,* 2017).

2.9.3. Molecular (genotypic) methods to detect MDR-TB

Molecular (genotypic) methods detect specific DNA mutations in the genome of the *M. tuberculosis*, which are associated with resistance to specific anti-TB drugs Molecular tests for detecting drug resistance to rifampicin alone or in combination with isoniazid have been recommended for use by WHO Xpert MTB/RIF and the next-generation assay Xpert MTB/RIF Ultra (Cepheid, Sunnyvale, CA, USA) are fully automated nucleic acid amplification assays that detect MTB and mutations affecting the rifampicin resistance determining region (RRDR, codons 426–452) of the *rpoB* gene directly from clinical specimens. Real-time polymerase chain reaction (PCR) and melting temperature-based analysis are used by the two assays, respectively, to target the RRDR wild-type sequence (no mutant probes targeted) (Cabibbe *et al*.,2017).

Line probe assays (LPAs) are based on the PCR amplification of specific fragments of the MTB genome, followed by reverse hybridisation of the PCR products to oligonucleotide probes immobilised on nitrocellulose strips. Resistance is detected by lack of binding to wild type probes and also by binding to probes targeting specific

mutations. Commercial LPAs include: GenoType MTBDR plus V2 (Hain Lifescience, Nehren, Germany) and Nipro NTM + MDRTB detection kit 2 (Nipro Corporation, Tokyo, Japan) for MTB rifampicin and isoniazid resistance determination (*rpoB* RRDR, *katG* region Ser315, *inhA* promoter); GenoType MTBDR*sl* V1 and V2 (Hain Lifescience) for identification of MTB mutations associated with fluoroquinolone (*gyrA* quinolone resistance determining region, QRDR, plus *gyrB* QRDR in version two) and second-line injectable drug (*rrs* region 1400, plus *eis* promoter in version two excluding the ethambutol resistance-conferring target *embB*, included in the previous version) resistance (Cabibbe *et al* .,2017).

2.9.3.1. DNA Line Probe Assays

Line probe assays (LPAs) are basically DNA–DNA hybridization assays that allow the simultaneous detection of different mutations by using multiple probes'After DNA extraction and target amplification, amplicons are hybridized to specific oligonucleotide probes that are complementary to the target sequences and are immobilized on the surface of a strip. After several post-hybridization washes to remove non-specific binding, the amplicon-probe hybrids are visualized by eye as colored bands on the strip (Nguyen *et al* ., 2019).

2.9.3.2. Real-Time PCR Assays

Two main approaches are commonly used in real-time PCR: (i) the use of non-specific fluorescent dyes to detect any double-stranded DNA generated by PCR amplification, and (ii) the use of sequence-specific probes tagged with a fluorescent reporter for the specific detection of the hybridization between probes and amplicons (Nuguyen *et al* .,2019*)*.

Each probe has a specific melting temperature (Tm), and a Tm change reflects the presence of mutations in the target. This feature has been used to develop real-time PCR tests for drug resistance screening for example of real time PCR assays which is used in this research The Xpert MTB/RIF assay uses semi-quantitative nested real-time PCR to amplify a fragment containing the 81 bp hotspot region of the rpoB gene (codons 507–533) that is then hybridized to five molecular beacon probes ,Each probe covers a separate sequence and is labeled with a fluorescent dye and the whole experiment is performed in a self-contained cartridge, like a mini-laboratory, to minimize cross-contamination between samples (Nuguyen *et al*., 2019).

2.10. Treatment and control of MDR -TB

Patients with MDR-TB and RR-TB (MDR/RR-TB) require radical changes in

treatment compared to those with drug-susceptible TB *(*Weyer *et al* .,2017) Patients infected with (MDR) TB strain do not respond to WHO standardized directly observed short-course chemotherapy and require longer, more toxic, and more expensive treatment that's why timely identification of patients with MDR TB enables rapid initiation of adequate treatment, (Mekonnen *et al* ., 2015) And need prolonged treatment (often up to two years) with costly (Weyer *et al* .,2017), highly toxic and much less effective second-line medicines, of which there is only a limited number (Weyer *et al*., 2017) treatment becomes extremely difficult The MDR/RR-TB crisis demonstrates many of the challenges because airborne transmission of the disease with explosive outbreaks should good quality surveillance done (Weyer *et al* .,2017)

The emergence of drug resistance mutations in *M. tuberculosis* during host infection either due to spontaneous errors during DNA replication and repair or to potentiate the activity of existing antituberculosis mostly due to defect in treatment regimen resulting in the emergence of drug resistant *M.tuberculosis* (McGrath *et al*.,2014).

Rapid diagnosis of drug resistance, appropriate treatment, improved infection prevention and control, and good care delivery systems with trained health personnel. Moreover, diagnostic platforms, logistics and digital technologies for sharing data can be used to link TB and AMR programmes at the country level (Weyer *et al* .,2017).

Existing regulatory frameworks, surveillance systems, infrastructure for laboratory services and infection control, and human resources already in place to manage drug resistance in tuberculosis, the WHO Global action plan on antimicrobial resistance calls for inclusive, multispectral and innovative partnerships to foster the development of antibiotics (Weyer *et al* ., 2017)

In addition to the affording of the data Ideally, routine drug susceptibility testing (DST) should be conducted before initiation of treatment in all patients with TB (Sanchez-Padilla *et al ., 2012)* (MDR-TB) and (XDR-TB) threat TB control programs globally *(*Calligaro *et al*., 2014).

Sub-Saharan Africa for example Multidrug-resistant tuberculosis (MDR-TB) did not receive major attention until recently where the tuberculosis incidence and risk factors are highest The most (Workicho *et al*. ,2017).

Table (1-3) Drug used for Tuberculosis treatment *(*Varine *et al* .,2016)

DESCRIPTION	DRUG
First-line oral anti-TB drugs	Isoniazid Rifampicin Ethambutol Pyrazinamide Rifabutin Rifapentine
Injectable anti-TB drugs (injectable agents or parenteral agents)	Streptomycin Kanamycin Amikacin Capreomycin
Fluoroquinolones (FQs)	Levofloxacin Moxifloxacin Gatifloxacin Ofloxacin
Oral bacteriostatic second-line anti-TB drugs	Ethionamide Prothionamide Cycloserine Terizidone *p*-aminosalicylic acid *p*-aminosalicylate sodium
Anti-TB drugs with limited data on efficacy and/or longterm safety in the treatment of drug-resistant TB (This group includes new anti-TB agents).	Bedaquiline Delamanid Linezolid Clofazimine Amoxicillin/Clavulanate Imipenem/Cilastatin Meropenem High-dose isoniazid Thioacetazone Clarithromycin

2.10 Previous studies

In a study carried in Sudan for susceptibility to isoniazid, rifampicin, ethambutol and streptomycin screened by the proportion method on Lowenstein Jensen media. 232 isolates were also genotyped by spoligotyping. In this study that (MDR-TB), being resistance to at least rifampicin and isoniazid, They were found in 5% (95% CI: 2,8) of new cases and 24% (95% CI: 14,34) of previously treated patients , And they conclude that emergence of drug resistant tuberculosis has the potential to be a serious public health problem in Sudan and that strengthened tuberculosis control and improved monitoring of therapy is needed (Eldin *et al.*,2011).

In study carried in Northern India focused on the prevalence of MDR-TB and pattern

of drug resistance among category II pulmonary TB patients from a tertiary care center and a primary care level center and the result were a total of 196 cases of sputum-positive category II pulmonary tuberculosis patients were included. Of these, 40 patients (20.4%) had MDR-TB. The mean age of MDR-TB patients was 33.25 ± 12.04 yr; 9 patients (22.5%) were female. Thirty six patients showed resistance to rifampicin and isoniazid; while 4 patients showed resistance to rifampicin, isoniazid and streptomycin. The prevalence of MDR-TB among category-II pulmonary tuberculosis patients was 20.4 %. (Sharma *et al* .,2011)

In the Kingdom of Swaziland in southern of Africa, which has the world's highest HIV and TB prevalences. Therefore, Astudy conducted to measure prevalence of drug-resistant TB. Of 988 patients screened, 420 new case-patients and 420 previously treated case-patients met the study criteria. Among culture-positive patients, 15.3% new case-patients and 49.5% previously treated case-patients harbored drug-resistant strains, MDR TB prevalence was 7.7% and 33.8% among new case-patients and previously treated case-patients, respectively. HIV infection and past TB treatment were independently associated with MDR TB. The findings assert the need for wide-scale intervention in resource-limited contexts such as Swaziland, where diagnostic and treatment facilities and health personnel (Sanchez-Padilla *et al* .,2012).

In other study was conducted in West Armachiho and Metema a total of 124 consecutive smear positive pulmonary tuberculosis patients were included in the study they found 117 (94.4 %) were susceptible to Rifampicin, while 7 (5.7 %) were confirmed to be resistant to Rifampicin and Isoniazid. The overall prevalence of MDR-TB was 5.7 % (2.3 % among new cases and 13.9 % among previously treated cases Were they found History of previous treatment was significantly associated risk factor for MDR-TBAnd they conclude that overall prevalence of MDR-TB of 5.7 % among all cases, with the prevalence of MDR-TB among previously treated cases being 13.9 % and among new cases only 2.3 %. History of previous anti TB treatment was the only statistically significant risk factor for MDR-TB

(Mekonnen *et al* .,2015).

In other study carried in Sudan an antimicrobial susceptibility patterns of 200 isolates of Mycobacterium tuberculosis, recovered from patients with pulmonary tuberculosis (PTB) in the Sudan, was determined against the first-line anti tuberculosis drugs Only 67 (33.5%) isolates were found sensitive to all drugs. Thirty-five (17.5%) isolates

were resistant to INH, 31 (15.5%) to RIF, 43 (21.5%) to STM and 24 (12%) to EMB. Twenty-one (10.5%) isolates were multidrug-resistant (at least to both INH and RIF), of which 9 (4.5%) were resistant to the four drugs. Five (2.5%) isolates were resistant to different combinations of three drugs. Conclusion (Nour *et al* .,2015).

In Ethiopia a cross-sectional study was conducted on 413 TB-positive clinical specimens 150 (36.3%) were multidrug-resistant and based on gene mutation analysis, failing of the *rpoB* WT8 gene with corresponding hybridization of *rpoB* MUT3 (S531L substitution) accounted for 85 (50.3%) rifampicin-resistant mutations. Among 176 isoniazid-resistant isolates, 155 (88.1%) strains had the Ser315Thr1 substitution, and they conclude the prevalence of multidrug-resistant *M. tuberculosis* was high in the study area. Ser531Leu and Ser315Thr1 substitutions were the highest gene mutations for rifampicin and isoniazid, respectively (Mekonnen *et al*., 2015).

In North bihar out of 256 sputum samples Line Probe Assay (LPA), 39(15%) samples was found to have resistance to both INH and Rifampicin. And they conclude the Prevalence of Multi drug resistant pulmonary tuberculosis in North Bihar is 15%. It needs early diagnosis by molecular diagnostic method and prompt treatment to reduce the spread of MDR TB cases (Tripathy *et al* .,2015).

Also in study done in Sudan with the concern that When first line drugs fail, second line drugs are used to treat MDR-TB, they investigated the initial second line drug resistance, The results of a total of 239 smear positive sputum were collected from retreatment TB patients One hundred and forty three *mycobacterium* isolates were successfully recovered from a total of 239 specimens (143/239; 59.8%). Fifty six strains were rifampicin resistant (RR); of these 54 were multi-drug resistant (MDR); two were RIF/INH-resistant mycobacterium other than tuberculosis (MOTT). Five of MDR (5/50; 10%) showed resistance to at least one second line drug and one isolate (1/50; 2%) was XDR. The XDR strain was concordantly detected by the two methods. And they conclude Initial resistance to second line anti-TB drugs among MDR-TB patients is at 10% levels and XDR-TB is prevalent at low levels (2%). Nevertheless; without great efforts from national tuberculosis control program (NTP) this figure can fuel the TB epidemics in Sudan (Adam *et al.*,2017) .

In other study done in Sudan Enan aimed to detect the frequency of drug resistance genes (rpoB, katG and pncA) in Sudanese tuberculosis patients. Seventy sputum samples were collected from Omdurman Teaching Hospital (Abu Anga) in Khartoum

state, Sudan. Sputum samples were disinfected and then DNA was extracted. Multiplex PCR was used to detect drug resistance genes (rpoB, katG and pncA) Fifty six sputum samples out of 70 were positive for the presence of drug resistance genes, drug resistance genes were detected in 22 (41.3%) for rifampicin, 29 (41.4%) for isoniazid and 33 (47.1%) for Pyrazinamide, also 16 (28.6%) samples had mono drug resistance, 29 (51.8%) had multi drug resistance and 11 (19.6%) had poly drug resistance. The ability of M. tuberculosis for the development of drug resistant gene were found to be associated with many factors, however in this study hypertension and diabetes mellitus were of no significant in the contribution of developing drug resistance *P. Value* > 0.574 (Enan .,2018)

In study carried in Botswana MDR TB was found in 139 (5.4%) cases with 1.3% among new cases and 7.7% among previously treated tuberculosis patients. (Tembo and Malangu ,2019) .

In Pakistan a study of 544 isolates from previously treated cases to detect the pattern of first and second line drug resistance 132 (24.3%) were MDR-TB, and they conclude that Prevalence of drug resistance in retreatment isolates was high. The alarmingly high prevalence of OFX resistance among MDR-TB isolates may threaten the success of efforts to control and treat MDR-TB (Javaid *et al.,* 2017)

CHAPTER III

MATERIAL AND METHOD

3.1. Study design

The study is prospective- cross sectional and laboratory based study.

3.2. Study area and duration

Study was conducted in Shendi city, River Nile State, Shendi Teaching Hospital, during the period from July 2019 to December 2019.

3.3. Study population

Fifty two positive patients involved and approved to participate.

3.3.1. Inclusion criteria

Patients who diagnosed with TB and patient with TB when fail in initial treatment and/or stopped the treatment - immunocompetent and immunocompromised patients.

3.3.2. Exclusion criteria

Inactive TB infected patients or LTBI patients were excluded.

3.4. Sample size

52 samples from TB positive patients used and enrolled in this study.

3.5. Data collection

Sample were collected convicted, Questionnaire was confirm to demographic, clinical and laboratory data.

3.6. Ethical consideration

Approved was taken from Ethical and Scientific Research Committee of Medical Laboratory Sciences colleague, Sudan university of Science and Technology and verbal consents was taken from patients also clinical approve was taken .

3.7. Lab processing

3.7.1. Sampling

Sputum sample was collected in wide mouth container after patients prepared by know instruction of sampling and set in specific collection place and gave them a wide mouth containers and asked them to cough deeply to expectorate sputum and sodium hydroxide 2:1 added then lid secured and samples labeled by names and serial numbers.

3.8. Principle and procedure

3.8.1. Principle

Xpert MTB/RIF is an integrated automated sample-processing and real-time PCR platform developed to simultaneously detect *M.tuberculosis* and rifampicin resistance in a single-use-cartridge hands-free step. The Xpert MTB/RIF assay consists of two main components, namely, a Xpert MTB/RIF plastic cartridge (containing the liquid sample processing and PCR buffers, and lyophilized real-time PCR reagents with internal sample processing and PCR probe quality controls) and the automated Xpert MTB/RIF machine (which controls the advanced automated portion of the procedure involving the engagement of the fluidics system within the cartridge, automated ultrasound lyses, and the performance of the real-time PCR analysis) (Patel *et al*. ,2013).

3.8.2. Procedure

Preparing the Sputum sample

1. Sputum has been processed for liquefaction of organic debris for liquefaction of organic debris anf bacterial decontamination other than mycobacteria.

2. The GeneXpert sample reagent was added into the sputum container (2:1 v/v), vigorously mixed and incubated for 15 minutes at room temperature.

Cartridge was prepared:

1. each XpertM.tb/RIF cartridges was labeled by attaching ID label on the writing side of the cartridge

2-Used sterile transfer pipette provided with the kit, the liquefied sample aspirated into the transfer pipette until the meniscus is above the minimum mark .

3. The cartridge lid opened and sample transferred into the open port of the XpertM.tb/RIF cartridge.

Sample was dispensed slowly to minimize risk of aerosol formation.

4. Closed cartridge lid after making sure the lid was snapped firmly into place.

5. Loaded cartridge into the GeneXpertDx (any test was started within 30 minutes of preparing the cartridge)

Test was started

1. The system was equipped with the GX2.1 software AND the XpertM.tb/RIF assay is imported into the software.

2. The computer turned on followed by the GeneXpertDx instrument

3. On the Windows™ desktop, the GeneXpertDx shortcut icon duple clicked

4. The GeneXpertDx System software Logged on.

5. In the GeneXpertDx System window, Creat test bar clicked ,and the Scan Cartridge Barcode dialog box appears.

6. The 2D barcode located on the XpertM.tb/RIF cartridge was scanned And the Create Test window appeared . The software automatically filled the boxes for the following fields: Select Assay, Reagent Lot ID, Cartridge SN, and Expiration Date based on the barcode information.

7. The sample ID box was typed (the sample lab accession number). Cross-checked to ensure

It is typed correctly. The sample ID/lab accession number is associated with the test results in the "View Results" window and all generated reports.

8. Test bar started after clicked on the dialog box that appeared and then lab password typed

9. Opened instrument module door with the flashing green light and the cartridge was loaded.

10 closed ye door and the green light was stopped flashing and became steady once the test started. When the test was finished, the green light turned off and the system released the door lock.

11. Released the system door lock at the end of run the module door opened and the cartridge removed.

12. Used cartridges are considered capable of transmitting infectious agents. The used cartridges disposed according to institution safety guidelines (because it considered capable of transmitting infectious disease.

3.9. Statistical analysesis

Data was analyzed by using SPSS - Statistical Package for the Social Sciences program_version 20 presented as "mean±SD frequency and *chi square p.value* was set at ± 0.05 level of significant.

CHAPTER IV

RESULT

4.1 Result

Fifty two sputum from patients referred to central laboratory either new cases of pulmonary tuberculosis detected for RIF as well or patients who diagnosed with tuberculosis and checked for MDR /TB as follow (43/52) (82.7 %) were active TB patients negative MDR using PCR Gene xpert test compare with (9/52) (17.3%) MDR TB patients ,17.3% (9/ 52) positive for MDR-TB as in Table 4.1

Male were 30/52 (56 %) compare with female 22/52 (44%) . MDR most frequently in male (56%) compared with female (44%) with insignificant statistical association between gender and MDR-TB with p *value 0.887* as explain in Table 4.2

The average ages varies from 2-90 years age with mean age 45± SD and grouped into 2 to 30 years, 31 to 60 and above 60 age grouped 31 to 60 years were most frequent 4 (45%) with MDR compared with 2-30 years group 3 (33%) and above 60years 2 (22%) they was insignificant in statistical association between age MDR-TB *p value 0.993* as shown in Table 4.3

Locality as one of risk factors to develop MDR-TB all participated who live in rural area 9/9 (100%) MDR positive with significant statistical association between area and MDR-TB *p value* 0.036 as shown in Table 4.4

Other educational level with insignificant statistical association between MDR-TB and education level with insignificant *p.value 0.619* in Table 4.5

People with free business was the most frequent MDR TB infected (45%) compared with other occupations and there was insignificantt statistical association between MDR-TB and occupation *p value 0.559* in Table 4.6

HIV co infection with MDR TB 2 patient (22%) were positive for both and 6 (67%) and 1 unknown (11%) the negative significant statistical association between MDR-TB HIV co infection denies any relation between HIV is a reason for developing of MDR TB with significant statistical association *p value* 0.002 in Table 4.7

Patient who previously treated from TB and recurrence with tuberculosis found positive for MDR with 8 (89%) compared to 1 (11%) who are not previously treated with significant statistical association between MDR-TB and previous treatment *p value 0.025* which reveal strong relation between MDR and failed previous treatment as shown in table 4.8

Regarding MDR distribution rate according to mycobacterium quantity found they was most frequently in Medium mycobacterium bacilli quantity, 5 (56%) compared to the low quantity 1 (11%) and high 3 (33%) with insignificant statistical association between MDR-TB and Mycobacterium bacilli quantity *p value 0.442* in Table 4.9

Table (4-1) Distribution of MDR patients among TB patients

Patients	Frequency	Percentage
TB (MDR Negative)	43	82,7%
MDR-TB	09	17,3%
Total	52	100%

Table (4-2) Relationship between gender and MDR-TB

Gender	Positive MDR	Negative MDR	*P.value*
Male	5 (56%)	25 (58%)	*0.887*
Female	4 (44%)	18 (42%)	
Total	9 (17.3%)	43(82.7%)	

Table (4-3) Association of age and MDR

Age group	Positive MDR	Negative MDR	*P.value*
2-30 years	3 (33%)	15 (35%)	*= 0.99*
31-60 years	4 (45%)	18 (42%)	
Above 60 years	2 (22%)	10 (23%)	
Total	9 (17.3%)	43(82,7%)	

Table (4-4) Relationship between MDR-TB and residence

Resident	Positive MDR	Negative MDR	*P.value*
Rural	9 (100%)	29 (67%)	*0.036*
Urban	0 (0%)	14 (33%)	
Total	9 (17.3%)	43(82,7%)	

Table (4-5) Association between MDR-TB and Educational levels

The educational levels	Positive MDR	Negative MDR	*P.value*
Illiterate	3 (33%)	13 (30%)	*0.619*
Primary	2 (22%)	14 (33%)	
Secondary	4 (45%)	12 (28%)	
Graduate	0 (0%)	4 (9%)	
Total	9 (17.3%)	43(82,7%)	

Table (4-6) Relationship between occupation and MDR-TB

Occupation	Positive MDR	Negative MDR	*P.value*
No job	3(33%)	19 (44%)	*0.559*
student	2 (22%)	7 (16%)	
Free business	4 (45%)	12 (28%)	
employee	0 (0%)	5 (12%)	
Total	9 (17.3%)	43(82, 7%)	

Table (4-7) Relationship between MDR-TB and HIV co-infection

Patients with HIV co-infection:	**Positive MDR**	**Negative MDR**	***P.value***
Positive HIV	2 (22%)	2 (5%)	*0.002*
Negative HIV	6 (67%)	10 (23%)	
Unknown HIV results	1 (11%)	31 (72%)	
Total	9 (17.3%)	43(82, 7%)	

Table (4-8)Association between Previous treatment and MDR-TB

Previous treatment	**Positive MDR**	**Negative MDR**	***P.value***
Yes	8 (89%)	17 (40%)	*0.025*
No	1 (11%)	19 (44%)	
unknown	0 (0%)	7 (16%)	
Total	9 (17.3%)	43(82, 7%)	

Table (4-9) Relationship between quantity of mycobacterium and MDR-TB

Quantity of mycobacterium	**Positive MDR**	**Negative MDR**	***P.value***
Low	1 (11%)	13 (30%)	*0.442*
Medium	5 (56%)	16 (37%)	
High	3 (33%)	14 (33%)	
Total	9 (17.3%)	43(82,7%)	

CHAPTER V

DISCUSSION

The rising incidence of (MDR-TB) have threatened the global measures to control the TB epidemic and the detection of such resistance at the earliest is essential to limit the spread of the evolving resistance and to initiate optimal therapy (Sethi *et al ., 2019).*

In this study, the overall frequency of MDR-TB was (17.3 %.) 9 positive MDR-TB which is less than other study done in Sudan, Omdurman Hospital in Khartoum's state were MDR –TB represent 51.6% (Enan ,2018) , and higher than other study done in Sudan were MDR –TB only represented 10.5% (Nour *et al* .,2015). And near to other study carried in Sudan were MDR- TB isolated 54/239 (22.5%) (Adam *et al.*,2017) this variation due to sample sizes and different geographical area .

The relation between gender and MDR-TB was insignificant which agreed with study done in South West Nigeria, that gender were not significantly associated with MDR-TB (Daniel & Osman., 2011)

The result According to the age; showed the highest frequency of MDR-TB in age group from 31 to 60 years with insignificant association between age and MDR-TB which disagreed to other study that found age group from 10 to 25 years was the most affected group with MDR-TB and there was statistical significant different between age and MDR-TB (Ullah *et al*., 2016).

According to the residence there was strong relation between MDR-TB and residence showed strong relation between MDR-TB and geographical area which matched with other study that found geography had positive relationships with the 'MDR'(Liu *et al* .,2015)

The present study considered high in compared with study done West Armachiho and Metema were MDR only represented (5.7%) (Mekonnen *et al.*,2015) and close to a result carried in northern India were prevalence of MDR-TB (20.4%) among TB patients (Sharma *et al* , 2011)

The HIV co infection and MDR-TB and HIV co infection in this study denies any relation between HIV and developing of MDR TB among TB patients with statistical significant harmonized with a study from Vietnam showed in which the rate of acquired MDR-TB was lower in HIV co-infected 11 (9.1%) than in 390 HIV-negative patients (25.9%)

(Suchindran *et al*., 2009) and disagree with data reported to the World Health

Organization (WHO) which found a HIV-positive TB patients had a significantly higher ($p<0.05$) of MDR-TB disease than HIV-negative TB patients *(*Dean *et al* ., 2014)

The present study show that Patient who previously treated from TB and recurrence with tuberculosis found positive for MDR by (89%) which is close Ullah and her group which they found previous TB treatment and MDR-TB relation significant ($P<0.001$)(Ullah *et al* ., 2016).

This is variation and disagreement in result may be due to sample size which other studies run by a larger sample of patients in comparison with this study or may be due to difference in the methods used for identification of *MDR- TB* from specimens, or may be due to the difference in geographical area .

5.2 Conclusion

-The present study showed low frequency of *MDR-TB* and this this frequent is considered obstacle for TB control program.

-*MDR-TB* mostly prevalent in rural area due to lack of health services

-There was significant association between *MDR-TB* and previous treatment and Geographic area also significant relation with HIV co infection

- There was no significant association between *MDR-TB* and gender, Occupation, level of education and *Mycobacterium tuberculosis* quantity and age.

5.3 Recommendations

. Tuberculosis treatment should be carefully managed and early detection of MDR-TB to control the prevalence of resistant type.

. TB /MDR-TB Control program should be concerned in rural area and TB-patients should be followed up to avoid treatment cut.

. More research on MDR-TB with genotyping needed for further information and more research should be conducted (include large sample size)to collect more data about the MDR-TB prevalence.

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Appendix (1) questionnaire

بسم الله الرحمن الرحيم

Sudan University of Sciences and Technology

College of Graduate studies

Prevalence of Multi Drug Resistant (MDR) among Pulmonary Tuberculosis in Shendi city River Nile State–Sudan 2019

انتشار المتفطرة السلية المتعددة المقاومة للأدوية
بين مرضى السل الرئوي بمدينة شندي ولاية نهر النيل السودان 2019

This questionnaire related to patients of tuberculosis.

Patient No:……..……..

1/ Gender:

A. Male ( ) B. Female ( )

2/ Age:

A. 2-30 Years ( ) B. 31-60 ( ) C. more than 60 Years ( )

3/Geographical area

A. Rural ( ) B. Urban ( )

4/ Education level;

A. Illiterate ( ) B. Primary ( ) C. Secondary ( )
D. Graduate ( )

5/ Occupation

A. No Job ( ) B. Student ( ) C. Free business ( )
D. Employee ( )

6/ HIV test

A. Positive ( ) B. Negative ( ) C. Unknown ( )

7/ Previous treatment

A. Yes ( ) B. No ( ) C. Unknown (

Appendix 2-A

GeneXpert Dx System

Appendix 2-B

GeneXpert cartridge