Sudan University of Sciences and Technology

College of Graduate Studies

Estimation of Interleukin 17 (IL17) Level among Sudanese Active pulmonary Tuberculosis Patients in Abo Anga Hospital Khartoum State

تقييم مستوى المادة الخلوية 17 البلازمية وسط مرضى السل الرئوي الحاد السودانيين بولاية الخرطوم

A Dissertation Submitted in Partial Fulfilment for the Requirement of M.Sc. Degree in Medical Laboratory Sciences (Microbiology)

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الأية

قال تعالى:

(لَا يُكَلِّفُ اللَّهُ نَفْسًا إِلَّا وُسْعَهَا ۚ لَهَا مَا كَسَبَتْ وَعَلَيْهَا مَا اكْتَسَبَتْ ۗ رَبَّنَا لَا تُؤَاخِذْنَا إِنْ نَسِينَا أَوْ أَخْطَأْنَا ۚ رَبَّنَا وَلَا تَحْمِلْ عَلَيْنَا إِصْرًا كَمَا حَمَلْتَهُ عَلَى الَّذِينَ مِنْ قَبْلِنَا ۚ رَبَّنَا وَلَا تُحَمِّلْنَا مَا لَا طَاقَةَ لَنَا بِهِ ۖ وَاعْفُ عَنَّا وَاغْفِرْ لَنَا وَارْحَمْنَا ۚ أَنْتَ مَوْلَانَا فَانْصُرْنَا عَلَى الْقَوْمِ الْكَافِرِينَ)

صدق الله العظيم

سورة البقرة الأية(286).

DEDICATION

To

My father and mother with all love and respect ..

My husband and my little baby ..

My brothers and my sisters ..

My best friends ..specially Alaa Kamal Taha , Hisham Mohamed

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First of all I thank great full to **Almighty ALLAH** for giving me ability and strength to complete this research.

I would like to thank my supervisor **Dr. Hind Haidar Ahmed who** gave me a lot of her time and helped me to complete this work. Special thanks to **Prof. Muawia Mukhtar** and medical staff of Ibn Sina hospital for their vital assistance and help.

ABSTRACT

Mycobacterium tuberculosis is widely distributed pathogen especially in developing countries which causes primary and secondary pulmonary tuberculosis and if untreated extra pulmonary tuberculosis.

This is case control study was aimed to evaluate the interleukin- 17(IL17) among Sudanese acute tuberculosis patients in Abo- Anga Hospital compared to healthy control group in Khartoum State during the period from June(2019) to June (2021) .

Blood specimens were collected from each patient and IL-17 concentration was measured by Enzyme Linked Immunosorbent Assay (ELISA) in Ibn Sina University Research Center. The data was analyzed using SPSS (Version 20).

The mean level of IL-17 in acute tuberculosis patient was higher (15.6± 8.1 pg/ml), compared to control group (10.6±9.1 pg/ml) with statistical significant difference between case and control (*p-value:* 0.007).

There was no statistical correlation between IL-17 level and age, sex, smoking status and socioeconomic status among cases subjects (*p*.value 0.2, 0.3, 0.8, 0.9) respectively.

This study concluded that: IL-17 concentration was high among active TB patients and may be useful diagnostic biomarker for acute tuberculosis infection.

المستخلص

بكتريا السل الرئوي هي بكتريا واسعة الانتشار وبالأخص في الدول النامية والتي تسبب السل الرئوي الأولي والثانوي وإذا لم يتم معالجته قد يؤدي إلى السل المنتشر خارج الرئة.

هدفت هذه الدراسة الحالة والضابطة إلى تقييم مستوى المادة الخلوية 17 لدى المرضى السودانيين المصابين بالسل الرئوي الحاد بمستشفى ابو عنجة مقارنة مع الافراد الطبيعيين بولاية الخرطوم في الفترة من يوليو(2019) إلى يوليو (2021).

سحبت عينة دم من كل مشارك. تم قياس تركيز المادة الخلوية 17 عن طريق فحص الممتز المناعي المرتبط بالإنزيم في مركز أبحاث جامعة ابن سينا.

كان هناك ارتفاع ذو دلالة إحصائية للمادة الخلوية 17 في مرضى السل الرئوي الحاد(15.6±8.1) مقارنة بالأفراد الطبيعيين(10.6±9.1) القيمة الاحتمالية (0.007)

لم يتأثر مستوى المادة الخلوية 17 بالفئة العمريه او الجنس أو حالة التدخين أو الحالة الاجتماعية علي مستوى الحالات المصابة والأفراد الطبيعيين (القيمة الاحتمالية: 0.2, 0.3, 0.8, 0.9) علي التوالي.

خلصت هذه الدراسه ان تركيز الماده الخلويه 17كان عاليا في مرضى السل الرئوي النشط و قد يكون مؤشرتشخيصي جيد لعدوى السل الحاد .

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LIST OF ABBREVIATIONS

Abbreviation	Full name
CD	Cluster of differentiation
GM-CSF	granulocyte-macrophage colonystimulating factor
INF	Interferon
IGRA	interferon-gama (IFN-γ) release assay
MDR	multidrug-resistant tuberculosis
MHC	Major histocompatibility complex
MTB	*Mycobacterium. Tuberculosis*
MTBC	*Mycobacterium. Tuberculosis* complex
NAAT	Nucleic acid amplification test
NGS	next-generation sequencer
NK	Natural killer
PAMPs	Pathogen-associated molecular patterns
PMN	Poly morph nuclear neutrophil
PPD	Purified protein derivative
TB	Tuberculosis
Th	T helper
TNF	Tumor necrosis factor
TST	tuberculin skin test
WGS	Whole-genome sequencing
XDR	Extensively drug-resistant

CHAPTER I

INTRODUCTION

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INTRODUCTION

1.1 Introduction

Despite recent advances in tuberculosis (TB) diagnosis and treatment, it still remains a major infectious disease killer in resource-poor settings. In 2015, 10.4 million new cases were diagnosed resulting in 1.8 million deaths and over 50 million new infections (WHO, 2017).

Adaptive immune responses mediated by CD_{4+} T cells and CD_{8+} T cells, and T helper (Th_1) cytokines characterized by interferon gama (IFN-γ) production are associated with a good prognosis and play an important role in countering the progression of *Mycobactrium tuberculosis*(MTB) infection (Fan *et al.*, 2015). However, Th1 cells (primarily CD_{4+} cells producing IFN-γ) alone are not capable of controlling the infection and other factors, including Th_2 cells, Th_{17} cells and regulatory T cells (Treg cells), are also involved in the progression of MTB infection (Abhimanyu *et al*., 2013).

Interleukin (IL)-17, also known as IL-17A, is a number of the IL-17 family which range from A to F. However, IL-17 is of particular importance as it is the cytokine primarily secreted by Th17 cells (Diao *et al*., 2012). Recent studies have shown that IL-17 plays an important role in the initial immune responses and is involved in both immune protection and immune pathology in MTB infection (Fan *et al*., 2015).

Vaccination, has been shown to inhibit bacterial growth in the lung after MTB infection, as well as promote the production of chemokines that recruit and activate neutrophils and IFN-γ producing CD_{4+} T cells (Ballester *et al*., 2011).

1.2 Rationale

MTB secretes a range of effector proteins to confuse the host immune system, thus promoting its intracellular survival and shaping its lifestyle to persist in granulomas during the latency phase of infection (Gröschel *et al*., 2016).

Results of several studies suggest possible role of IL-17 in the modulation of immune response in autoimmunity, cancer and infections (bacterial, fungal, viral) but its role in tuberculosis infection and progression of the infection from acute into chronic infection have not been well established. Some studies demonstrated that IL-17 played an essential role in granuloma formation in the lung, and was involved in the pathological damage mediated by the initial neutrophil recruitment following MTB infection (Cruz *et al*., 2010). To my knowledge there was no published data about the evaluation of IL17 among Sudanese TB patients, therefore this study will evaluate the utility of using Interleukin-17 as biomarker for monitoring the progression and treatment of TB.

1.3 Objectives

1.3.1 General objective

1. To assess IL-17 level among Sudanese Active pulmonary Tuberculosis Patients in Abo Anga Hospital Khartoum State, Sudan.

1.3.2 Specific objectives

1. To measure IL-17 level among acute tuberculosis patient and apparently healthy individuals using ELISA technique.
2. To compare between IL-17 level between TB patients and healthy controls.
3. To detect the association of possible risk factors affecting IL-17 level in acute tuberculosis patients (age, gender, smoking status and socioeconomic status).

CHAPTER II

LITERATURE REVIEW

CHAPTER II

LITERATURE REVIEW

2.1 Tuberculosis

2.1.1 Historical Background

Robert Koch, (1882), a German Microbiologist, was the first who discovered the tubercle bacillus or Koch's bacillus; named *Mycobacterium. tuberculosis* as a causative agent of the disease. At the earliest times of disease discovery, the only available advice for a consumptive patient who contracted infection was to take a rest and consume nutritious food (Palomino, 2007). However, subsequent prominent work carried out by scientists and researchers have paved the way to nearly consider TB a disease of the past. Such revolutionary period, along with later great discoveries initially marked by tuberculin test in 1890 facilitating early diagnosis of the disease followed by development of Bacillus-Calmette Guerin (BCG) vaccine in 1908 and use of anti-tuberculosis drugs in 1943, offered hope for eradication of a major fatal disease and limiting its spread (Palomino *et al.*, 2007; WHO, 2014).

2.1.2 Epidemiology

In 2015 the largest number of new TB cases occurred in asia, with 61% of new cases followed by Africa, with 26% of new cases. Also in 2015 10.4 million people fell ill with TB and 1.8 million death from the disease (including 0.4 million among people with HIV) and over 95% of TB occur in low and middle income countries (WHO, 2017).

In 2016, there were still _10.4 million TB cases, including 600,000 rifampicin-resistant TB (RR-TB) and 490,000 multidrug-resistant TB (MDR-TB) cases (WHO, 2017). Different from many other bacterial pathogens that secrete various toxins to cause acute inflammation and severe tissue damage (Ramachandran, 2014).

2.1.3 Causative agent

TB is caused by members of the specie *Mycobacterium tuberculosis* complex (MTBC), which includes: *Mycobacterium tuberculosis*, the etiologic agent of TB in humans; *M. africanum*, that causes TB in humans only in certain regions of Africa; *M. bovis*, *M. caprae* and *M. pinnipedii*, causing TB in wild and domesticated mammals; *M. microti*, that causes TB only in voles (Cole *et al*., 2011).

2.1.4 *Mycobaterium tuberculosis*

A major feature of MTB is the peculiar cell wall structure, that provides an exceptionally strong impermeable barrier to noxious compounds and drugs and that plays a fundamental role in virulence (Hoffmann *et al*., 2013). Non motile, non–spore-forming, aerobic rods that are 0.2 to 0.6 × 1 to 10μm in size. The rods occasionally form branched filaments, but these can be readily disrupted. The cell wall is rich in lipids, making the surface hydrophobic and the mycobacteria resistant to many disinfectants and common laboratory stains. Once stained, the rods also cannot be decolorized with acid solutions; hence the name acid-fastbacteria. Most mycobacteria divide slowly, and cultures require incubation for up to 8 weeks before growth is detected because the structure of the cell wall is complex and the organisms have fastidious growth requirements (Murray *et al*., 2013).

2.1.5 Disease transmission

TB is an air-borne disease due to droplet infection from those who suffer from active pulmonary TB through cough, sneeze, speak or spit.One sneeze can release about 40,000 droplets (Cole and Cook, 1998). Each one of infectious droplets may induce the disease, since the infectious dose of TB is quite low (inhaling less than ten bacteria) may cause an infection (Nicas *et al.*, 2005). People with prolonged, frequent, or close contact with patients (co-patients) are at a particular high risk of being

infected, with an estimated 22% infection rate. A person with active, but untreated TB can infect 10–15 people every year (WHO, 2007). Transmission can only occur from people with active, latent TB (Kumar *et al*., 2007). It depends upon the number of infectious droplets expelled by a carrier, the effectiveness of ventilation, the duration of exposure and the virulence of the MTB strain (CDC, 2003).

2.1.6 Risk factors for TB

A number of factors make people more susceptible to TB infections. The most important risk factor globally is human immunodeficiency virus (HIV); 13% of all people with TB are infected by the virus. This is a particular problem in sub-Saharan Africa, where rates of HIV are high. Of people without HIV who are infected with tuberculosis, about 5–10% develop active disease during their lifetimes; in contrast, 30% of those co-infected with HIV develop the active disease (Chaisson *et al*., 2008). Chronic lung disease is another significant risk factor. Silicosis increases the risk about 30-fold. Those who smoke cigarettes have nearly twice the risk of TB compared to non smokers. Other disease states can also increase the risk of developing TB. These include alcoholism and diabetes mellitus (three-fold increase). Certain medications, such as corticosteroids and in fliximab (an anti-tumor necrosis factor α (TNFα) monoclonal antibody), are becoming increasingly important risk factors, especially in the developed world. Genetic susceptibility also exists, for which the overall importance remains undefined (Vanzylsmit, 2010)

2.1.7 Tuberculosis Pathogenicity

MTB is exposed to the air as droplet nuclei from coughing, sneezing, shouting or singing of individuals with pulmonary or laryngeal TB. Transmission occurs through inhalation of these droplet nuclei which passes through the mouth or nasal cavities, the upper respiratory tract, bronchi and finally reaches the alveoli of the

lungs (CDC,2016). TB infection begins when the mycobacteria reach the pulmonary alveoli, where they invade and replicate within endosomes of alveolar macrophages. During this process, the bacterium is enveloped by the macrophage and stored temporarily in a membrane-bound vesicle called a phagosome (Yon, 2015).

The phagosome then combines with a lysosome to create a phagolysosome. MTB can reproduce inside the macrophage and will eventually kill the immune cell (Yon, 2015). The primary site of infection in the lungs, known as the "Ghon focus", is generally located in either the upper part of the lower lobe or the lower part of the upper lobe. Tuberculosis of the lungs may also occur via infection from the bloodstream. This is known as Simon's focuses and is typically found at the top of the lung. This hematogenous transmission can also spread the infection to more distant sites, such as peripheral lymph nodes, the kidneys, the brain, and the bones. All parts of the body can be affected by the disease, though for unknown reasons it rarely affects the heart, skeletal muscles, pancreas, or thyroid (Ian, 2015).

2.1.8 Intracellular survival of MTB with counterbalanced host immune defenses

The host exerts both innate and adaptive immune functions to protect against MTB infection. Initially, the innate immune cells rapidly respond by direct recognition of conserved pathogen-associated molecular patterns (PAMPs) like lipoproteins, glycolipids, and carbohydrates on the MTB cell surface (Killick *et al*., 2013). Subsequently, a variety of immune mechanisms, such as phagocytosis, autophagy, apoptosis, and inflammasome assembly, are evoked to efficiently control M.*tuberculosis* survival (Liu *et al*., 2017). Finally, adaptive immunity such as Th_1/Th_{17} responses mediated by MTB-specific CD_{4+} T cells become involved, which plays a pivotal role in the control of TB progression (Jasenosky *et*

al., 2015). host adaptive immunity to MTB is activated after a considerably longer interval compared to other pathogen infections. Several studies demonstrate that the CD_{4+} T cell response does not initiate until 10–14 days after infection and peaks at nearly 3 weeks after infection by MTB in mice (Wolf *et al.*, 2008). This delayed response has not yet been entirely explained, though one possibility is that MTB may suppress the function of dendritic cells (DCs) and limit their migration from the lungs to lymph nodes for activation of initial T cells (Griffiths *et al*., 2016).

2.1.9 Pathogenesis

2.1.9.1 Pulmonary TB

2.1.9.1.1 Primary pulmonary

The site of the initial infection is usually the lung, following the inhalation of bacilli. These bacilli are engulfed by alveolar macrophages in which they replicate to form the initial lesion. Some bacilli are carried in phagocytic cells to the hilar lymph nodes where additional foci of infection develop. The initial focus of infection together with the enlarged hilar lymph nodes forms the primary complex. In addition, bacilli are seeded by further lymphatic and haematogenous dissemination in many organs and tissues, including other parts of the lung (Greenwood *et al*., 2012).

The activated T cells release cytokines, notably interferon-γ (INFγ), which, together with calcitriol, activate macrophages and cause them to form a compact cluster, or granuloma, around the foci of infection. Granuloma formation is usually sufficient to limit the primary infection: the lesions become quiescent and surrounding fibroblasts produce dense scar tissue, which may become calcified. Programmed cell death (apoptosis) of bacteria-laden macrophages by cytotoxic T cells and

natural killer (NK) cells contributes to protective immunity by generating a metabolic burst that kills tubercle bacilli (Greenwood *et al*., 2012).

2.1.9.1.2 Secondary (reactivated) tuberculosis

In about 10% of infected persons the primary tuberculosis reactivates to become an organ tuberculosis, either within months (5%) or after a number of years(5%). Reactivation begins with a caseation necrosis in the center of the granulomas (also called tubercles) that may progress to cavitation (formation of caverns). Tissue destruction is caused by cytokines, among which tumor necrosis factor alpha (TNFα) appears to play an important role. This cytokine is also responsible for the cachexia associated with tuberculosis (Kayser *et al.,* 2005).

2.1.9.1.3 Clinical feature

Primary tuberculosis is either asymptomatic or manifest only by fever and malaise. In secondary TB Cough is the universal symptom. It is initially dry, but as the disease progresses, sputum is produced, which even later is mixed with blood (hemoptysis). Fever, malaise, fatigue, sweating, and weight loss all progress with continuing disease. Less commonly, reactivation tuberculosis can also occur in other organs, such as the kidneys, bones, lymph nodes, brain, meninges, bone marrow, and bowel. Disease at these sites ranges from a localized tumor like granuloma (tuberculoma) to a fatal chronic meningitis. Untreated, the progressive cough, fever, and weight loss of pulmonary tuberculosis creates an internally consuming fire that usually takes 2 to 5 years to cause death. The course inquired immunodeficiency syndrome (AIDS) and other cell mediated immunity-compromised patients is more rapid (Rayan and Ray, 2004**).**

2.1.9.2 Extra-pulmonary TB

2.1.9.2.1 Tuberculous Lymphadenitis

It presents most commonly in the cervical lymph nodes, followed by the mediastinal and axillary nodes (Geldmacher *et al*., 2011).

2.1.9.2.2 Pleural TB

Accounting for roughly 4% of all TB cases, pleural TB is the second leading cause of extra-pulmonary TB (Baumann *et al*., 2007)

2.1.9.2.3 Central Nervous System TB

A devastating manifestation of the disease, central nervous system TB occurs in approximately 1% of all TB cases. Tuberculous meningitis is clinically heralded by a 2- to 3-week prodrome of malaise, headache, low-grade fever, and personality changes. This prodrome is followed first by a meningitic phase that mimics bacterial meningitis (fever, nuchal rigidity, altered mental status) and then by a paralytic phase characterized by rapid progression to stupor, coma, seizures, paralysis, and death. (Phypers *et al*., 2006)

2.1.9.2.5 Skeletal TB

Skeletal TB occurs in 1% to 5% of patients with TB and presents most commonly in the thoracolumbar spine (Sharma *et al*., 2005).

2.1.9.2.6 Miliary TB

The lymphatic and hematogenous spread of TB is referred to as miliary *TB*. Patient presentation is variable, and systemic symptoms (fever, weight loss, night sweats) are common (Sharma *et al*., 2005).

2.1.10 Diagnosis of tuberculosis

2.1.10.1 Microscopic examination of tuberculosis

Confirmation of the diagnosis of TB requires laboratory identification of MTB by AFB smear microscopy with nucleic acid amplification test and/or culture. The diagnosis of latent tuberculosis infection is established by a positive result on either a tuberculin skin test (TST) or an interferon-gama (IFN-γ) release assay (IGRA), in the absence of active TB(CDC, 2013).

2.1.10.2 Culture of MTB

According to WHO standards for the diagnosis of pulmonary TB, acid-fast smears are first obtained from patient's phlegm and for negative results, specific culturing environments will be used as the golden standard (WHO, 2004). Culture is traditionally performed on solid egg-based media, such as Lowenstein-Jensen (L-J) media, which is composed of egg proteins, potato flour, salts, and glycerol. L-J media supports good growth of MTBC, but is not as reliable for all mycobacterial species including *M. bovis* and *M. genavense* (Robbe-Austerman *et al*., 2013). Most *Mycobacterium* species, including MTBC, grow best at a temperature of 35–37 °C. Like many aerobic bacteria, mycobacterial growth is stimulated by incubation in air containing 5–10% CO2. Mycobacteria typically grow more slowly on solid media as compared to broth culture. MTBC colonies are characteristically dry with a rough texture and a cream/tan color, and are colloquially described as "rough and buff." Several *Mycobacterium* species also produce pigments that can range from yellow to orange in color. Photochromogenic species only produce pigment after exposure to light, while scotochromogens produce pigment regardless of light exposure. All members of the *M. tuberculosis* complex are non-pigmented. Growth of MTBC from clinical specimens

takes an average of 10 days by automated broth systems versus 20–25 days on solid media (Moreira *et al*., 2015)

2.1.10.3 Molecular Approaches for TB diagnosis

2.1.10.3.1 Nucleic acid amplification test (NAAT)

Most NAAT assays detect the mycobacterial insertion element IS6110 for the identification of the MTB complex organisms.12 NAAT detects MTB ribosomal RNA or DNA directly from sputum specimens, both the(AFB) smear-positive and AFB smear-negative.13 The NAAT systems, with rapid turn-around times, facilitate testing and treatment initiation in the same visit and, therefore, loss to follow-up cases can be reduced. The NAAT showed very high sensitivity in sputum smear-positive patients and around 61 to 76% sensitivity in patients with smear-negative sputum (Sia and Wieland, 2011).

2.1.10.3.2 Xpert MTB/RIF assay

The Xpert MTB/RIF assay is a nuclear acid amplification- based test using a cartridge based on the Gene Xpert Instrument System.The basis of the Xpert MTB/RIF as say is a real-time PCR that can be used to detect DNA sequences specific to the MTB in sputum samples. The Xpert MTB/RIF test directly from sputum can detect 99% of smear-positive patients and more than 80% of smear-negative cases (Friedrich *et al*., 2013). According to the WHO in 2013, a Xpert MTB/RIF assay could be used for: an additional test following microscopic TB examination; a replacement examination for AFB smear microscopy; detection of MTB in both AFB smear-positive and smear-negative culture-positive cases; detection of MTB in pleural in pleural fluid; detection of MTB in lymph node in samples from biopsy or fine-needle aspiration; detection of MTB in gastric fluid; detection of MTB in samples of cerebrospinal fluid; and detection of MTB in tissue samples (WHO, 2013).

2.1.10.3.3 Whole-genome sequencing (WGS)

Whole-genome sequencing (WGS) is becoming an affordable and accessible method that can identify microevolution within MTB lineages as they are transmitted between hosts. The WGS can detect various types of mutations better than the Xpert MTB assay. There are two classes of sequencers that exist: the first generation sequencer and the second generation (widely known as the next-generation sequencer [NGS]) (Köser *et al*., 2014).

The first generation sequencer is relatively slow, but has a high throughput and low cost. The second generation has a lower throughput, higher cost and is able to sequence multiple genomes in less than a day (Köser *et al*., 2014).

2.1.11 Tuberculin Sensitivity and Testing

Because infection with the TB bacillus can lead to delayed hypersensitivity to tuberculoproteins, testing for hypersensitivity has been an important way to screen populations for tuberculosis infection. The tuberculin test, called the Mantoux test, involves local injection of purified protein derivative (PPD), a standardized solution taken from culture fluids of MTB. The injection is done intradermally into the forearm to produce an immediate small bleb. After 48 and 72 hours, the site is observed for a red wheal called an induration, which is measured and classified according to size (Talaro, 2012).

2.1.12 Treatment

TB is an extremely stubborn disease especially with the development of antibiotic resistance. TB has been traditionally treated with such first-line drugs as isoniazid and rifampin. Ethambutol, pyrazinamide, and streptomycin also are used to help delay the emergence of resistant strains. Unfortunately, the appearance of MDR-TB has occured and now accounts for 5% of new TB cases. This has necessitated a switch to a

group of second-line drugs, including fluoroquinolones and kanamycin. If drug therapy is effective for pulmonary TB, patients usually become noninfectious within three weeks as determined by bacteria free sputum samples. Still, for such individuals, antimicrobial drug therapy is intensive and must be extended over a period of six to nine months or more (Pommerville and Jeffrey, 2011).

2.1.12.1 Multi drug resistance tuberculosis (MDR-TB)

Is defined as tuberculosis caused by mycobacterium tuberculosis showing in vitro resistance to isoniazid and rifampicin with or without resistant to any other drugs (WHO, 2010).

MDR-TB results from either infection with organisms which are already drug resistant or may develop in the course of a patient's treatment. (WHO, 2013).

2.1.12.2 Extensively drug-resistant TB (XDR-TB)

Is a form of TB caused by organisms that are resistant to isoniazid and rifampicin (i.e. MDR-TB) as well as any fluoroquinolone and any of the second–line anti- TB injectable drugs(amikacin, kanamycin or capreomycin)(WHO, 2013).

2.1.13 Prevention

Vaccination against TB can sometimes be rendered by intradermal injections of an attenuated strain of *Mycobacterium bovi*s, a species that causes TB in cows as well as humans. The weakened strain is called Bacille Calmette-Guérin (BCG), after Albert Calmette and Camille Guerin, the two French investigators who developed it in the 1920s. Though the vaccine is used in parts of the world where the disease causes significant mortality and morbidity, health officials in the United States generally do not recommend the BCG vaccine because it has limited effectiveness for preventing TB in adults and is only moderately effective in children for 10 years. New vaccines consisting of subunits, molecules

of DNA, and attenuated strains of mycobacteria are currently being developed (Pommerville and Jeffrey, 2011). BCG should never be given to persons known to be HIV positive. Many attempts are currently being made to develop alternative vaccines, particularly nonviable subunit ones (Greenwood *et al*., 2012).

2.2Cytokines

2.2.1 Roles and Nomenclature

Cytokines are soluble molecules plays an extremely important role in clinical immunology. They are act as stimulatory or inhibitory signals between cells. Cytokines that initiate chemotaxis of leucocytes are called chemokines (Horton-Szar *et al,* 2012). It is Stimulate growth and differentiation of lymphocyte, activates immune cells to eliminate microbes and antigen (Ag), Stimulate hematopoiesis and used in medicine as therapeutic agent (Abbas *et al.,* 2015). It nomenclature is according to producing cell is divided into: monokines produced by macrophage/monocyte, lymphokines produced by lymphocyte, Interleukins produced by leucocytes and act on other leucocytes eg IL-1and IL-2 and IL-3 and biologic response modifier which used clinically to increase or reduce immunity (Hawas, 2016).

2.2.2 Properties of cytokines

Cytokines are produced transiently in response to antigen and usually acts on same cell that produces the cytokine (autocrine) or nearby cells (paracrine) and each cytokine has multiple biologic actions (Pleiotropism) Multiple cytokines may share the same or similar biologic activities (Redundancy) (Reche, 2019).

2.2.3 Cells produce cytokines

Cytokines are a cell-signaling group of low molecular weight extracellular polypeptides /glycoprotiens synthesized by different immune cells, mainly, by T cells, neutrophils and macrophages (Ma *et*

al., 2018). The production and release of cytokines from innate immune cells are critical responses to inflammation and infection in the body. Innate immune cells comprise populations of white blood cells such as circulating DCs, neutrophils, NK cells, momocytes, eosinophils, and basophils, along with tissueresident mast cells and machrophages (Lwasaki and Medzhitov, 2010).

Residing at the frontline of defence in immunity, these cells control opportunistic invasion by a wide range of viral, fungal, bacterial and parasitic pathogens, in part by releasing a plethora of cytokines and chemokines to communicate with other cells and thereby to orchestrate immune response (Vazquez *et al*., 2015). This array of soluble mediators secreted by different innate immune cells includes TNF, IFNγ, interleukins IL-1β, IL-4, IL-6, IL-10, IL-12, IL-18, CCL/4RANTES, and transforming growth factor beta (TGFβ) (Lacy and Stow , 2011).

Th1 subset secreted IL-2, IFNγ, and TNF, and is responsible for many classic cell mediated function including activation of cytotoxic T lymphocytes and macrophages (Siransy *et al* ., 2018).

The main function is cellular immune system maximizes the killing efficacy of the macrophages and the proliferation of cytotoxic CD_{8+} T cells. Also promotes the production of immunoglobulin G (IgG), an opsonizing antibody. Other functions IFNγ increases the production of IL-12 by DCs and macrophages, and via positive feedback, IL-12 stimulates the production of IFN-γ in Th cells, thereby promoting the Th1 profile. IFN-γ also inhibits the production of cytokines such as IL-4 (Christensen *et al*., 2018).

The Th_2 subset secreted IL-4, IL-5 , IL-6 , IL-9 , IL-10 , IL-13 and IL-25 .The main functions Humoral immune system Stimulates B-cells into proliferation, to induce B cell antibody class switching, and to increase neutralizing antibody production (IgG, IgM and IgA as well as IgE

antibodies (Owen *et al*., 2013). Also IL-4 acts on Th cells to promote the production of Th_2 cytokines (including itself; it is auto-regulatory), while IL-10 (IL-10) inhibits a variety of cytokines including IL-2 and IFN-γ in Th cells and IL-12 in DCs and macrophages (Christensen *et al*., 2018).

The Th_{17} are a subset of pro-inflammatory T helper cells involved in recruiting leukocyres and incducing inflammation (Hammerich, 2019).

The main effector cytokines are IL-17A, IL-17F, IL-21, and IL-22, and granulocyte-macrophage colonystimulating factor (GM-CSF). IL-17 family cytokines (IL-17A and IL-17F) target innate immune cells and epithelial cells, among others, to produce granulocyte- colonystimulating factor (G-CSF) and IL-8 (CXCL8), which leads to neutrophil production and recruitment (Zambrano and Zaragoza, 2017).

The Th_9 subsets secreted IL-9 which have a role in the induction and the pathogenesis of atopic disease, antiparasite immunity and immune pathological disease of the gut (Kaplan *et al*., 2015). Th_9 cells have also shown both pro- and anti-tumorigenic activity, depending on the type of cancer (Tan *et al*., 2017).

2.2.4 Types of cytokines according to T helper

2.2.4.1 Tumor necrosis factor (TNF)

It is produced chiefly by activated macrophages, although it can be produced by many other cell types such asCD_{4+} lymphocytes, NK cells, neutrophils, mast cells, eosinophils, and neurons. TNF is a member of the TNF superfamily, consisting of various transmembrane proteins with a homologous TNF domain (Swardfager *et al*., 2010)

2.2.4.2 Interferons (IFN)

Interferons (IFN) are a group of signaling proteins made and released by host cells in response to the presence of several viruses. In a typical scenario, a virus-infected cell will release interferons causing nearby cells to heighten their anti-viral defenses (Levy *et al*., 2011).

Interferons are named for their ability to "interfere" with viral replication by protecting cells from virus infections. It is also have various other functions: they activate immune cells, such as natural killer cells and macrophages; they increase host defenses by up-regulating antigen presentation by virtue of increasing the expression of major histocompatibility complex (MHC) antigens (Espinosa *et al*., 2017).

2.2.4.3 Interleukins

Interleukins (ILs) are a group of secreted proteins with diverse structures and functions. These proteins bind to receptors and are involved in the communication between leukocytes. They are intimately related with activation and suppression of the immune system and cell division. The interleukins are synthesized mostly by helper CD_{4+} T lymphocytes, monocytes, macrophages and endothelial cells (Seyfizadeh and Babaloo, 2015).

2.2.5 IL-17

The interleukin-17 (IL-17) family is the most recently described subclass of cytokines. Since 2000, we have started to gain an understanding of IL-17 family members and their corresponding receptors, which has led to new insights into how immunity to infections and autoimmunity are governed. To date, there are six IL-17-family ligands [IL-17A, IL-17B, IL-17C, IL-17D, IL-17E (IL-25) and IL-17F], and five receptors (IL-17RA, IL-17RB/ IL-25R, IL-17RC, IL-17RD/SEF and IL-17RE) (Gaffen, 2009). Interleukin- 17A (hereafter referred to as IL-17) is the most intensively studied, but interest in the rest of the family is growing. Interleukin 17 (IL-17) is a pro-inflammatory cytokine that plays an important role in autoimmunity , graft rejection , and immune responses against intracellular pathogens (Torrado *et al*., 2011) By inducing the recruitment and activation of poly morph nuclear neutrophils (PMN) and triggering the production of pro-inflammatory cytokines and chemokines

, IL-17 is also associated with tissue damaging inflammation (Cooper, 2010). The human IL-17 gene was mapped on human chromosome 6p12. The gene product is a protein of 150 amino acids with a molecular weight of 15 kDa and is secreted as a disulfide linked homodimer of 30–35 kDa glycoproteins. IL-17 polypeptide comprises a 19-amino-acid signal sequence followed by a 136-amino-acid mature fragment. It comprises at least one N-linked glycosylation site and biochemically, IL-17 A migrates as a dimer, apparently through six cysteine residues that form intermolecular bond interactions (Awasthi and Kuchroo, 2010)

2.2.5.1The cellular source of IL-17

IL-17 cytokines are now known to be secreted by many cells types other than Th_{17} ; apart from CD_{8+} T cells, gama delta (γδ)T cells, natural killer NK cells, monocytes, macrophages, DCs, microglia, neutrophils, eosinophil, astrocytes, and oligodendrocytes. Thus, cells of both the innate and the adaptive immune systems as well as non-immune cells produce IL-17A and IL-17F (Hisakata Y, 2010).

Pathogens can induce Th_{17} response, including gram-positive *Propionibacterium acnes*, gram-negative *Citrobacter rodentium*, *Porphyromonas gingivalis, Klebsiella pneumoniae,* bacteroides species and *Borrelia* species, *Mycobacterium tuberculosis,* parasitic infections like *Toxoplasma .gondii*, and fungi such as *Candida albicans* (Koll, 2010).

2.3 IL-17 and tuberculosis

Following deposition of MTB in the lung, it is likely that DCs migrate to the draining lymph nodes (dLN) where T cells are primed. In the dLN, both IFN-γ and IL-17 producing T cells are induced and these cells then migrate to the lung where they exert effector function (Cooper, 2010).

Although we are still learning how mycobacteria stimulate innate immune receptors, cytokines produced by MTB-primed DCs are likely to

be crucial for balanced acquired cellular responses (Cooper, 2010). The development of Th_{17} cells is initiated by IL-6 or IL-21 in the presence of low amounts of transforming growth factor beta; IL-1β and TNF may also act as cofactors for Th_{17} differentiation (Van Beelen *et al.*, 2007). IL-17 producing CD_{4+} T cells, activated in response to vaccination, has been shown to inhibit bacterial growth in the lung after MTB infection, as well as promote the production of chemokines that recruit and activate neutrophils and IFN-γ producing CD_{4+} T cells (Ballester *et al*., 2011). Moreover, IL-17 is essential for the vaccine-induced protection against MTB infection by inducing the localization of the proinflammatory cytokine producing C-X-C motif chemokine receptor 5-positive (CXCR5+) T cells, thereby promoting early macrophage activation and the control of MTB (Gopal *et al*., 2013) In contrast, other studies demonstrated that IL-17 played an essential role in granuloma formation in the lung, and was involved in the pathological damage mediated by the initial neutrophil recruitment following MTB infection (Cruz *et al*., 2010).

2.4 Previous studies

A study conducted by Fatemeh *et al* (2016) in Iran aimed to evaluate the level of IL17 in patients with tuberculosis infection, found that the mRNA levels of IL-17 were significantly higher in patients after PPD stimulation (1.45±0.47) compared to non-stimulated Peripheral blood mononuclear cells (0.03±0.01) (*P*=0.00) Also the study showed that there is no significant differences in age and sex were observed between the two groups (*P. value*>0.05). Other study by Mohammed *et al* (2018) aimed to evaluate serum level of IL-17 among pulmonary tuberculosis patients in Babylon province, found that the median serum level of IL-17 in pulmonary tuberculosis patients was 303.42pg/ml compared with 207.37 pg/ml for control group with *p*-value (0.0008). Another study

conducted by Jayne. *et al*(2010) in west Africa aimed to discriminate between active TB disease and latent infection by evaluating of TNF-a, IL-12(p40) and IL-17 level found that following stimulation with PPD, production of all cytokines except IL-18 was significantly higher from TB cases compared to TST2 contacts while production of IFN-c, IL-13 and IL-17 were all significantly higher in TST+ compared to TST2 contacts (p= 0.0027; p= 0.0266 and p= 0.0105 respectively). In another study of Yung-Che Chena, *et al* (2011) in china was aimed to show the prognostic values of serum IL-17 in patients with pulmonary tuberculosis found that there was lowering in IL17 level in TB patient before and after treatment (. 24.6 ± 8.2 pg/ml vs. 15.7 ± 2.9 pg/ml *p value* = 0.001). Another study conducted by Lichen *et al* (2016) was aimed to study the decreased IL‑17 during treatment of sputum smear‑ positive pulmonary tuberculosis, found that plasma IL17 level was significantly elevated in the AFB-positive group compared to healthy controls (*p.value* < 0.001) After the effective treatment, the patients achieved AFB-negative status have plasma levels of IL-17 was remarkably decreased in comparison with the smear-positive group (*p.value* < 0.001).

CHAPTER III

MATERIALS AND METHODS

CHAPTER III

MATERIALS AND METHODS

3.1. Study design

This study is an analytical case-control study.

3.2. Study area and duration

This study was conducted in Abo Anga Hospital in Khartoum State during the period from June (2019) to June (2021).

3.3. Study population

Study population consisted of 88 Sudanese individuals of age between 17-65 years, divided into 2 groups as follows: Acute TB infected made up of 44 patients and 44 apparently healthy control group.

3.3.1. Inclusion criteria

Patients with acute TB infection positive with gene Xpert, not under treatment, and had symptoms of TB less than three months, control group was apparently healthy sub

3.3.2. Exclusion criteria

Immune compromised patients and any disease can affect IL17 level like (diabetic, renal failure, hypertension chronic TB, extra pulmonary TB and auto immune diseases) were excluded.

3.4. Ethical consideration

The study was approved by Scientific Research Committee of College of Medical Laboratory Science, Sudan University and Technology, and written consent was obtained from participants before collection of the blood specimens.

3.5. Sample size

A total of 88 subjects were enrolled in this study. 44 samples were collected

from active tuberculosis patients and 44 samples were collected from healthy volunteer.

3.6. Sampling technique

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

3.7. Data collection

A structured questionnaire was used to collect demographic data.

3.8. Specimen collection

Under sterile condition five ml of venous blood sample was withdrawn from each participant, then waited until sample clotted. Serum was separated by centrifugation at 5000 rpm for five minutes, and collected into plain container then stored at -20C˚ until analysis.

3.9. Laboratory investigation

IL17 level was measured by – Enzyme linked immunosorbent assay Kit (Sandwich Technique), BioLegand, U.SA.

3.9.1 ELISA Procedure

In day one**,** a 100 µL of diluted capture antibody solution was added to each well, the plate was sealed and incubated overnight between 20˚C and 80˚C.

In Day two, the plate was washed 4 times with at least 300µL of wash buffer per well and blotted any residual buffer by firmly tapping the plate upside down on clean absorbent paper. To block the plate by adding 200 µL 1X assay diluents A to each well, The plate was sealed and incubated at room temperature for 1 hour with shaking at approximately 500 rpm (with a 0.3 cm circular orbit). All subsequent incubation with shaking were performed similarly. The plate washed 4 times; A 100 µL of diluted standards and samples were added to the appropriate wells. The plate was sealed and incubated at room temperature for 2 hours with shaking. Then the plate was washed 4 times. A 100 µL of diluted detection antibody solution was added to each well. The plate was sealed and incubated at room temperature for 1 hour with shaking. The plate was washed 4 times; 100 µL of diluted Avidin-HRP solution was added to each well. The plate

was sealed and incubated at room temperature for 30 minutes with shaking. The plate was washed 5 times; soaked for 30 seconds to 1 minute per wash. A 100μL of freshly mixed TMB substrate solution was added to each well and incubated in the dark for 30 minutes. A 100μL of stop solution was added to each well. The optical density was read at 450 nm and 570 nm within 15 minutes.

3.10. Statistical analysis

Data were analyzed using statistical package for social science (SPSS version 20).Frequencies and Mean ± SD were measured. One-way ANOVA test, paired sample T test and independent T test for testing significant difference and correlation test to find out correlation. Probability value ≤0.05 was considered statistically significant.

CHAPTER III

RESULTS

CHAPTER IV

RESULTS

TB acute patients and apparently healthy control were enrolled in this study their ages varied from 15-70 years, 44 subjects were acute tuberculosis, 26 of them, (59 %) were males and 18 of (41 %) were females, with mean age of cases (41±14.3) years. The other 44 subjects were apparently healthy control, 22/44 (50%) were males and 22/44 (50 %) were females with mean age of control group 34.3±12.2 S.D years.

Table (4-1): Distribution of gender among case and control groups

Gender	**Cases(N)**	**Controls(N)**
Male	26 (59 %)	22 (50%)
Females	18 (41%)	22 (50%)
Total	44 (100%)	44 (100%)

Table (4-2): Distribution of age groups among case and control groups

Age groups /years	**Cases(N)**	**Controls(N)**
11-30	16(36%)	15(34%)
31-50	21(47%)	18(41%)
51-70	7(15%)	11(25%)
Total	44(100%)	44(100%)

The results showed that IL-17 level did not affected by the socio-economic status of case subject with *p.value* showed insignificant correlation (*P-value* 0.5).

Table (4-3): Distribution of socio-economic status among Acute TB patients:

Socio-economic status	Frequencies	Percentage%
Low	31	70%
Moderate	12	27%
High	1	2%
Total	44	100%

The mean level of IL-17 in acute tuberculosis patient (15.6± 8.1 pg/ml), in control group (10.6±9.1 pg/ml) with statistical significant difference between case and control (*p-value* 0.007) (Table 4-5).

Tables (4-4): Association of IL-17 Level among case and control groups

Subjects	Mean	*p-value*
Cases(N=44)	15.6± 8.1	0.007
Controls(=44)	10.6±9.1	

The mean level of IL-17 was higher in males (16.5±8.3 pg/ml) than females (14.4±1.8 pg/ml) and showed no statistical significant difference (*P-value* 0.3) (Table 4-6).

Table (4-5): Association between IL-17 and gender among TB patients

Gender	Mean ±STD	*p-value*
Male (N=26)	16.5±8.3	0.3
Female (N=18)	14.4±7.9	

Table (4-7) showed no statistical significant difference of IL-17 level among TB smoking patients (16.01±8.6.5pg/ml) and non-smoking TB patients (15.5±8.0 pg/ml) (*p. value* 0.847).

Table (4-6): Association between IL-17 and smoking among TB patients

Smoking status	Mean ±STD	*p-value*
Smoking (N=23)	16.5±8.6	0.84
Nonsmoking (N=21)	15.5±8.0	

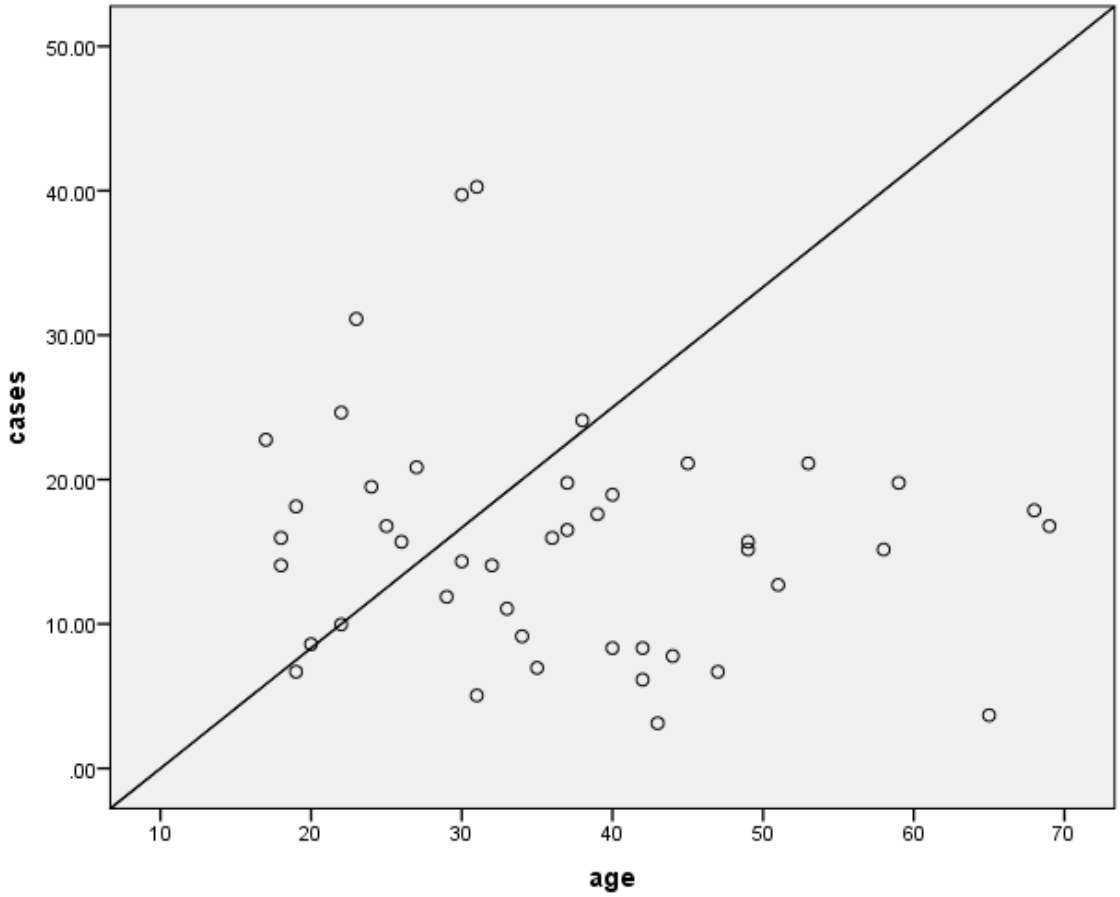
Table (4-7): Association between IL-17 and Socio-economic status among TB patients

Socio-economic status	Mean ±STD	*P.value*
Low	16.0±8.7	0.59
Moderate	13.9±6.7	
High	21.0±8.7	

The results also showed IL-17 level did not affected with age group among case subject and *p.value* showed insignificant correlation (*P-value* 0.25) (Table 4-8).

Table (4-8): Association between IL-17 and age groups among TB patients

Age groups/ year	Mean ±STD	*P.value*
11-30 (N=16)	17.4±7.5	0.25
31-50 (N=21)	13.2±7.6	
51-70 (N=7)	14.9±5.6	



*P.value:*0.25

Figure (4-1): Correlation between (IL17) level and age among Acute TB patients

CHAPTER V

DISCUSSION, CONCLUSION ANDRECOMMENDATION

CHAPTER V

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1. Discussion

IL-17 plays a key role in the maintenance of tissue integrity and the generation of protective immune responses to infectious microorganisms, especially at epithelial barrier sites (Veldhoen , 2017).

In the present study serum level of IL-17(pg/ml) is significantly higher in acute tuberculosis patients compared to healthy control group *(p value* 0.007) , This finding was supported by Thomas *et al* (2008), who demonstrate that a significant increases of IL-17 in acute tuberculosis patients compared with healthy control group (*P.value* <0.05), also Nathella Kumar *et al* (2021) showed that significantly elevated of serum level of IL-17 level in acute tuberculosis patients compared to healthy control (*p.value* <0.05).

This study also demonstrate that there is no association between IL-17 and gender and age among cases (*p.value*: 0.3, 0.4 respectively). This finding was matched with Fatemeh *et al* (2016) whom demonstrated that there is no statistical difference of IL-17 in gender and age in case and control group.

The present study found there was no association in IL-17 level among smoking and non-smoking TB patients (*p.value* 0.84). In contrast, the study carried out by Olsson *et al* (2018) did not support the finding of the present study and demonstrate that there was statistically significant increase of IL-17 level in smoking patients (*p.value*: 0.002). A difference in the populations and geographical area between the two studies may account for these differences.

5.2 Conclusion

This study concluded that;

A significant increase in serum IL-17 level was observed in TB patients compared to healthy control group.

There was no association between IL-17 level among active TB patients with gender, age, smoking and socioeconomic status.

IL-17 can be considered useful as diagnostic biomarker for tuberculosis infection.

5.3 Recommendations

Further studies may be conducted considering:

Studying the role of IL-17 in the progressing of secondary and extra pulmonary tuberculosis.

Studying the role of IL-17 in latent TB infection.

Studying the effect of TB treatments on IL-17 level.

Studying the role of the other types of IL-17 family (B, C, D, E and F) on TB infection.

Finally, taking this work into the next levels the genomic levels and estimating the effects of different mutation and gene polymorphisms using a number of molecular and bioinformatics tools will expanse our knowledge about the role of IL-17 in the pathogenesis of TB infection and so many other diseases.

REFERENCES

Abbas, A.K, Lichtman . A.H and Pillai. S. (2015). Cellular and molecular immunology. 8th ed . Elsevier Saunders. Canda, 469-472.

Abhimanyu, M, Komal, Varma-Basil M. (2013). Lack of association between IL17A and IL17F polymorphisms and related serum levels in north Indians with tuberculosis. *Gene*.**529**:195–8.

American Thoracic Society; Centers for Disease Control and Prevention (CDC); Infectious Diseases Society of America. Treatment of tuberculosis [published correction appears in *MMWR Recomm Rep*. 2005; 53(51):1203. Dosage error in article text]. *MMWR Recomm Rep*. 2003; 52(RR-11):1-77annurev.*immunol*.19.1.93).

Awasthi, A. Kuchroo V. (2010) .Th17 cells: from precursors to players in inflammation and infection. Interl *Immunol*; **21**: 489-98.

Ballester M, Nembrini C, Dhar N, de Titta A, de Piano C, Pasquier M *et al*.(2011). Nano `particle conjugation and pulmonary delivery enhance the protective efficacy of Ag85B and CpG against tuberculosis. *Vaccine*.; **29**: 6959–66.

Baumann MH, Nolan R, Petrini M, Lee YC, Light RW, Schneider E.(2007). Pleural tuberculosis in the United States: incidence and drug resistance. *Chest*; 131:1125-1132.

Bell, L. C. K., and Noursadeghi, M. (2017). Pathogenesis of HIV-1 and Mycobacterium tuberculosis co-infection. Nat. *Rev. Microbiol*. **16**, 80–90.

CDC, (2003). Division of Tuberculosis limination. Core Curriculum on Tuberculosis: What the Clinician Should Know.4th edition.

CDC. Chapter 2: Transmission and Pathogenesis of Tuberculosis (August 15 2016). Available online: https://www.cdc.gov/tb/education/corecurr/pdf/chapter2.pdf.

CDC. How TB Spreads. CDC; 2016 (August 12 2016). Available online: https://www.cdc.gov/tb/topic/basics/ howtbspreads.htm.

Chaisson. R.E, Martinson, N.A (2008). "Tuberculosis in Africa—combating an HIV-driven crisis".*NEJM*; **358** (11): 1089–92.

Christensen, Saraiva, Veldhoen and Murphy. (2018). Interleukin-10 production by Th1 cells requires interleukin-12-induced STAT4 transcription factor and ERK MAP kinase activation by high antigen dose. *Immunity*, **31** (2): 209–219.

Cole, E and Cook, C. (1998). "Characterization of infectious aerosols in health care facilities: an aid to effective engineering controls and preventive strategies". *Am J Infect Control***26**: 453–64.

Cole, S.T., Brosch, R., Parkhill, J., Garnier, T., Churcher, C., Harris, D *et al*.,(2011) Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence . *Nature,* **393**: 537-544. http://dx.doi.org/10.1038/31159 PMid: 9634230.

Cooper. A. M. (2009). Cell mediated immune responses in tuberculosis. Ann *Rev Immunol*; **27**:393– 422.

Cooper, A. M. (2009). IL-17 and anti-bacterial immunity: protection versus tissue damage. *Eur J Immunol*; **39**:649–52.

Cruz, A., Fraga, A.G., Fountain, J.J., Rangel-Moreno, J., Torrado, E., Saraiva, M., Pereira, D.R., Randall, T.D., Pedrosa ,J., Cooper, A.M., Castro, A.G.(2010). Pathological role of interleukin 17 in mice subjected to repeated BCG vaccination after infection with *Mycobacterium tuberculosis*. J Exp Med, **207**:1609–16.

Diao H. Liu, X. Wu, Z. Kang, L. Cui, G. Morimoto, J. Denhardt, DT. Rittling, S. Iwakura, Y. Uede, T. Li, L.(2012) Osteopontin regulates interleukin-17 production in hepatitis. *Cytokine*, **60**:129–37.

Espinosa. V, Dutta. O, McElrath .C, Du. P, and Chang. Y.J.(2017). Type III interferon is a critical regulator of innate antifungal immunity . *Science Immunology*, **2** (16).

Fan L, Xiao H, Mai G, Su B, Ernst J, Hu Z.(2015). Impaired *M. tuberculosis* antigen-specific IFN-gamma response without IL-17 enhancement in patients with severe cavitary pulmonary tuberculosis. *PLoS ONE*, **10**:e0127087.

Fatemeh Heidarnezhad , Amir Asnaashari , Seyed Abdolrahim Rezaee (*et al*) (2016) Evaluation of Interleukin17and Interleukin 23 expression in patients with active and latent tuberculosis infection. Iran J Basic Med Sci. *Ijbms.***19**:844-850.

Fernandes. P, Ma. y, Gaeddert. M (et al). (2019) Sex and age differences in Mycobacterium tuberculosis infection in Brazil. *Epidemiol Infect*. **146**(12).p 4.

Friedrich. **S.O**, Rachow, A., Saathoff, E., Singh, K., Mangu, C.D., Dawson R, *et al*. (2013). Assessment of the sensitivity and specificity of Xpert MTB/RIF assay as an early sputum biomarker of responseto tuberculosis treatment. *Lancet Respir Med*, **1**:462-70.

Flynn, J. L. and Chan, J. (2001). Immunology of tuberculosis. Annu. Rev. *Immunol*. **19**, 93–129.

Gaffen, S.L. (2009).Structure and signalling in the IL-17 receptor family. Nat Rev *Immunol*, **9**:556–67.

Geldmacher H, Taube C, Kroeger C, Magnussen H, Kirsten DK.(2011).Assessment of lymph node tuberculosis in northern Germany: a clinical review. *Chest*, **121**:1177-1182.

Gopal R, Rangel-Moreno J, Slight S, Lin Y, Nawar HF, Fallert Junecko BA, Reinhart TA, Kolls J, Randall TD, Connell TD, Khader SA.(2013). nterleukin-17-dependent CXCL13 mediates mucosal vaccine-induced immunity against tuberculosis. *Mucosal Immunol*.;**6**:972–84.

Greenwood, D., Barer, M., Slack, R and Irving, W. (2012). Medical microbiology. 18th edition. *Elsevier*. China**.**

Griffiths, K. L., Ahmed, M., Das, S., Gopal, R., Horne, W., Connell, T. D., *et al*. (2016). Targeting dendritic cells to accelerate T-cell activation overcomes a bottleneck in tuberculosis vaccine efficacy. *Nat. Commun*. **7**:13894. doi: 10.1038/ncomms13894. **15**.

Gröschel, M. I., Sayes, F., Simeone, R., Majlessi, L., and Brosch, R. (2016). ESX secretion systems: mycobacterial evolution to counter host immunity. Nat. Rev. *Microbiol*. **14**, 677–691. doi: 10.1038/nrmicro.131.

Hammerich.l (2019).Role of IL-17 and TH17 cells in liver diseases. .*Immunology research,* **9**:41-43.

Hawas, I.L., Hoegh ., M, Ladelund., S, Niesters., H.G. and Hogh, B. (2016). Hepatitis B virus DNA in saliva from children with chronic hepatitis B as potential mode of horizontal transmission. *Jornal of pediatric infection*. **29**(5):65-67.

Hisakata, Y. (2010). Current perspectives on the role of IL-17 in autoimmune disease. *J Inflam Res*, **3**: 33-44

Hoffmann, C., Leis, A., Niederweis, M., Plitzko, J.M. and Engelhardt, H. (2013) Disclosure of the mycobacterial outer membrane: cryo-electron tomography and vitreous sections reveal the lipid bilayer structure. *Proc. Natl. Acad. Sci*. U. S. A **105**, 3963-3967.

Horton-Szer.D., Shiach. C. and Helber. M. (2012). Haematology and Immunology, 4th ed, China, Mosby *Elsevier*, p82.

Ian A., Oumou B. (2015). Pulmonary tuberculosis: diagnosis and treatment. *BMJ*. 2015 May 20; **332(**7551): 1194–1197.

Im JG, Itoh H, Shim YS, et al. Pulmonary tuberculosis: CT findings–early active disease and sequential change with antituberculous therapy. *Radiology*. 1993; 186: 653-660.

Jasenosky, L. D., Scriba, T. J., Hanekom, W. A., and Goldfeld, A. E. (2015). T cells and adaptive immunity to Mycobacterium tuberculosis in humans. *Immunol. Rev*. **264**, 74–87.

Jayne S. Sutherland, Bouke C. de Jong, David J. Jeffries, Ifedayo M. Adetifa, Martin O. C. Ota. (2010) Production of TNF-a, IL-12(p40) and IL-17 Can Discriminate between Active TB Disease and Latent Infection in a West African Cohort. *PLOS one.* **5**(8).p 2.

Kaplan, M.H. (2013) Th9 cells Differentiation and disease. *Immunological Reviews*, 252: 104-115.

Kayser, F. H., Bienz, K. A., Eckert, J and Zinkernagel, R. M. (2005). Medical Microbiology**.** Thieme. New York.

Killick, K. E., Ni Cheallaigh, C., O'Farrelly, C., Hokamp, K., MacHugh, D. E., and Harris, J. (2013). Receptor-mediated recognition of mycobacterial pathogens. Cell. *Microbiol.* **15**, 1484–1495.

Kolls J. (2010) The role of Th17 cytokines in primary mucosal immunity. *Cyt Gro Fact Rev*; **21**: 443-8.

Köser. C.U. (2014), Ellington MJ, Peacock SJ. Whole-genome sequencing to control antimicrobial resistance. *Trends Genet*; **30**:401-7.

Kumar, Vinay. Abbas, Abul, K.Fausto. Nelson and Mitchell. Richard, N. (2007). Robbins Basic Pathology. *Saunder Elsevier*,**8.** p. 516–522.

Lacy. P and Stow. L. (2011). Cytokines release from innate immune cells: associated with diverse membrane trafficking pathways. *Blood journal*, **118**(1):9-18.

Levy. D.E, Marié. I.J and Durbin. J.E. (2011). Induction and function of type I and III interferon in response to viral infection. *Current Opinion in Virology*, **1** (6): 476 86.

Lichen Xu, Guangying Cui, Hongyu Jia, Yunan Zhu (*et al*) Decreased IL-17 during treatment of sputum smear-positive pulmonary

tuberculosis due to increased regulatory T cells and IL-10. *J Transl Med*.**14**:179.

Liu, C. H., Liu, H., and Ge, B. (2017). Innate immunity in tuberculosis: host defense vs pathogen evasion. Cell. Mol. *Immunol*. **14**, 963–975. doi: 10.1038/cmi.2017.88.

Lwasaki . A and Medzhitov. R. (2010). Regulation of adaptive immunity by the innate immune system. *Science*, **327**(5963):291-295.

Ma. K, Zhang. H and Baloch. Z. (2018). Pathogenetic and therapeutic applications of tumor necrosis factor-alpha (TNF-alpha) in major depressive disorder: A systematic review. *International Journal of Molecular Sciences*, **17**:733.

Mohammed A. AL-Saadi1, Ifad Kerim Abd Al-Shibly, Mustafa Jawad AL-Imari. (2018). *Researh Gate*. [Online] Available from**: https://www.researchgate.net/publication/333667047.** [Accessed: 9th June 2021.].

Moreira Ada S , Huf G , Vieira MA , Costa PA , Aguiar F , Marsico AG , et al.(2015). Liquid vs solid culture medium to evaluate proportion and time to change in man- agement of suspects of tuberculosis-a pragmatic randomized trial in secondary and tertiary health care units in Brazil. *PLoS One*, **10**(6):e0127588.

Murray, P. R., Rosenthal, K. S and Pfaller, M. A. (2013) Medical Microbiology. 7th Edition. *Elsevier Saunders*. Philadelphia.

Nathella Pavan Kumar, Kadar Moideen, VaithilingamV.Banurekha, Dina Nair, and Subash Babu (2021). Plasma Proinflammatory Cytokines Are Markers of Disease Severity and Bacterial Burden in Pulmonary Tuberculosis. *IDSA*. **1**(5) p2-3.

Nicas, M. Nazaroff, W. Hubbard, A. (2005). "Toward understanding the risk of secondary airborne infection: emission of respirable pathogens". *J Occup Environ Hyg*, **3**: 143–54.

Owen. J.A, Punt. J and Stranford. S.A. (2013). KUBY immunology, 7th ed ,W.H . Freeman and company: New York, p. **370**.

Palomino, J.C. (2007). Non-conventional and new methods in the diagnosis of tuberculosis: feasibility and applicability in the field. **26**: 1-12.

Pandapat, M.C.(1990). Mishra BM, Dash SP, Kar PK. Peripheral lymph node tuberculosis: a review of 80 cases. *Br J Surg*.; **77**:911-912.

Peter Olsson, Kristin Skogstrand, Anna Nilsson *et al* (2018) Smoking, disease characteristics and serum cytokine levels in tuberculosis patients. *Rheumatology International*. **38**(5):1503-1510.

Phypers, M. Harris, T. Power, C. (2006). CNS tuberculosis: a longitudinal analysis of epidemiological and clinical features. *Int J Tuberc Lung Dis*, **10**:99-103.

Pommerville, Jeffrey C. (2011), Alcamo's fundamentals of microbiology / Jeffrey C. Pommerville. — 9th ed, Jones

Poulsen A.(1957) Some clinical features of tuberculosis [concl]. *Acta Tuberc Pneumol Scand*.; **33**(1-2):37-92.

Ramachandran, G. (2014). Gram-positive and gram-negative bacterial toxins in sepsis: a brief review. *Virulence* **5**, 213–218. doi: 10.4161/viru.27024.

Reche .P. (2019). The structures of cytokine and receptors. *Cytokine*, **116**:161-168.

Robbe-Austerman, S., Bravo. D.M., Harris, B. (2013). Comparison of the MGIT 960, BACTEC 460 TB and solid media for isolation of *Mycobacterium bovis* in United States veterinary specimens. *BMC Vet Res*; **9**: 74.

Seyfizadeh, N. and Babaloo, Z. (2015).Interleukin. *The international jornal of biochemistry and cell biology*, **29**: 59-62.

Sharma. SK. (2005) Mohan A, Sharma A, Mitra DK. Miliary tuberculosis: new insights into an old disease. *Lancet Infect Dis*; **5**:415-430.

Ryan, K. J. and Ray, C. G. (2004). Sherris Medical Microbiology. An Introduction to Infectious Diseases. 4th edition. McGraw-Hill Companies. USA**.**

Shim, T. S. (2014). Diagnosis and treatment of latent tuberculosis infection due to initiation of Anti-TNF therapy. *Tuberc. Respir. Dis*.**76**, 261–268. doi: 10.4046/trd.2014.76.6.261.

Sia. I.G, Wieland. M.L. (2011). Current concepts in the management of tuberculosis. *Mayo Clin Proc*; **86**:348-61.

Siransy, L.K., Yapo-Crezoit, C.C, Maxime, K., Diane, M.K., Goore, S and Kabore, S. *et al*. (2018). Th1 and Th2 cytokines pattern among Sickle cell Disease patients in Cote d'ivoire. *Journal of clinical immunology*, **2**(1):1-4.

Swardfager, W., Lanctôt, K., Rothenburg, L., Wong, A., Cappell, J. and Herrmann. N (2010). A meta-analysis of cytokines in Alzheimer's disease. *Biol Psychiatry*; **68** (10): 930–941.

Talaro, Kathleen P. (2012) Foundations in microbiology, edition 8th, published by McGraw-Hill, United States, New York, pp: 763-772.

Tan, Hongwu, Wang, Shuyun, Zhao and Ludong. (2017). A tumour-promoting role of Th9 cells in hepatocellular carcinoma through CCL20 and STAT3 pathways. *Clinical and Experimental Pharmacology and Physiology*, **44** (2): 213–221.

Thomas J, Barbara Kalsdorf, Deborah-Ann Abrahams (*et al*).(2021) Distinct Specific IL17 and IL22 producing CD4+ T cell subsets contribute to the human anti Mycobacterial immune response.*Immunol*.**180**(3) 1962-1970.

Torrado, E., Robinson, R.T. and Cooper, A.M. (2011). Cellular response to mycobacteria: balancing protection and pathology. *Trends Immunol*; **32**:66–72.

Van Beelen, A.J., Zelinkova, Z., Taanman-Kueter, E.W., Muller, F.J, Hommes, D.W., Zaat, S.A, et al.,(2007) Stimulation of the intracellular bacterial sensor NOD2 programs dendritic cells to promote interleukin-17 production in human memory T cells. *Immunity*; **27**:660–9. [PubMed: 17919942]

Van zylSmit, R.N., Pai, M., Yew, W.W., Leung, C.C., Zumla, A., Bateman, ED. and Dheda, K. (2010). Global lung health: the colliding epidemics of tuberculosis, tobacco smoking, HIV and COPD. *European Respiratory Journal*; **35** (1): 27–33.

Vazquez, M, Dibene, J.C. and Zlotnik, A. (2015). B cells responses and cytokines production are regulated by their immune microenvironment. *Cytokines*, **74**(2):318321.

Veldhoen, M. (2017). Interleukin 17 is a chief orchestrator of immunity. *NATURE IMMUNOLOGY*.**18** (6). P 616.

Wolf, A. J., Desvignes, L., Linas, B., Banaiee, N., Tamura, T., Takatsu, K., et al. (2008). Initiation of the adaptive immune response to Mycobacterium tuberculosis depends on antigen production in the local lymph node, not the lungs. *J. Exp. Med*. **205**, 105–115. doi: 10.1084/jem.20071367.

World Health Organization (WHO, 2004) /IUATLD Global Project on Anti-Tuberculosis Drug Resistance Surveillance. Anti-tuberculosis drug resistance in the world 2004. Available from: http:// whqlibdoc.who.int/publications/2004/9241562854.pdf.

World Health Organization, (WHO). (2007) Tuberculosis. Fact sheet No 104.

World Health Organization, (WHO). (2010) treatment of Tuberculosis: 19 guidelines. 4th edition. *Geneva*, (WHO/HTM/TB/2009. **420**.

World Health Organization (WHO). (2013) Global TB Programme. Automated real-time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and rifampicin resistance: xpert MTB/RIF assay for the diagnosis of pulmonary and extrapulmonary TB in adults.

World health Organization (WHO). (2013): www.who. int/tb/ challenges / mdr WHO 2013.

World Health Organization (WHO). (2014). Global Tuberculosis Control, WHO Report. Geneva: (cited 2014 November 11). Available at: http://apps.who.int/iris/ bitstream/10665/137094/1/9789241564809_eng.pdf.

World Health Organization (WHO). (2017). Global Tuberculosis Report 2017. Geneva: WHO press. Wick, C., Onestingel, E., Demetz, E., Dietrich, H., and Wick, G. (2017). Oral tolerization with mycobacterial heat shock protein 65 reduces chronic experimental atherosclerosis in aged mice. *Gerontology*; **64**, 36–48.

World Health Organization (WHO). (2017). *Global Tuberculosis Report 2015*. (2017): http://www.who.int/tb/publications/global_report/en/

Yon, J. (2015) Diagnosis of Pulmonary Tuberculosis: Recent Advances and Diagnostic Algorithms. *Tuberc Respir Dis (Seoul).* **78**(2): 64–71.

Chen, Y.C., Chin, C.H., Liu, S.F., Wu, C.C., Tsen, C.C., Wan,g Y.H., Chao, T.Y., Lie C.H., Che,n C.J., Wang, C.C. and Lin, M.C. (*et al*). Prognostic values of serum IL-17 in patients with pulmonary tuberculosis found that there was lowering in IL17 level in TB patient before and after treatment. *Dis Markers*, **31**(2): 101-110.

Zambrano –Zaragoza, J.F., Romo-Martínez, E.J., Durán-Avelar Mde, J., García-Magallanes, N., Vibanco-Pérez, N. (2017). Th17 cells in autoimmune and infectious diseases. *International Journal of Inflammation*: 651503.

APPENDIXES I

Questionnaire

Sudan University of Science and Technology

College of Graduate Studies

Assessment of Interleukin 17 (IL17) Level in Sudanese Acute Tuberculosis Patients in Khartoum State

-Date:……

- Patient No……..

-Age: |___| years

-Gender: Male { } Female{ }

-Socioeconomic status: Poor{ } Medium{ } Rich{ }

-Smoking: Yes { } No { }

- Duration of symptoms:……

- Method by which TB diagnosed

ZN stain{ } ELISA{ } Genetic{ }

-Result:

IL-17 level…… pg/ml

APPENDIXES II

Human IL-17A ELISA MAX™ Deluxe Set

tificate of Analysis

uct Name: Human IL-17A ELISA MAX™ Deluxe Set

uct Cat. No: 433914 (5 plates) / 433915 (10 plates) / 433916 (20 plates)

lo: 251472

ation Date: 31-MAR-2020

tents Description	Quantity (5 plates)	Volume (per bottle)	Part No.	Lot No.
nan IL-17A ELISA MAX™ ture Antibody (200X)	1 vial	300 µL	79665	B250911
nan IL-17A ELISA MAX™ ection Antibody (200X)	1 vial	300 µL	79667	B250917
man IL-17A Standard	2 vials	12 ng	79055	B257171
din-HRP (1,000X)	1 vial	60 µL	79004	B254792
strate Solution A	1 bottle	30 mL	78570	B256929
strate Solution B	1 bottle	30 mL	78571	B256930
ating Buffer A (5X)	1 bottle	30 mL	79008	B255601
say Diluent A (5X)	1 bottle	60 mL	78888	B256744
inc™ MaxiSorp™ ELISA ates, Uncoated	5 plates	-	423501	-

rage Conditions

Unopened set: Store set components between 2°C and 8°C. Do not use this set beyond its expiration date.

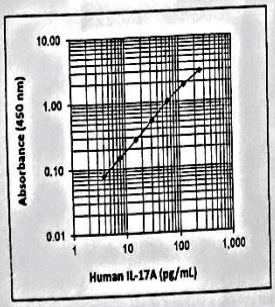
Opened or reconstituted components:

2.1. Reconstituted standard stock solution can be aliquoted into polypropylene vials and stored at -70°C for up to one month. Avoid repeated freeze/thaw cycles.

2.2 Other components: Store opened reagents between 2°C and 8°C and use within one month.

ote: Precipitation of Assay Diluent A (5X) may be observed when stored long term between 2°C and 8°C. The precipitation does not alter the performance of the assay. If heavy precipitation is observed, it can be filtered to clarify the solution.

Lot #:B251472



This standard curve is for demonstrative purposes only. A standard curve must be run with each assay.

This is to certify that the product was manufactured under stringent process controls to ensure lot to lot consistency and complete lot traceability. The product has been tested and meets quality control specifications.

Signature: _____ (Quality Control) Date: 3/22/18

BioLegend is ISO 9001:2008 and ISO 13485:2003 Certified

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BioLegend | 9727 Pacific Heights Blvd | San Diego, CA 92121 U.S.A.
Phone: (858)-768-5800 | Fax: (877)-455-9587 | biolegend.com

ELISA MAX™ Deluxe Set Protocol

Materials to be Provided by the End-User

1. Phosphate-Buffered Saline (PBS): 8.0 g NaCl, 1.16 g Na_2HPO_4, 0.2 g KH_2PO_4, 0.2 g KCl, add deionized water to 1.0 L, pH to 7.4, 0.2 µm filtered.
2. Wash Buffer: BioLegend Cat. No. 421601 is recommended, or PBS + 0.05% Tween-20.
3. Stop Solution: BioLegend Cat. No. 423001 is recommended, or acid solution, e.g. 2N H_2SO_4.
4. Plate Sealers: BioLegend Cat. No. 423601 is recommended.

Reagent Preparation

Reagents Description	Dilute with	Dilution for 1 plate
Coating Buffer A (5X)	Deionized Water	2.4 mL in 9.6 mL DI H_2O
Capture Antibody (200X)	1X Coating Buffer A	60 µL in 12 mL Buffer
Assay Diluent A (5X)	PBS	12 mL in 48 mL PBS
Detection Antibody (200X)	1X Assay Diluent A	60 µL in 12 mL Buffer
Avidin-HRP (1,000X)	1X Assay Diluent A	12 µL in 12 mL Buffer

Standard reconstitution : Reconstitute the lyophilized Human IL-17A Standard by adding 0.2 mL of 1X Assay Diluent A to make the 60 ng/mL standard stock solution. Allow the reconstituted standard to sit at room temperature for 15-20 minutes, then briefly vortex to mix completely.

Prepare 1,000 µL of the top standard at 250 pg/mL by adding 4.2 µL of reconstituted standard stock solution to 995.8 µL 1X Assay Diluent A Perform six two-fold serial dilutions of the 250 pg/mL top standard with 1X Assay Diluent A in separate tubes. 1X Assay Diluent A serves as the zero standard (0

Samples: For cell culture supernatant samples, the end user may need to determine the dilution factors in a preliminary experiment. Serum or plasma samples should be tested initially without any dilution. If dilution is required, samples should be diluted in 1X Assay Diluent A before adding to the wells.

TMB Substrate Solution is a mixture of equal volumes of Substrate Solution A and Substrate Solution B. Mix the two components immediately prior to use. For one plate, mix 5.5 mL Substrate Solution A with 5.5 mL of Substrate Solution B in a clean container (solution should be clear and colorless).

ELISA Procedure Summary

Day 1

Add 100 µL diluted Capture Antibody solution to each well, seal the plate and incubate overnight between 2°C and 8°C.

Day 2

1. Wash plate 4 times*, block the plate by adding 200 µL 1X Assay Diluent A to each well, seal the plate and incubate at room temperature for 1 hour with shaking at approximately 500 rpm (with a 0.3 cm circular orbit). All subsequent incubation with shaking should be performed similarly.
2. Wash plate 4 times*, add 100 µL diluted standards and samples to the appropriate wells.
3. Seal the plate and incubate at room temperature for 2 hours with shaking.
4. Wash plate 4 times* , add 100 µL diluted Detection Antibody solution to each well, seal the plate and incubate at room temperature for 1 hour with shaking.
5. Wash plate 4 times*, add 100 µL diluted Avidin-HRP solution to each well, seal the plate and incubate at room temperature for 30 minutes with shaking.
6. Wash plate 5 times*, soaking for 30 seconds to 1 minute per wash. Add 100 µL of freshly mixed TMB Substrate Solution to each well and incubate in the dark for 30 minutes.
7. Add 100 µL Stop Solution to each well. Read absorbance at 450 nm and 570 nm within 15 minutes. The absorbance at 570 nm can be subtracted from the absorbance at 450 nm.

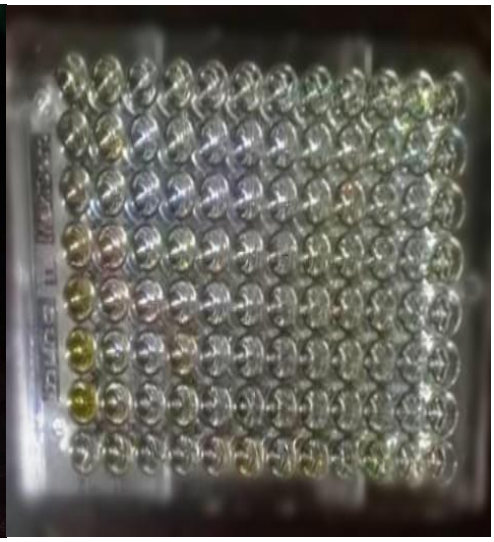
***Plate Washing:** Wash step is crucial to assay precision. Wash the plate with at least 300 µL of Wash Buffer per well and blot any residual buffer by firmly tapping the plate upside down on clean absorbent paper.

For more detailed set information, please refer to the online manual at: **www.biolegend.com/media_assets/pro_detail/datasheets/433914.pdf**

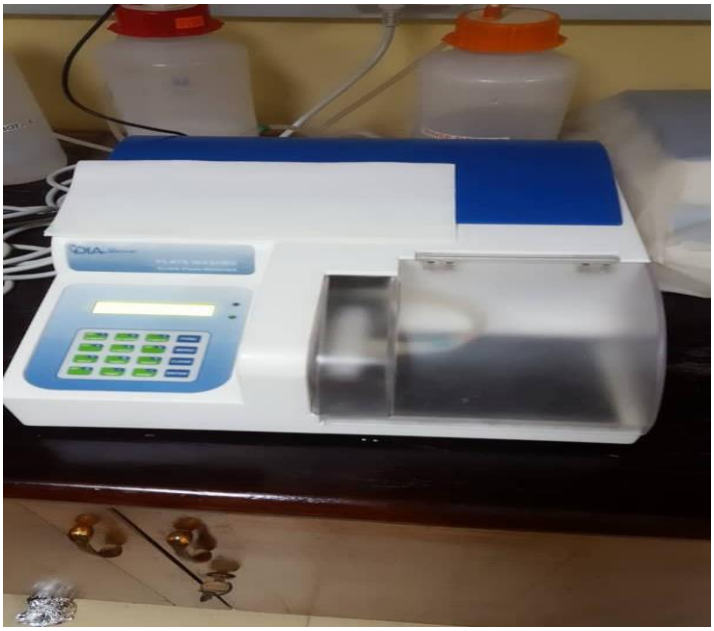
BioLegend®

Part No. 79671_V02

Color plate (1) ELISA kits.



Color plate (2) Micro titer plate.



Color plate (3) ELISA reader