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Serodetection of Human Immunodeficiency Virus among Tuberculosis Patients at Abu Anja teaching Hospital in Khartoum State.

A dissertation submitted in partial fulfillment for the requirements of M.Sc. degree in medical laboratory sciences (Microbiology)

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آية:

قَالَ تَعَالَىٰ: ﴿ ٱللَّهُ نُورُ ٱلسَّمَٰوَٰتِ وَٱلۡأَرۡضِۚ مَثَلُ نُورِهِۦ كَمِشۡكَوٰةٖ فِيهَا مِصۡبَاحٌۖ ٱلۡمِصۡبَاحُ فِي زُجَاجَةٍۖ ٱلزُّجَاجَةُ كَأَنَّهَا كَوۡكَبٞ دُرِّيّٞ يُوقَدُ مِن شَجَرَةٖ مُّبَٰرَكَةٖ زَيۡتُونَةٖ لَّا شَرۡقِيَّةٖ وَلَا غَرۡبِيَّةٖ يَكَادُ زَيۡتُهَا يُضِيٓءُ وَلَوۡ لَمۡ تَمۡسَسۡهُ نَارٞۚ نُّورٌ عَلَىٰ نُورٖۚ يَهۡدِي ٱللَّهُ لِنُورِهِۦ مَن يَشَآءُۚ وَيَضۡرِبُ ٱللَّهُ ٱلۡأَمۡثَٰلَ لِلنَّاسِۗ وَٱللَّهُ بِكُلِّ شَيۡءٍ عَلِيمُۢ ﴾

سورة النور الآية (35)

Dedication

I dedicate this research to

My family, friends, colleges, and to all people who make this study possible.

Acknowledgment

Thanks to Allah would be before anything as without Allah will, this work would not be completed.

We are heartily thankful to my supervisor, **Dr. Waseem Sameer Kwami**, for his encouragement, guidance and support from the initial to the final level enabled me to develop and of the understanding of the subject.

My deep and great thanks will not complete without mentioning the staff of Microbiology department at Shendi University.

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ABSTRACT:

Tuberculosis (TB) is a major cause of morbidity and mortality and the control of the co-infection of HIV and tuberculosis epidemics is a major challenge facing many countries, especially developing countries that involve the Sudan.

Early detection of HIV among TB patients is very important for preventive purposes; it offers an opportunity to introduce prophylactic therapy and antiretroviral treatment that reduces the morbidities and mortality. Also support the policy of treatment for TB-AIDS co-infection.

This is descriptive cross sectional study conducted at Abu-anja Teaching Hospital Khartoum state, in period from October 2018 toMarch2019, the objective of study was to determine the serodetection of Human Immunodeficiency Virus infection among tuberculosis patients.

A total of 90venous blood samples were collected from the all study participants, serum was obtained by centrifugation at (3000 rpm) for 5 minutes. The presence or absence of antibody for HIV1/2 was determined by using sandwich ELISA Assay, by comparing the absorbance measured for each sample with the cut-off-value.

The study revealed that the serodection of HIV infection among tuberculosis patients was 5.6%, and the serodetection was found to be in male's tuberculosis patients only.

Also serodetection of HIV was found in all age groups and TB status (new cases and follow-up)

The study concluded that all TB cases need to be screened for HIV so that HIV/TB is detected early and managed promptly.

المستخلص:

مرض الدرن الرئوي يعتبر من الأسباب الأساسية في زيادة معدلات الإصابة والوفيات والعدوى المزدوجة بين مرض الدرن الرئوي وفيروس نقص المناعة المكتسبة تشكل تحدى لمعظم الدول خاصة الدول النامية بما فيها السودان.

الاكتشاف المبكر لفيروس نقص المناعة المكتسبة بين المصابون بالدرن الرئوي يعتبر مهما في عمليات الوقاية لتقليل عدد الإصابات والوفيات وذلك باستخدام العلاج المبكر للعدوى المزدوجة.

أجريت هذه الدراسة الوصفية في مستشفي ابوعنجة للأمراض الصدرية التعليمي ولاية الخرطوم في الفترة من شهر أكتوبر 2018 إلى مارس 2019 .

الهدف من هذه الدراسة هو تحديد نسبة وجود فيروس نقص المناعة المكتسبة في الأشخاص المصابون بمرض الدرن الرئوي .

تم جمع عدد 90 عينة دم من المشاركين في الدراسة وتم فصل المصل من هذه العينات عن طريق استخدام جهاز الطرد المركزي بسرعة 3000 دورة في الدقيقة . وتم تحديد مستويات العيار الكمي لهذه الأجسام المضادة عن طريق استخدام جهاز الاليزا (نوعية الساندوتش). وذلك عن طريق قياس درجة الامتصاصية لكل عينة ومقارنتها مع الامتصاصية المرجعية.

توصلت هذه الدراسة إلى أن 5.6 % من المشاركين يحملون فيروس نقص المناعة المكتسبة وجميعهم من الذكور. وكذلك تشمل الإصابة كل المجموعات العمرية وحالات الدرن الرئوي الجديدة والمتابعة.

خلصت الدراسة إلى أن نسبة الذين يحملون الفيروس تعتبر نسبة عالية. وكذلك أوصت الدراسة بان يجب علي كل المصابون بالدرن الرئوي عمل اختبار لفيروس نقص المناعة المكتسب في الإنسان. وكلما كان تشخيص العدوى المزدوجة مبكرا كلما كان العلاج أفضل.

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List of abbreviations

Abbreviation	Full name
AIDS	Acquired immunedeficiency syndrome
ART	Antiretroviral therapy
BCG	Bacille Calmette-Guérin
CD	Cluster of differentiation
CNS	Central nervous system
DNA	Deoxy ribonucleic acid
ELA	Enzyme-linked immunoassay
ELISA	Enzyme linked immunosorbent assay
HAART	Highly active antiretroviral therapy
HIV	Human immunodeficiency virus
ICT	Immunochromatograprhic test
INH	Isonicotinolhyrazine
IV	Intravenous
LTBI	Latent tuberculosis infection
MDR-TB	Multi-drug resistant tuberculosis
MTB	*M .tuberculosis*
PCR	Polymerase chain reaction
RT	Retroviral therapy
SPSS	Statistical Package for the Social Sciences
TB	Tuberculosis
TST	Tuberculin skin test
WHO	World Health Organization
XDR-TB	Extensively drug- resistant tuberculosis
ZN	Ziehl-neelsen

CHAPTER ONE

INTRODUCTION and OBJECTIVES

CHAPTER ONE

INTRODUCTION and OBJECTIVES

1.1. Introduction:

Tuberculosis (TB) is caused by bacteria (Mycobacterium tuberculosis) that most often affect the lungs. The lifetime risk of developing TB for those infected with the organism is 5-15%, however, that risk is substantially increased in individuals who are immunocompromised such as those living with Human Immunodefiency Virus (HIV).[1]

Most human cases of TB are caused by *Mycobacterium tuberculosis*, which is an estimate to infect 1.7 Billion people around the world. Of these, eight million new people are affected by active tuberculosis. In total, TB is responsible for around three million fatalities each year.[2]

Human Immunodefiency Virus (HIV) infects cells of the immune system; specifically CD4 +ve cells including helper T lymphocytes leading to progressive loss of cellular and eventually all immune mechanisms. This eventually leads to the development of acquired immunodeficiency syndrome (AIDS) which is characterized by susceptibility to infection by opportunistic and virulent pathogens and AIDS-related malignancies.[3]

The relative risk of HIV TB among HIV individuals is 20 to 30. [1]Among children under 14 years of age, one million were affected with TB in 2017 with 230.000 fatalities [1] .This figure includes children who are co-infected with HIV and TB.[1]

In the context of co-infection, disease progress is accelerated for both pathogens, with higher mortality than infection of either HIV of MTB. Three-hundred-thousand people died from HIV-associated TB in 2017, with approximately one million new cases of TB among HIV positive individuals, the majority of whom (72%) live in Africa.[1]

TB has a global distribution affecting the population of every continent. However, the vast majority of TB cases occur in South-East Asia and Western Pacific; which account for 62%. Africa is comes second with 25% of global TB cases.[1]

In 2017, 87% of new TB cases occurred in the 30 high TB burden countries. Eight countries accounted for two thirds of the new TB cases: India, China, Indonesia, the Philippines, Pakistan, Nigeria, Bangladesh and South Africa.[1]

HIV/TB co-infection management is a complicated endeavor, with high treatment failure and antimicrobial drug resistance on the rise. More than a million people currently live with HIV/AIDS typically affecting the poor, the homeless, intravenous (IV) drug users, alcoholics, the elderly or those with debilitating illnesses.[2]

Tuberculosis (TB) is a major cause of morbidity and mortality and the control of the concomitant of HIV and tuberculosis epidemics is a major challenge facing countries in sub Saharan Africa.[4]

In Sudan tuberculosis-related mortality rate is estimated at 25.0 per 100 000 population. A total of 20181 detected tuberculosis cases were reported in 2013, of which 5980 (30%) were new sputum smear-positive cases. Collaboration of tuberculosis and HIV is the one of the most important challenges to the national tuberculosis programme in Sudan.[5]

The first case of HIV and AIDS in Sudan was reported in 1986 and Sudan is an endemic area of tuberculosis however there are few published data -none is available in Sudan- concerning TB –HIV co -infection.[4]

Early detection of HIV among TB patients is very important for preventive purposes; it offers an opportunity to introduce prophylactic therapy and antiretroviral treatment that reduces the morbidities and mortality. Also support the policy of treatment for TB-AIDS co-infection.[4]

1.2. Rationale:

HIV is a risk factor for many diseases including TB. In Sudan, there is no regular screening for early HIV detection among TB patients due to social and economic issues. AIDS is a chronic disease; the patient can carry the virus for long time without an established diagnosis. Early detection of HIV among TB patients is very important for preventative purposes; it offers an opportunity to introduce prophylactic therapy and antiretroviral treatment that reduces the morbidities and mortality. Moreover, support the policy of treatment for MTB/HIV co-infection.

1.3. Objectives:

1.3.1. General objective:

To determine the serodetection of human immunodeficiency virus infection among tuberculosis patients at Abu- anja teaching hospital in Khartoum state.

1.3.2. Specific objectives:

1. To detect positive HIV cases among reported TB patients at Abu-Anja teaching hospital by using ELISA technique.
2. To correlate between positive HIV cases and patients demographic data.

CHAPTER TWO

LITERATURE REVIEW

CHAPTER TWO

LITERATURE REVIEW

2.1. Background:

Tuberculosis (TB) is an old disease – studies of human skeletons show that it has affected humans for thousands of years. The cause remained unknown until 24March 1882, when Dr.Robert Koch announced that he had discovered the bacillus *Mycobacterium tuberculosis*, an event that is now commemorated every year as World TB Day.[6]

TB is a chronic infectious disease caused by bacteria known as *Mycobacterium tuberculosis* and occasionally by *Mycobacterium bovis* that causes TB of the bone (bovine TB)**.** Tuberculosis disease mostly affects the lungs, though other parts of the body can be infected (except teeth, hair, and nails).[7]

Worldwide, TB is one of the top 10 causes of death and the leading cause from a single infectious agent (above HIV/AIDS). Millions of people continue to fall sick with TB each year.[6]

TB is a curable disease, though it still remains a major public health problem worldwide, especially in developing countries. Globally, it ranks as the second leading cause of death from an infectious disease.[8]

The "end" of TB as an epidemic and major public health problem is still a distant reality. This is despite the fact that, with a timely diagnosis and correct drug treatment, most people who develop the disease can be cured. Twenty–five years ago, in 1993, WHO declared TB a global health emergency.[6]

Diagnosis and successful treatment of people with TB averts millions of deaths each year (an estimated 54 million over the period 2000–2017), but there are still large and persistent gaps in detection and treatment. [6]

The situation has become alarming due to co-infection with HIV and the development of drug resistance. The emergence of resistance to drugs used for TB treatment, and particularly multidrug-resistant TB (MDR-TB), has become a significant public health problem in a number of countries and an obstacle to effective global TB control.[8]

The main health-care interventions to prevent new infections of *Mycobacterium tuberculosis* and their progression to TB disease are treatment of latent TB infection and vaccination of children with the bacille Calmette-Guérin (BCG) vaccine.[6] TB preventive treatments for a latent TB infection is expanding, but most of those for whom it is strongly recommended are not yet accessing care, whereas coverage of BCG vaccination is high. WHO has strongly recommended treatment for latent TB infection in two priority groups: people living with HIV, and children aged less than 5 years who are household contacts of someone who has bacteriologically confirmed pulmonary TB. [6]

There is strong need to address the tuberculosis (TB) and human immune deficiency virus (HIV) co-infection.[4]

In 2017, TB caused an estimated 1.3 million deaths (range, 1.2–1.4 million) among HIV-negative people and there were an additional 300 000 deaths from TB (range, 266 000–335 000) among HIV-positive people. [6]

Although HIV markedly increases the risk of progression latent TB to active TB and increases the mortality associated with TB, TB also hastens the progression of HIV disease, increasing the risk of developing other opportunistic infections.[9]

In the absence of antiretroviral therapy (ART) the risk of an HIV-infected person progressing from latent TB infection (LTBI) to active TB is more progressive than the person used ART.[10]

2.2. Different forms of tuberculosis:

2.2.1. Pulmonary tuberculosis:

In the lunge, *M. tuberculosis* causes an inflammatory reaction leading to liquefied destruction of lung tissue with caseation, i.e. breakdown of the diseased tissue into a cheesed-like mass. The yellow pieces of caseous material contain tubercle bacilli and are oven coughed-up by the patient in sputum. Patients with advanced infection have difficulty in breathing due to cavities in their lungs.[11]

2.2.2. Renal and urinogenital tuberculosis:

Organism reaches the kidney and genital tract by way of blood circulation.

There is no organism on urine culture but contain pus cells.[11]

2.2.3. Miliary tuberculosis:

Patients often acutely ill with fever but a chronic form of disease can also occur. The disease is usually recognized by seeing wide spread fine nodules on the chest X-rays.[11]

2.2.4. Tuberculosis meningities:

The tubercle bacilli reach the meninges in the blood. Usually occur due to complication of primary tuberculosis, more frequency in infants and young children.[11]

2.2.5. Pulmonary tuberculosis:

Pulmonary tuberculosis (TB) is a contagious, airborne infection that destroys body tissue. Pulmonary TB occurs when *M. tuberculosis* primarily attacks the lungs. However, it can spread from there to other organs. Pulmonary TB is curable with

an early diagnosis and antibiotic treatment. If left untreated, the disease can cause life-threatening complications like permanent lung damage.[12]

2.2.6. Latent TB Infection:

People with latent TB infection have TB germs in their bodies, but they are not sick because the germs are not active. These people do not have symptoms of TB disease, and they cannot spread the germs to others. However, they may develop TB disease in the future. They are often prescribed treatment to prevent them from developing TB disease.[10]

2.3.7. TB Disease:

People with TB disease are sick from TB germs that are active, meaning that they are multiplying and destroying tissue in their body. They usually have symptoms of TB disease. People with TB disease of the lungs or throat are capable of spreading germs to others. They are prescribed drugs that can treat TB disease.[13]

M.tuberculosis becomes dormant before it progresses to active TB. It most commonly involves the lungs and is communicable in this form, but may affect almost any organ system including the lymph nodes, CNS, liver, bones, genitourinary tract, and gastrointestinal tract.[13]

2.3. *Mycobacterium tuberculosis:*

2.3.1. Species:

The most important mycobacterium species which causes tuberculosis is: *Mycobacterium tuberculosis* (Koch bacillus).

Tuberculosis is also caused by *M. bovis*, *M. africanum*, and occasionally by opportunistic mycobacterium.[11]

2.3.2. Normal habitat:

The main reservoirs of *Mycobacterium tuberculosis* are infected humans .The organism can also occur as a pathogen in animals. It is mainly transmitted by infected persons coughing and spitting out bacilli which are then inhaled by others.[11]

M. bovis is found mainly as a pathogen in cattle and occasionally in other animals. Human can become infected by close contact with infected animals or by ingestion the bacilli in milk from infected animals. Person to person transmission of bovine strains may also occur. [11]

2.3.3. General characteristics of *M. tuberculosis*:

2.3.3.1. Microscopy:

Mycobacterium tuberculosis is a non-spore forming, non-capsulated, straight or slightly curved rod measuring 1-4x0.2-0.5 µm. [11]

Although they do not stain readily, after being stained by gram stain, they resist decolorization by acid or alcohol and are therefore called "acid-fast" bacilli. Instead of gram stain used Acid-fast stains such as Ziehl-Neelsen (ZN), or fluorescent stains such as auramine.[3]The cell wall of *Mycobacterium* consist from a thick layer of peptidoglycan and mycolic acid layer. [3]

2.3.3.2. Culture:

M. tuberculosis will grow aerobically in a protein-enriched medium. The optimal temperature for growth is 35-37°C the organism is slow -growing, and pigment is not produced. [11]

Several media are available for culturing Mycobateria.Commonly used media include liquids such as middle brook 7H9 or 7H12, egg-based solid media such as Lowenstein-Jensen, and solid agar-based such as Middle brook 7H11 or 7H10.[14]

Mycobacterial culture on liquid medium is significantly faster (10 to 14 days) than that on solid medium. Automatic detection tools, such as the Bactec Mycobacterial Growth Indicator Tube 960 (MGIT 960, Becton-Dickinson, and Sparks, MD, USA) or BacT/ALERT (bioMérieux S.A., Marcy l'Etoile, France) have been used, but they need a stable electricity supply, technical support, and expensive reagents.[12]

Many mycobacteria produce carotenoid pigments under appropriate conditions. Some organism produces pigment in dark (scotochromogens) and others only on exposure to light (photochromogens). Thus, pigment production can be a useful aid to identification.[15]

When cultured on Lowenstein-Jensen medium at 35-37°C, *M .tuberculosis*
Produces raised, dry, cream (buff) coloured colonies and produced within 2-3 weeks after incubation, but cultures must be incubated for up to 6 weeks before being discarded. [11]

2.3.3.3. Pathogenesis:

Mycobacteria are emitted in droplets smaller than 25 μm in diameter when infected persons cough, sneeze, or speak. The droplets evaporate, leaving organisms that are small enough, when inhaled, to be deposited in alveoli. Inside the alveoli, the host's immune system responds by release of cytokines and lymphokines that stimulate monocytes and macrophages. Mycobacteria begin to multiply within macrophages. Some of the macrophages develop an enhanced ability to kill the organism, but others may be killed by the bacilli. One to 2 months after exposure, pathogenic lesions associated with infection appear in the lung.[3]

The ability of *M. tuberculosis* to grow even in immunologically activated macrophages and to remain viable within the host for decades is a unique characteristic of the pathogen.[16]

2.4. Transmission:

Person can get TB by breathing in air droplets from a cough or sneeze of an infected person. The resulting lung infection is called primary TB.[17]

Most people recover from primary TB infection without further evidence of the disease. The infection may stay inactive (dormant) for years. In some people, it becomes active again (reactivates). [17]

Most people who develop symptoms of a TB infection first became infected in the past. In some cases, the disease becomes active within weeks after the primary infection. [17]

There are many factors that increase the risk of active TB or reactivation of TB, this include, Person with weakened immune systems, for example due to HIV/AIDS, chemotherapy, diabetes, Poor nutritional status/poverty, or medicines that weaken the immune system. Also age factor like older adults and infants.[18]

2.5. Symptoms:

Symptoms of TB disease depend on where in the body the TB bacteria are growing. TB bacteria usually grow in the lungs (pulmonary TB). TB disease in the lungs may cause symptoms such as, a bad cough that lasts 3 weeks or longer, pain in the chest, coughing up blood or sputum (phlegm from deep inside the lungs).[2]

Other symptoms of TB disease are weakness or fatigue, weight loss, no appetite, chills, fever, sweating at night. Symptoms of TB disease in other parts of the body depend on the area affected.[18]

People who have latent TB infection do not feel sick, do not have any symptoms, and cannot spread TB to others.[18]

2.6. Diagnosis:

Diagnosis of active pulmonary tuberculosis includes demonstration of clinical symptoms and abnormal chest radiographs and confirmation by isolation of *M. tuberculosis* from relevant clinical material. [16] Diagnosis of latent TB relies on the tuberculin skin test (TST) or blood tests. [16]

2.7. Treatment:

Treatment requires the use of multiple antibiotics over a long period of time. [16]Antibiotic resistance is a growing problem with increasing rates of multiple drug-resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB). [16]

Therapy directed against *M. tuberculosis* depends on the susceptibility of the isolate to various antimicrobial agents.[2]To prevent the selection of resistant mutants, treatment of tuberculosis requires four drugs: isoniazid, rifampin, ethambutol, and pyrazinamide. Initial therapy includes all four drugs for 8 weeks. However, if drug susceptibility is determined for isoniazid, rifampin, and pyrazinamide, ethambutol may be discontinued. This is the preferred therapy for initial treatment, followed by isoniazid and rifampin for an additional 18 weeks.

The most common two-drug regimen is isoniazid (INH, also known as isonicotinoylhydrazine) and rifampin.[2] the combination is administered for 9 months in cases of uncomplicated tuberculosis; if pyrazinamide is added to this

regimen during the first 2 months, the total duration of therapy can be shortened to 6 months. Ethambutol may also be added to the regimen. INH prophylaxis is recommended for individuals with a recent skin test conversion who are disease free.[2]

2.8. Prevention:

Prevention of TB involves screening those at high risk, early detection and treatment of cases, and vaccination of children with the bacillus Calmette-Guérin (BCG) vaccine.[6] Those at high risk include household, workplace, and social contacts of people with active TB.[6]

The vaccine is not suitable for use in HIV-positive children. New vaccines that are safe and effective against all forms of TB in all age groups, including the HIV-positive population, are needed.[19]

At this time, at least four types of anti-tuberculosis vaccines are currently being evaluated in experimental studies in animals.[2]

2.9. Prognosis:

Tuberculosis is a curable disease. Progress of tuberculosis from infection to frank illness involves overcoming of the immune system defenses by the bacteria. As the bacteria start to multiply, it affects the immune system and finally overwhelms it to cause the disease.[8]

Once diagnosed, with effective, adequate and appropriate therapy with anti-tubercular drugs, treatment is possible and so is cure.[6]

2.10. Human immunodeficiency virus (HIV):

Human immunodeficiency virus (HIV) causes progressive impairment of the body's cellular immune system, leading to increased susceptibility to infections and

tumors, and the fatal condition AIDS (acquired immunodeficiency syndrome). There are two main types of the virus, HIV-1 which causes most HIV infections worldwide and HIV-2 which is found mainly in West Africa. HIV-2 is less easily transmitted than HIV-1 and the period between initial infection and illness is longer than with HIV-1.[20]

HIV types, derived from primate lent viruses, are the etiologic agents of AIDS. The illness was first described in 1981, and HIV-1 was isolated by the end of 1983. Since then, AIDS has become a worldwide epidemic, expanding in scope and magnitude as HIV infections have affected different populations and geographic regions. Millions are now infected worldwide; once infected, individuals remain infected for life. Within a decade, if left untreated, the vast majority of HIV-infected individuals develop fatal opportunistic infections as a result of HIV induced deficiencies in the immune system. AIDS is one of the most important public health problems worldwide at the start of the 21st century. The development of highly active antiretroviral therapy (HAART) for chronic suppression of HIV replication and prevention of AIDS has been a major achievement in HIV medicine.[3]

2.10.1. Structure:

Structurally HIV consists of:

– An inner core containing two copies of single stranded RNA, viral enzymes and capsid protein p24 (group specific core antigen which does not vary).

– Double layered lipid envelope, derived from the membrane of the host cell. The envelope contains virus specific glycoproteins gp120 (protrudes from the surface) and gp41 (embedded in the envelope). These enable the virus to attach to and infect host cells. The gene that encodes gp120 mutates rapidly, producing many antigenic variants.[20]

2.10.2. Transmission:

HIV is present in semen, vaginal/cervical secretions and blood, and these are the main vehicles by which the virus is transmitted. The virus may also be Present in saliva, tears, urine, breast milk, cerebrospinal fluid and infected discharges.[20]

The stage of illness of an infected person influences the probability of HIV transmission. The risk of transmitting the virus is highest in the early (seroconversion) and late stages of disease when viral numbers are at their highest.

In tropical and developing countries, HIV is mainly transmitted by:

– Heterosexual sexual intercourse (85–95% transmission).

– Mother to child transmission during pregnancy,

Labour and delivery or breast-feeding.

– Transfusion of infected blood or blood products.

– Use of contaminated syringes and needles.[20]

2.10.3. Laboratory Diagnosis:

In the laboratory, HIV infection can be detected by three ways:

(1) Virus isolation; (2) serologic determination of antiviral antibodies; and (3) measurement of viral nucleic acid or antigens.[3]

Virus Isolation: HIV can be cultured from lymphocytes in peripheral blood (and occasionally from specimens from other sites).[3]

The most sensitive virus isolation technique is to cultivate the test sample with uninfected, mitogen stimulated peripheral blood mononuclear cells. Primary isolates of HIV grow very slowly compared with laboratory adapted strains. Viral growth is detected by testing culture supernatant fluids after about 7–14 days for viral reverse transcriptase activity or for virus-specific antigens (p24). Virus isolation techniques are time-consuming and laborious and are limited to research studies. [3]

Serology: Test kits are commercially available for measuring antibodies by enzyme-linked immunoassay (EIA). If properly performed, these tests have a sensitivity and specificity exceeding 98%. When EIA-based antibody tests are used for screening populations with a low prevalence of HIV infections (e.g., blood donors), a positive test in a serum sample must be confirmed by a repeat test. If the repeat EIA test is reactive, a confirmation test is performed to rule out false-positive EIA results. [3]

The most widely used confirmation assay is the Western blot technique, in which antibodies to HIV proteins of specific molecular weights can be detected. Antibodies to viral core protein p24 or envelope glycoproteins gp41, gp120, or gp160 are most commonly detected. [3]

Simple, rapid tests for detecting HIV antibodies are available for use in laboratories like immunochromatographictest (ICT). [3]

Anti-HIV is usually present within 3–6 weeks following infection. [20]

Most individuals will have detectable antibodies within 6–12 weeks.[3]

Detection of Viral Nucleic Acid or Antigens: Amplification assays such as the RT-PCR, DNA PCR, and bDNA tests are commonly used to detect viral RNA in clinical specimens. [3]

2.10.4. Treatment:

Treatment of HIV by ART to all HIV-infected individuals with *M. tuberculosis* infection would reduce the number of people who develop active TB and are vital to prevent the two epidemics fueling each other. Numerous cohort studies have shown that in HIV-infected persons, ART reduces TB risk in adults as well as in children. There is recent evidence that earlier initiation of ART in HIV-infected patients with newly diagnosed TB reduces mortality significantly.[9]

2.11. Previous studies:

Study carried out in public DOTS clinics in Sindh, Pakistan at 2008 – 2012, estimated that out of 9655 TB patients tested for HIV 28 were positive giving a prevalence of 0.29%. And the majority of them were males.[21]

Study carried out in a rural tuberculosis referral clinic in northern Nigeria at 2010, estimated that 257 TB patients tested for HIV infection, 106 were positive giving a prevalence of 41.2% infection in this group. In relation to gender, 44.83% (52/116) of the female patients tested positive while 38.30% (54/141) of the male patients tested positive for HIV infection.[22]

Study conducted in a national prevalence survey setting in Zambia, 2013–2014. Estimated that out of 151 PTB patients tested for HIV 36 were positive giving a prevalence of 23.8%, and there were no significant differences in the prevalence of HIV among TB by sex.[23]

Study carried out in Kassala Teaching Hospital, eastern Sudan, during January 2008– through December 2010, estimated that out of 109 TB patients tested for HIV 20 was positive giving a prevalence of 18.3%. Out of 20, 17(85%) were pulmonary TB and 3(15%) were extra pulmonary TB. 17 pulmonary TB giving prevalence 15.5% .And the majority of them are males.[4]

CHAPTER THREE

MATERIALS and METHODS

CHAPTER THREE

MATERIALS and METHODS

3.1. Study design:

The study is descriptive cross sectional hospital base study conducted in period from October 2018 to March 2019.

3.2. Study area:

This study is conducted at Abu anja Teaching Hospital which is institute fully dedicated to thoracic diseases; the Hospital is located in Omdurman city, near to Omdurman passports office.

3.3. Study Population:

3. Sudanese pulmonary tuberculosis patients arrived to Abu-Anja Teaching Hospital and by obtaining the demographic data (gender, age, the duration of TB infection).

3.4. Inclusion criteria:

Patients who were confirmed by laboratory methods to be TB positive including those under treatment (follow-up), (appendix V).

3.5. Exclusion criteria:

Patients with Extra -pulmonary tuberculosis or those who already completed Tb treatment regimens.

3.6. Ethical considerations:

The ethical consideration of this study was approved by ethics committee, faculty of graduate studies, Shendi University, Khartoum state ministry of health research department, and from Abu anja Teaching Hospital.

The participants were informed about the purpose of the research before sample collection, and verbal consent obtained from them. Privacy and confidentiality of participants were ensured.

3.7. Sample size:

Convenience sampling technique was used; 90 pulmonary tuberculosis patients were enrolled after they agreed to participate in this study.

3.8. Data collection:

An interview with structured questionnaire (appendix I) was done for all participants in this study for obtaining the demographic and clinical data.

3.9. Data analysis:

Data were analyzed by statistical package for social sciences (SPSS) version 16.

3.10. Study procedure:

3.10.1. Blood Sample Collection:

Venous blood sample was collected in plain container, from each individual included in this study; the serum was obtained by centrifugation at (3000 rpm) for 5 minutes.

3.10.2. Procedure of ELISA Technique:

The reagents were allowed to reach room temperature (18-30°C) for 25 minutes, and the wash buffer was checked for the presence of salt crystals then diluted 20 times with distilled water.[24]

The strips were set in strip-holder and numbered with sufficient number of wells included three negative control (B1, C1, D1), three positive control (E1, F1, G1), and one Blank (A1). [24]

20µl of conjugate reagent (biotinylated anti-HIV p24 antibodies) was added into each well except the blank.

100µl of positive control, negative control, and specimen were added into their respective wells by separate disposable pipette tip.[24]

The plate was covered by its cover and incubated for 60 minutes at 37°C in thermostat-controlled water tank. After the end of the incubation, the plate cover was removed and discarded. And the wells were washed, each well 5 times with diluted Wash buffer. After the final washing cycle, the plate was turned down onto blotting paper and taped. And any remain liquids was removed.

100µl of HRP-Conjugate Reagent was added into each well except the blank, and mixed gently.[24]

The plate was covered with its cover and incubated for 30 minutes at 37°C.After the end of the incubation, the plate cover was removed and discarded. And the wells were washed, each well 5 times with diluted Wash buffer. After the final washing cycle, the plate was turned down onto blotting paper and taped. And any remain liquids was removed.[24]

50µl of chromogen A and 50µl chromogen B solution were dispensed into each well included the blank, and the plate was covered by its cover and mixed gently by tapping. The plate was incubated at 37°C for 15 minutes and the light was avoided.

Blue colour was produced in positive control and HIV1/2 positive for antibodies sample wells.[24]

The plate cover was removed and discarded. 50µl Stop Solution was added into each well by multichannel pipette and mixed gently. Intensive yellow colour was developed in positive control and HIV1/2 positive for antibodies sample wells.[24]

3.9.3. Reading and Interpretation of the Results:

The plate reader was calibrated with the blank well and the absorbance was read at 450nm within 15 minutes after the reaction stop. Cut-off- value (C.O) was calculated and the results evaluated.[24]

The presence or absence of antibody for HIV1/2 was determined by comparing the absorbance measured for each sample with the cut-off-value.[24]

Samples give absorbance equal more than cut-off-value indicate that theHIV1/2 antibodies are present (positive result), while the samples give absorbance less than cut-off-value indicate that the HIV1/2 antibodies are absence (negative result).[24]

CHAPTER FOUR

RESULTS

CHAPTER FOUR

RESULTS

In this study a total of 90 participants were included the majority of them 66(73.3%) were males and only 24(26.7%) were females (Table 4.1).The age of study participants ranged from 18 to 65 years, 60 of them were less than 40 years old and only 30 were above than 40 years old (Table 4.2).

In this study out of 90 study participants 67(74.4%) were follow up cases and 23(25.6%) were new cases (Table 4.3).

Out of the 90 participants of the tuberculosis patients, 5(5.6%) were HIV positive, and 85(84.4%) were HIV negative (Table 4.4).

In this study out of all 66 tuberculosis male patient 7.5% (5 cases) showed HIV positive result and all the 24 female participants showed HIV negative result(p-value = 0.003) (Table 4.5).

Out of 23 new tuberculosis cases only 1(4.3%) showed HIV positive result, and out of 57tuberculosis follow up cases 4(5.9%) showed HIV positive result (p-value = 0.773) (Table 4. 6).

In this study only 2 cases (3.3%) out of 60 tuberculosis patients they were less than 40 years old were HIV positive and 3 cases (10%)out of 30 tuberculosis patients who were above 40 years old were HIV positive (p-value = 0.173) (Table 4.7).

Table (4.1) Shows distribution of study population according to sex.

Sex	Frequency	Percentage
Male	66	73.3%
Female	24	26.7%
Total	90	100

Table (4.2) Shows distribution of study population according to age.

Age group	Frequency	Percentage
Less than 40 years	60	66.7%
Above 40 years	30	33.3%
Total	90	100%

Table (4. 3) shows the distribution of study population according to TB status.

TB Status	Frequency	Percentage
New cases	23	25.6%
Follow-up cases	67	74.4%
Total	90	100 %

Table (4.4) shows the frequency of HIV infection among study population.

Study population	Frequency	Percentage
HIV Positive	5	5.6%
HIV Negative	85	94.4%
Total	90	100 %

Table (4.5) shows the frequency of HIV infection among the gender.

Sex	Frequency	Percentage	P-value
Males (n=66)	5	7.5%	0.003
Females (n=24)	0	0%	

Table (4.6) shows the frequency of HIV infection among the different TB Status group.

TB Status	Frequency	Percentage	P-value
New cases (n=23)	1	4.3%	0.773
Follow-up(n=67)	4	5.9%	

Table (4.7) shows the frequency of HIV infection among the age group.

Age group	Frequency	Percentage	P-value
Less than 40 years (n=60)	2	3.3%	0.173
Above 40 years(n=30)	3	10%	

CHAPTER FIVE

DISCUSSION, CONCLUSSION and RECOMMENDATIONS

CHAPTER FIVE

DISCUSSION, CONCLUSSION and RECOMMENDATIONS

5.1. DISCUSSION:

The present study involved 90 Sudanese TB patients; the study was conducted to determine the serodetection of Human Immunodeficiency Virus infection among tuberculosis patients at Abu-anja teaching hospital in Khartoum state.

The current study showed that out of 90 tuberculosis patients 5.6% (5) showed HIV positive result, this finding is different with the study conducted in Kassala Teaching Hospital in eastern Sudan, by Abdallah*etal*. (2010) who indicated that the seroprevalence of HIV among tuberculosis patients in Kassala is 15.5% which is higher than the current study. The higher prevalence may be attributed to location of Kassala in margin of eastern part of Sudan which allow interference with other countries, and also the eastern part of Sudan suffering from malnutrition which weakening the immunity of individuals there .[4]

The present study showed that out of 66 males tuberculosis patients 7.5% (5) showed HIV positive result which is significantly higher than in females (p-value=0.003).This result is in agreement with the study conducted in Pakistan by Hasnain *et al*. (2012) who indicated that the seroprevalence of HIV among males tuberculosis patients is higher than females tuberculosis patients. Males are more active in work field and this can increase chances of expoture.[21] Males have the tendency of migrating from one place to another searching for better work. As a result of this, they are in contact with more people increasing the chances of exposure to the infection.

The current study showed that out of 67 TB follow up cases 5.9% (4) showed HIV

positive result, and out of 23 new TB cases 4.3% (1) showed HIV positive result, the serodetection of HIV infection among new cases and follow-up cases was insignificantly statistically different (p-value = 0.773) .This may be due to nature of HIV can infect the human in any time.

The current study showed that out of 30 TB patient who were above 40 years 10% (3) showed HIV positive result, and out of 60 TB patient who were less than 40 years 3.3% (2) showed HIV positive result, the serodetection of HIV infection among those above 40 years and those less than 40 years was in significantly statistically (p-value 0.173).This result is in agreement with the study conducted in Kassala Teaching Hospital in eastern Sudan, by Abdallah*etal*. (2010) who indicated that the HIV infection among TB patients was observed in all age groups. This might indicate the possibility of gap between initial willingness for the screening and actual attendance for the test.[4]

5.2. Conclusion:

- The serodetection of HIV infection among tuberculosis patients was 5.6%.
- The serodetection of HIV infection among tuberculosis patients was found to be in males only.
- The serodetection of HIV infection among new cases and follow-up tuberculosis patients was insignificantly statistically different.
- The serodetection of HIV infection among tuberculosis patients who were above 40 years and less than 40 years was insignificantly statistically different.

5.3. Recommendations:

1. Studies to document HIV/TB co-infection should be implemented across the country.

2. All TB cases need to be screened for HIV so that HIV/TB is detected early and managed promptly.

3. Public awareness, community mobilization through different interventions should be encouraged and stepped up especially in areas where there is high prevalence of HIV and TB. This will help in the control and prevention of the dual co-infection which is challenging the health system.

4. This study was a hospital-based one; which might not reflect what was at the community level thus more research is needed.

5. More studies should be conducted on different age groups including children less than five years.

6. Delivery of children's should be occurring at a hospital to grantee the take of vaccine within suitable time.

7. This study was concerned on pulmonary tuberculosis, more studies is needed to covering the extra-pulmonary tuberculosis.

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24. Fortress diagnostics sealing.

APPENDIX I

بسم الله الرحمن الرحيم

جامعة شندي

كلية الدراسات العليا

كلية علوم المختبرات الطبية

استبيان Questionnaire

عن دراسة الحالات المشابهة لمرضك

رقم المريض.....Patient number:

التاريخ \ \.....Date / /

تاريخ المرض .....Date of disease (TB)

النوع.....Sex

العمر.....Age

APPENDIX II

بسم الله الرحمن الرحيم

جامعـــــة شنـــــدي

كلية الدراسات العليا والبحث العلمي

مركز الخرطوم

النمرة : ج ش/ك د ع/م خ/ | 2018/8/6م

إلى من يهمهم الأمر بوزارة الصحة – إدارة البحوث

المحترمين

السلام عليكم ورحمة الله وبركاته

الموضوع : الطالب/ عباس أحمد علي

بالإشارة إلى الموضوع أعلاه نفيدكم بأن الطالب المذكور أعلاه من ضمن طلاب الكلية بالفصل الدراسي الرابع – ببرنامج ماجستير علوم المختبرات الطبية ، تخصص الأحياء الدقيقة، وهو بغرض توزيع استبانة لدراسة الحالة وجمع العينات للبحث التكميلي والذي عنوانه :

Prevalence of HIV among TB patients in Khartoum state

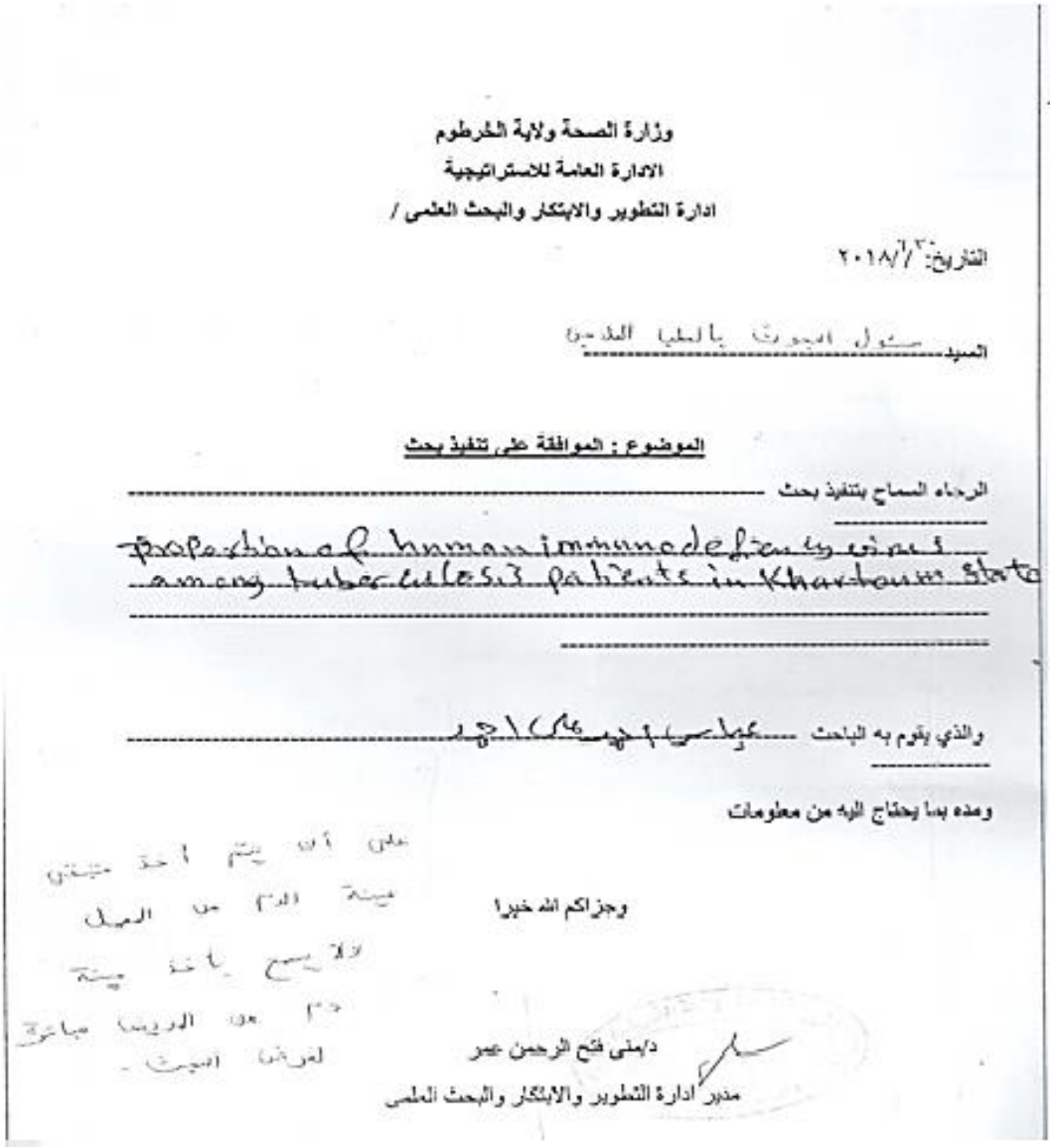
نرجو تيسير مهمته البحثية .

ولكم فائق الشكر والتقدير،،،،

الصادق أحمد عبدالقادر

مسجل المركز

APPENDIX III



وزارة الصحة ولاية الخرطوم

الادارة العامة للاستراتيجية

ادارة التطوير والابتكار والبحث العلمى /

التاريخ: ٢٠١٨/٦/٣٠

السيد مسئول المعمل بالمعمل المركزي

الموضوع : الموافقة على تنفيذ بحث

الرجاء السماح بتنفيذ بحث

Prevalence of human immunodeficiency virus among tuberculosis patients in Khartoum State

والذي يقوم به الباحث عباس عبدالله عباس

ومده بما يحتاج اليه من معلومات

على أن يتم أخذ عينة الدم من المعمل المركزي وليس بأخذ عينة من الوريد مباشرة لعرض البحث .

وجزاكم الله خيرا

د/منى فتح الرحمن عمر

مدير ادارة التطوير والابتكار والبحث العلمى

APPENDIX IV

وزارة الصحة – ولاية الخرطوم

الإدارة العامة للطب العلاجي

وحدة التخطيط والتدريب

النمرة: وص / و ح ت/ ع ط ع/144

التاريخ: ‎/ ‎/2018م

السيد / مدير عام مستشفى ....

المحترم

السلام عليكم ورحمة الله تعالى وبركاته

الموضوع/الموافقة على تنفيذ بحث

بالاشاره الى الموضوع اعلاه ، نفيد سيادتكم بأن الباحث /الباحثة / ....

بصدد أجراء بحث بعنوان : ....

proportion of Human Immunodeficiency Virus among tuberculosis patients in Khatoum State

وعليه نرجو التكرم بمد الطالب/الطالبة بمتطلبات البحث .

مع مراعاة الاتي:

1/ عدم السماح بأخذ العينات من المريض مباشرة (يتم اخذ متبقي عينة الدم من المعمل)

2/ فترة اجراء البحث اسبوعين فقط من تاريخه

3/غير مسموح للباحث ان يستخدم اجهزة المستشفى.

4/ غير مسموح للباحث استخدام المواد المستهلكة بمعمل المستشفى كالمحاليل والمحاقن واداة جمع العينة (Containers).

وجزاكم الله خيــــــراً ....

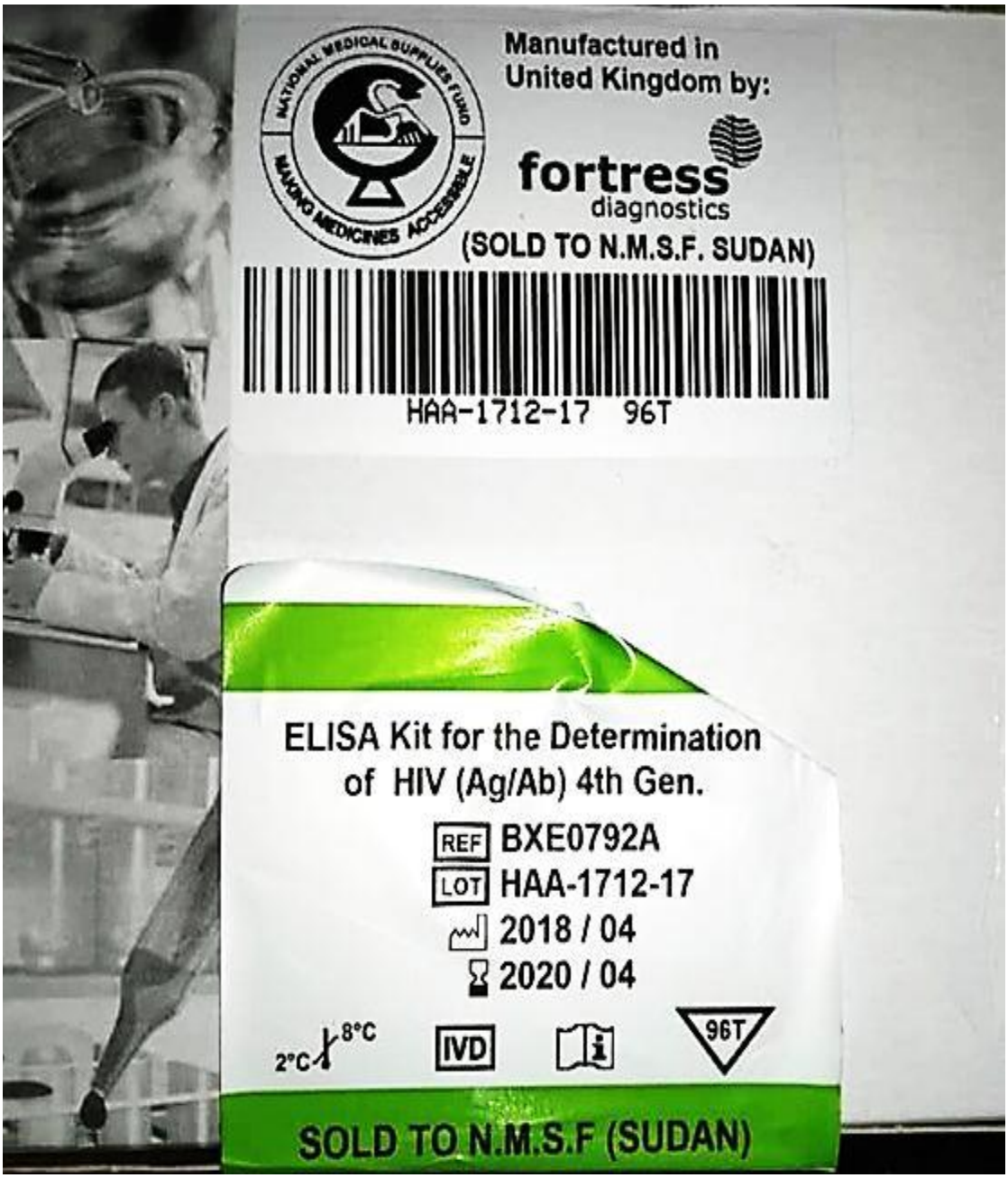
د. يوسف تين محمد نور

مدير الإدارة العامة للطب العلاجي

APPENDIX V

Patient No.	Age by years	Sex	TB Duration			TB Status	Date of specimen collection	HIV result
			Week	Month	Year			
1	38	Male	—	3	—	Follow up	15/10/2018	
2	26	Male	2	—	—	New case	15/10/2018	
3	30	Male	—	—	1	Follow up	15/10/2018	
4	28	Male	—	4	—	Follow up	15/10/2018	
5	40	Female	—	2	—	Follow up	15/10/2018	
6	25	Female	—	4	—	Follow up	15/10/2018	
7	32	Male	—	4	—	Follow up	15/10/2018	
8	25	Female	—	2	—	Follow up	16/10/2018	
9	27	Female	—	2	—	Follow up	16/10/2018	
10	52	Female	—	—	2	Follow up	16/10/2018	
11	55	Male	—	2	—	Follow up	17/10/2018	
12	43	Male	—	4	—	Follow up	17/10/2018	
13	28	Male	—	5	—	Follow up	17/10/2018	
14	42	Male	—	2	1	Follow up	17/10/2018	
15	27	Male	—	—	—	Follow up	17/10/2018	
16	50	Male	1	—	—	New case	17/10/2018	
17	32	Male	2	—	—	New case	17/10/2018	
18	24	Male	—	3	—	Follow up	17/10/2018	
19	26	Female	—	3	—	Follow up	17/10/2018	
20	19	Female	1	—	—	New case	17/10/2018	
21	30	Male	—	2	—	Follow up	17/10/2018	
22	40	Female	2	—	—	New case	17/10/2018	
23	25	Male	1	—	—	New case	17/10/2018	
24	30	Female	—	3	—	Follow up	17/10/2018	
25	63	Male	—	—	3	Follow up	17/10/2018	
26	54	Male	—	3	—	Follow up	17/10/2018	
27	54	Male	—	2	—	Follow up	17/10/2018	
28	49	Male	—	—	3	Follow up	17/10/2018	
29	47	Male	—	7	—	Follow up	17/10/2018	
30	49	Male	—	6	—	Follow up	21/10/2018	

APPENDIX VI

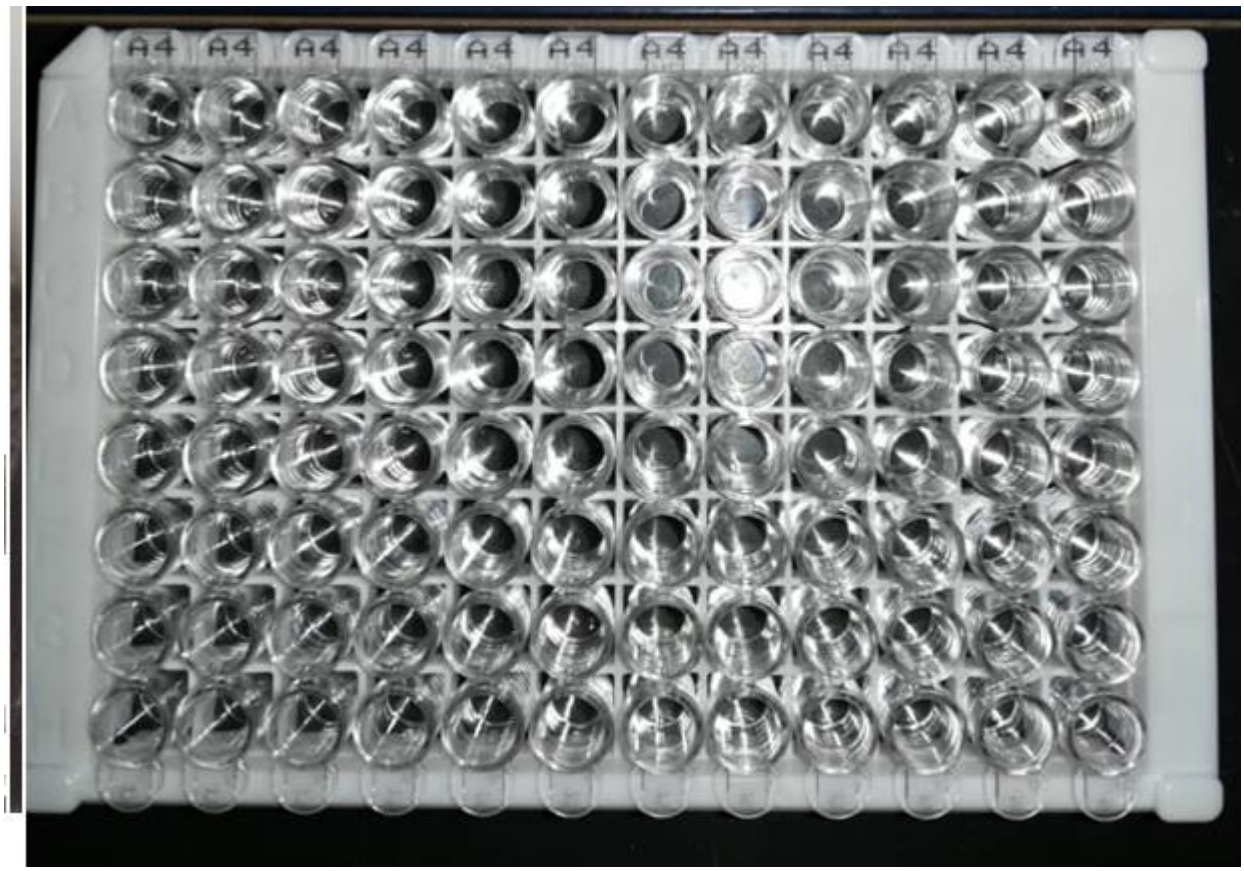


Fortress diagnostics HIV (Ag/Ab) ELISA kit

APPENDIX VII

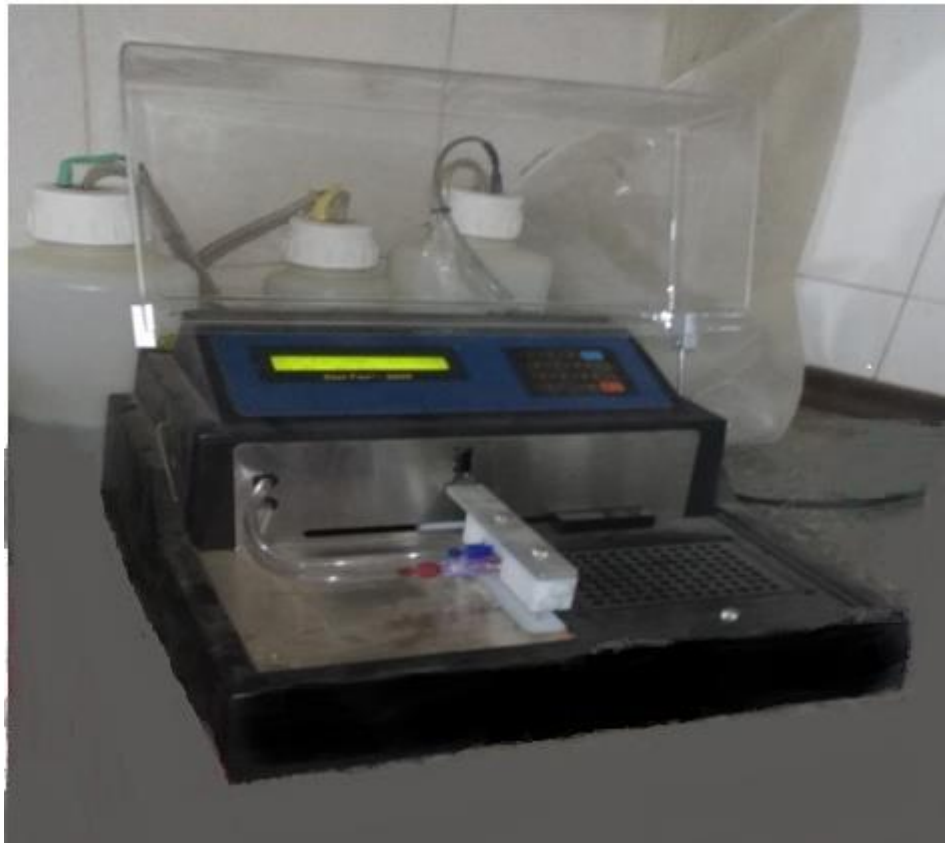
ELISA kit components

APPENDIX VIII



ELISA kit Plate

APPENDIX IX



ELISA washer apparatus

APPENDIX X

ELISA reader apparatus

APPENDIX XI

Equipments and materials used for this study

-ELISA apparatus and their components.

-ELISA Kit components.

- Calculator.

- Centrifuge.

- Water bath.

- Automatic pipette.

- Tube rack.

- Tourniquet.

- Sterile 5ml syringe.

- 70% alcohol.

- Cotton.

- Gloves.

- Face mask.