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Determination of Mycobacterium Tuberculosis among Sudanese Diabetic Patients by Cytokines and Compare by Molecular Techniques in Khartoum State- Sudan

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Abstract:-

➢ ***Background***

Pulmonary tuberculosis is the one of the main health problem among Sudanese diabetic patients who are considered as immune-compromised patients.

➢ ***Objectives of this study***

To evaluate the different cytokines among study groups (diabetic and tuberculosis patients, non diabetic tuberculosis patients, diabetic and cardio vascular disease patients and healthy individuals). And detection of Mycobacterium tuberculosis using Real Time PCR.

➢ ***Methodology***

This study was designed as descriptive a prospective, analytical case-control study. This study was enrolled 402 individuals' 40 were diabetic and tuberculosis patients, 41 non diabetic tuberculosis , diabetic CVD were 160 and 161 healthy control. Five ml of blood withdraw in plain vaccutainor after informed consent from individuals whom participate in this study . We measured the cytokines,(IL6 ,IL10 and TNFα) levels from all participant using direct ELISA technique . Then the Real time PCR was done for all participants.

➢ ***Results***

The mean levels of IL6 was recorded in 161 healthy persons 94.80.while recorded 196.27 in 41 tubercles non diabetic patients 210.27. in 40 tuberculous diabetic patients and 297.42 . in 160 patients with CVD, TB and diabetic199.33. The mean levels of IL10 was recorded in 161 healthy persons mean 4.21. while recorded mean 8.84 in 41 tubercles non diabetic patients , mean 8.61 in 40 tuberculosis diabetic patients and mean 7.90 in 160 patients with CVD, TB and diabetic.

The mean levels of TNFα was 81.59 pg/ml in 161 healthy persons. while it was 232.29 pg/ml in 40 tubercles non diabetic patients , 261,78 in 40 tuberculosis diabetic patients and 297.42 in 160 patients with CVD, TB and diabetic. Real time PCR was positive among T.B patients and it was strong in 192 patients group, while it was negative It was a weak positive in 49 patients and negative in 10 cardio vascular disease. It was negative in all healthy control 161 individuals.

➢ ***Conclusion***

TNF was elevated in all groups of tuberculosis patients regardless they were diabetic or not. All T.B patients showed positive result by Real time PCR .63 % from CVD with T.B and DM showed positive result. The estimation of cytokines with combination with real time PCR showed strong correlation.

I. INTRODUCTION

A. Diabetes Mellitus

➢ *Definition*

Diabetes mellitus is not a single disease entity, but rather a group of metabolic disorders sharing the common underlying feature of hyperglycemia (Mitchell *et al.,* 2006).

➢ *Incidence*

Worldwide, over 140 million people suffer from diabetes making this one of the most common diseases. In the Western population the prevalence of DM has been estimated to be 3-5% and the incidence is rapidly growing up and will be more than doubled within 15 years. Type II DM accounts

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for more than 80 % cases of DM and is slow-onset, heterogeneous disorder, resulting from interactions between environmental factors and polygenetic inheritance (Ostenson, 2001).

➢ *TB and Diabetes*

Diabetes is a chronic (long-lasting) disease that affects how the body turns food into energy. Tuberculosis (TB) is a serious health threat, especially for people living with diabetes. Two TB-related conditions exist: latent TB infection and TB disease. People with latent TB infection are not sick because the body is able to fight the bacteria to stop them from growing. People with TB disease are sick and have active TB because the body cannot stop the bacteria from growing. People living with diabetes who are also infected with TB are more likely to develop TB disease and become sick with TB (CDC, 2019).

Someone with untreated latent TB infection and diabetes is more likely to develop TB disease than someone without diabetes. Without proper treatment, diabetes and TB can increase health complications.

- In 2018, 9,029 new TB cases were reported in the United States.
- In 2017, 20% of persons with TB in the United States also had diabetes, as reported to the National TB Surveillance System.
- 30.3 million U.S. adults have diabetes.
- In the last 20 years, the number of adults diagnosed with diabetes has more than tripled.

Untreated latent TB infection can progress to TB disease. TB disease, without treatment, can progress from sickness to death (CDC, 2019).

Fortunately, treatment options are available for people with diabetes who also have either latent TB infection or TB disease. If a person is diagnosed with TB infection, further testing is required to rule out TB disease. People with either latent TB infection or TB disease can be effectively treated.

Before beginning treatment for TB disease or for latent TB infection, TB patients should talk to their doctor about any other medication they are taking, including medicine for diabetes. Some medications used to treat TB might interact with medicine used treat diabetes (CDC, 2019).

➢ *Impact of diabetes mellitus and tuberculosis on each other:*

There is strong evidence that DM increases the risk of TB disease two- to three- fold. This association may be even stronger in the presence of other risk factors. The increased risk occurs in both type 1 DM and type 2 DM. However, type 2 DM accounts for over 95% of patients with DM worldwide and therefore the public health burden of comorbid disease from type 2 DM is much greater (WHO, 2006).

The increase risk of TB has mainly been described for patients with smear-positive and culture-confirmed pulmonary disease, with little published evidence so far associating risk with extra-pulmonary TB (EPTB). There is recent evidence to show that DM is an important risk factor for MDR-TB (WHO, 2006).

➢ *The effects of DM on response to TB treatment?*

DM has several adverse effects on TB treatment.

➢ *Sputum bacteriological conversion:*

There is some evidence that DM prolongs smear and culture positivity at 2-3 months of treatment. Poor glycemic control may be an important factor in this delay.

➢ *Adverse drug reactions:*

DM is probably associated with a higher risk of hepatitis and renal drug toxicity. It is also associated with gastrointestinal and other side effects that may overlap between TB drugs and glucose lowering drugs used by persons with DM.

➢ *TB treatment outcomes:*

DM adversely affects TB treatment outcomes. The reasons are not completely understood but include the immunosuppressive effects of DM itself, drug-drug interactions, adverse effects from medications, suboptimal adherence to medication, reduced bio-availability of the drugs and other unlisted factors. The evidence points to an almost doubling of the risk of death during TB treatment among those with DM with the risk increasing to about five times when adjustments are made for age and other potential confounders.

Cardiovascular deaths could explain an increased rate of deaths within months after starting TB treatment and the much higher death rates among DM patients who smoke.

DM also increases the risk of TB treatment failure and losses to follow-up. It is not clear whether the poorer TB treatment outcomes described among those with worse glycemic control are due to existing DM-related complications or the hyperglycemia itself. The risks of relapse and recurrent TB in those who have completed anti-TB treatment are also higher among those with DM compared with those without: whether this is due to reactivation of disease from the original Mycobacterium tuberculosis or reinfection from another strain of Mycobacterium tuberculosis is not known. Some preliminary evidence suggests that improving glycemic control can lead to better TB treatment outcomes and reduced risk of relapse and recurrence (WHO, 2011).

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Tuberculosis is a widespread, and in many cases fatal, infectious disease caused by various strains of mycobacteria, usually Mycobacterium tuberculosis. Tuberculosis typically attacks the lungs, but can also affect other parts of the body. It is spread through the air when people who have an active TB infection cough, sneeze, or otherwise transmit respiratory fluids through the air. Most infections do not have symptoms, known as latent tuberculosis. About one in ten latent infections eventually progresses to active disease which, if left untreated, kills more than 50% of those so infected (Konstantinos, 2010).

Tuberculosis (TB) has been still a major public health problem in most developing countries and its incidence is rising in many developed countries. TB is a re-emerging infectious disease caused by a bacterium called Mycobacterium tuberculosis (Konstantinos, 2010).

Cytokines are soluble, small proteins that are produced by cells and act in a largely paracrine manner to influence the activity of other cells. Currently, the term "cytokine" describes proteins such as the tumor necrosis factor family, the interleukins, and the chemokine's. Virtually every nucleated cell can produce and respond to cytokines placing these molecules at the centre of most of the body's homeostatic mechanisms . Much of our knowledge of the function of cytokines has been derived from studies wherein homeostasis has been disrupted by infection and the absence of specific cytokines results in a failure to control the disease process. In this context, infection with Mycobacterium tuberculosis (Mtb) has proven to be very informative and has highlighted the role of cytokines in controlling infection without promoting uncontrolled and damaging inflammatory responses. Herein we focus on the key cytokine and chemokine's that have been studied in the context of human TB using experimental medicine as well as Mtb infection of various animal models, including non-human primates, mice and rabbits. Perhaps the most important message of this chapter is that in a complex disease such as tuberculosis (TB) the role of any one cytokine cannot be designated either 'good' or 'bad' but rather that cytokines can elicit both protective and pathologic consequences depending upon context.

Why is TB such an informative probe allowing for detailed investigation of the function of cytokines and chemokine's in immunity? One recent development in our understanding of TB stems from theories of co-evolution between modern humans and Mtb (Racquel *et al.*, 2016).

B. Cytokines:-

➢ *Tumor Necrosis Factor alpha (TNFα)*

TNFα is a cytokine that is released following activation of the immune system. Although it is primarily produced by macrophages, TNFα can also be secreted by lymphocytes, mast cells, endothelial cells, and fibroblasts. Because most cells exhibit responsiveness to TNFα, it is considered a major proinflammatory mediator (Racquel *et al.*, 2016).

➢ *The Interferon's:-*

The interferon family demonstrates the potential for similar cytokines to play protective and pathological roles in TB disease. Based on receptor specificity and sequence homology, the interferons (IFNs) are classified into two types. IFNγ is the only type II interferon and while structurally related to the type I interferon's IFNα and IFNβ, these cytokine use different receptors and have distinct chromosomal locations. Unlike type I IFNs that bind to a common heterodimer receptor comprised of IFNAR1 and IFNAR2 chains, IFNγ binds to the IFNγ receptor (IFNGR) which is comprised of two ligand binding IFNGR1 chains that associate with two signal transducing IFNGR2 chains. In addition while IFNγ is essential for survival following Mtb infection the type I IFNs appear to be largely detrimental to the host during TB and may be co-opted by the bacterium for its own ends (Racquel *et al*., 2016).

➢ *IL-12 Cytokine Family*

The IL-12 family of cytokines belongs to the IL-6 super family and is the only family composed of heterodimer cytokines and this unique feature bestows diverse and pleiotropic functions due to promiscuous chain pairing . The alpha chains of the IL-12 family (p19, p28 and p35) contain four-helix bundle structures and pair with one of two beta chains (either p40 or Epstein-Barr virus induced gene 3 (Ebi3)) (164–166). IL-12 is composed of the subunits p35/p40, IL-23 of p19/p40, IL-27 of p28/Ebi3, and IL-35 of p35/Ebi3 with expression of the distinct subunits being regulated independently (166). In addition, IL-12p40 can also be secreted both as a homodimer (IL-12p80 or IL-12p(40)2) and as a monomer (IL-12p40) . Both macrophages and dendritic cells are major producers of IL-12p40, IL-12, IL-23 and IL-27 . These cytokines are largely associated with the induction and regulation of cytokine expression within antigen-stimulated T cell populations (Racquel *et al.*,2016).

➢ *Chemokine's*

Chemokine's and cytokines are critical for initiating and coordinating the organized and sequential recruitment and activation of cells into Mtb-infected lungs. Correct mononuclear cellular recruitment and localization are essential to ensure control of bacterial growth without the development of diffuse and damaging granulocytic inflammation. An important block to our understanding of TB pathogenesis lies in dissecting the critical aspects of the cytokine/chemokine interplay in light of the conditional role these molecules play throughout infection and disease development (Tables I and II). Much of the data highlighted in this chapter appears at first glance to be contradictory but it is the balance between the cytokines and chemokine's which is critical and the 'goldilocks' (not too much and not

too little) phenomenon is paramount in any discussion of the role of these molecules in TB. Determination of how the key chemokine's/cytokines and their receptors are balanced and how the loss of that balance can promote disease is vital to understanding TB pathogenesis and to identifying novel therapies for effective eradication of this disease (Racquel *et al.*, 2016).

➢ *Detection and Quantification of Cytokines and Other Biomarkers*

Accurate measurement of cytokine concentrations is a powerful and essential approach to the study of inflammation. The enzyme-linked immunosorbent assay (ELISA) is a simple, low-cost analytical tool that provides both the specificity and sensitivity required for the study of cytokines in vitro or in vivo. This communication describes a systematic approach to develop an indirect sandwich ELISA to detect and quantify cytokines, or other biomarkers, with accuracy and precision. Also detailed is the use of sequential ELISA assays to analyze multiple cytokines from samples with limited volumes. Finally, the concept of a multiplex ELISA is discussed with considerations given to cost and additional time required for development (Evan *et al.*, 2012).

Cytokines are a cornerstone of any study that deals with inflammation, whether it is an in vitro cell culture system or an in vivo animal model . The cytokine profile as a whole and the relative abundance of one cytokine, and the endogenous inhibitors, define an inflammatory process that is in motion . Cytokines may be used to describe the nature of the insult, infection, or injury , and may even be used to stage the disease process (4). These studies revolve around the ability to detect, quantify, and discriminate a single cytokine from a multitude of biomolecules present in any given sample. One such method that is routinely used is the indirect sandwich enzyme-linked immunosorbent assay (ELISA) (Evan *et al.*, 2012).

The ELISA exploits the specificity of antibodies (Abs) and uses them to capture and quantify an analyte of interest from a given volume of sample, and it does this with remarkable sensitivity (pg/mL or ~0.5 pM for a 15 kDa protein) (Evan *et al.*, 2012).

C. Polymerase chain reaction (PCR)

➢ *Introduction*

The polymerase chain reaction (PCR) is a technique widely used in molecular biology. It drives its name from one of its key components, a DNA polymerase used to amplify a piece of DNA by in vitro enzymatic replication. As PCR progresses, the DNA thus generated is itself used as a template for replication. This sets in motion a chain reaction in which the DNA template is exponentially amplified. With PCR it is possible to amplify a single or few copies of a piece of DNA across several orders of magnitude, generating millions or more copies of the DNA piece. PCR can be extensively modified to perform a wide array of genetic manipulations. Almost all PCR applications employ a heat-stable DNA polymerase, such as Taq polymerase, an enzyme originally isolated from the bacterium Thermos aquatic us. This DNA polymerase enzymatically assembles a new DNA strand from DNA building blocks, the nucleotides, by using single-stranded DNA as a template and DNA oligonucleotides (also called DNA primers), which are required for initiation of DNA synthesis. The vast majority of PCR methods use thermal cycling, i.e., alternately heating and cooling the PCR sample to a defined series of temperature steps. These thermal cycling steps are necessary to physically separate the strands (at high temperatures) in a DNA double helix (DNA melting) used as template during DNA synthesis (at lower temperatures) by the DNA polymerase to selectively amplify the target DNA. The selectivity of PCR results from the use of primers that are complementary to the DNA region targeted for amplification under specific thermal cycling conditions (Garibyan, 2013).

➢ *Introduction to Real Time PCR*

As the name suggests, real time PCR is a technique used to monitor the progress of a PCR reaction in real time. At the same time, a relatively small amount of PCR product (DNA, cDNA or RNA) can be quantified. Real Time PCR is based on the detection of the fluorescence produced by a reporter molecule which increases, as the reaction proceeds. This occurs due to the accumulation of the PCR product with each cycle of amplification. These fluorescent reporter molecules include dyes that bind to the double-stranded DNA (i.e. SYBR® Green) or sequence specific probes (i.e. Molecular Beacons or TaqMan Probes). Real time PCR facilitates the monitoring of the reaction as it progresses. One can start with minimal amounts of nucleic acid and quantify the end product accurately. Moreover, there is no need for the post PCR processing which saves the resources and the time. These advantages of the fluorescence based real time PCR technique have completely revolutionized the approach to PCR-based quantification of DNA and RNA. Real time PCR assays are now easy to perform, have high sensitivity, more specificity, and provide scope for automation. Real time PCR is also referred to as real time RT PCR which has the additional cycle of reverse transcription that leads to formation of a DNA molecule from a RNA molecule. This is done because RNA is less stable as compared to DNA(El-Dawi et al., 2004)

➢ *Rationale*

Pulmonary tuberculosis is the one of the main health problem among Sudanese diabetic patients who are considered as immune-compromised patients.

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This research used to facilitate the early detection of the disease in order to prevent further serious complications of tuberculosis. By the techniques of real time PCR in combination with cytokines can aid to diagnose of pulmonary tuberculosis which may play an important role in treatment.

II. OBJECTIVES

A. General objective:-

- To evaluate the different cytokines among study groups (diabetic tuberculosis patients, non-diabetic tuberculosis patients, diabetic, and healthy individuals).
- To compare between different cytokines and Real Time PCR in detection of Mycobacterium tuberculosis.

B. Specific objectives:-

- To compare IL-6, IL10 and TNFα between the following groups:
- diabetic and healthy controls.
- diabetic with and without tuberculosis.
- patients with pulmonary tuberculosis and healthy controls.
- diabetic patients with pulmonary tuberculosis and diabetics without.
- diabetic with CVD and those without.
- poorly controlled and good controlled diabetics.

All compaition were done by using ELISA assay.

- To correlate the results of IL-6,IL10 and TNF with duration of T2DB, by using SSPS, ONE way and Two way ANOVA.
- To study the association of IL-6,IL10 and TNF with age and gender.
- To confirms diagnostic tuberculosis by Real Time PCR.
- To compare between different cytokines and Real Time PCR.

III. MATERIALS AND METHODS

A. Study design

This study was designed as a descriptive prospective, analytical case-control study. Cross sectional hospital based study. Which concerned to detect the cytokines (IL-6, IL10 and TNFα) by ELISA and detection of Pulmonary Tuberculosis (AAFB mycobacterium tuberculosis) by Real time PCR. The tests of ELISA and PCR were performed for all study groups.

B. Study area

This study was conducted in different hospitals (Alribat Teaching hospital where located in the centre of Khartoum and served the governmental persons and their relatives. Also some patients were enrolled in the study from Jaber Abu Elizz center for diabetic patients. Lastly Fadial private hospital was involved. During the period from May 2015 to Nov 2015.

C. Study duration

This study was started in September 2014 and ended in 2020

D. Study population:-

- *Sample size calculation*

This study was enrolled 402 individuals' 40 were diabetic and tuberculosis patients, 41 tuberculosis and non-diabetic ,tuberculosis diabetic and CVD were 160 and 161 healthy control. This number of patients was calculated according to the equation of sample size determination which expresses as follows.

- *Sampling*

Five ml of blood withdraw in plain vacationer after informed consent from individuals to participate in this study. The selected diabetic and non-diabetic subjects were aged between 35-70 years.

E. Data collection

A coded enrollment number was given for each enrolled patient. The data were collected by using a direct interviewing questionnaire. Medical information was collected from the patients with help of the physician. The questionnaire was used to collect data regarding name, age, gender, residence, duration of diabetes, presence of CVD complications, history of systemic diseases and medication.

IV. RESULTS

- *IL6 , IL10 and TNFα distribution of study groups:-*

In the group statistics regarding both gender male and female crossing the different agents

- TNF in female number 191 the mean = 199.91, std deviation =180.716, std error = 13.076, in male number 209 the mean = 202.53, std deviation = 180.400, std error = 12.479. the P. value in both genders was = 0.885.
- IL6 in female number 191 the mean = 165.81, std deviation = 125.108, std error = 9.053, in male number 209 the mean = 151.99, std deviation = 116.699, std error = 8.072. The P. value in both genders was = 0.254.
- IL10 in female number 191 the mean = 6.52, std deviation = 4.684, std error = 0.339, in male number 209 the mean = 6.64, std deviation = 6.321, std error = 0.437. The P. value in both genders was = 0.833.
- HBA1C in female number 191 the mean = 7.25, std deviation = 2.632, std error = 0.190, in male number 209 the mean = 7.19, std deviation = 2.582, std error = 0.179. The P. value in both genders was = 0.833.
- This is one-way anova test: - P. value = 0.000 which is a significant.

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- *Real time PCR result:-*

It was positive among T.B strong 192 P.T ,while it was negative in healthy control 161 patients ,weak positive 49 patients (figure -1)

TNF as dependent variable and PCR different results: -

- When PCR was positive in TB non-diabetic the mean = 389.87, std. deviation = 78.539, the number of populations was 15.
- When PCR was positive in tuberculosis + diabetic the mean = 386.71, std. deviation = 83.851, the number of populations was 17.
- When PCR was positive in CVD + TB + Diabetic the mean = 297.42, Std. Deviation = 198.423, the number of populations was 160.
- Totally in TNF as dependent variable and PCR when positive the mean = 312.55, Std. Deviation = 186.995, the number of populations was 192.
- When PCR was negative in healthy the mean = 81.59, Std. Deviation = 91.466, the number of populations was 161.
- Totally in TNF as dependent variable and PCR when negative the mean = 81.59,
- Std. Deviation = 91.466, the number of populations was 161.
- When PCR was weak positive in TB non-Diabetic, the mean = 141.38, Std. Deviation = 63.397, the number of populations was 26.
- When PCR was weak positive in TB + Diabetic, the mean =169.43, Std. Deviation = 59.888, the number of populations was 23.
- Totally in TNF as dependent variable and PCR when weak positive the mean =154.55, St. Deviation =62.747, the number of populations was 49.
- Total results of TNF as dependent variable and PCR different results in healthy the mean = 81.59, std deviation = 91.466, the number of populations was 161.
- Total results of TNF as dependent variable and PCR different results in TB non-Diabetic the mean = 232.29, std deviation = 139.118, the number of populations was 41.
- Total results of TNF as dependent variable and PCR different results in TB + Diabetic the mean = 261.78, std deviation = 129.382, the number of populations was 40.
- Total results of TNF as dependent variable and PCR different results in CVD +TB + Diabetic the mean =297.42, std deviation =198.423, the number of populations was160.
- Total results of TNF as dependent variable and PCR different results the mean =200.79, std deviation = 180.029, the number of populations was 402.

- *IL6 as dependent variable and PCR different results: -*

- When PCR was positive in TB non-diabetic the mean = 263.20, std.deviation = 91.793, the number of population was 15.
- When PCR was positive in tuberculosis + diabetes the mean = 266.00, std.deviation = 79.428, the number of population was 17.
- When PCR was positive in CVD+TB+Diabetes the mean = 199.33, std.deviation = 133.084, the number of population was 160.
- Totally in IL6 as dependent variable and PCR when positive the mean = 210.22, std.deviation = 128.401, the number of population was 192.
- When PCR was negative in healthy the mean = 94.80, std.deviation = 84.786, the number of population was 161.
- Totally in IL6 as dependent variable and PCR when negative the mean = 94.80, std.deviation = 84.786, the number of population was 161.
- When PCR was weak positive in TB non-diabetic the mean = 157.65, std.deviation = 101.232, the number of population was 26.
- When PCR was weak positive in tuberculosis + diabetes the mean = 168.83, std.deviation = 73.552, the number of population was 23.
- Totally in IL6 as dependent variable and PCR when weak positive the mean = 162.90, std.deviation = 88.593, the number of population was 49.
- Total results of IL6 as dependent variable and PCR different results in healthy the mean = 94.80, std.deviation = 84.786, the number of population was 161.
- Total results of IL6 as dependent variable and PCR different results in TB non-diabetic the mean = 196.27, std.deviation = 109.559, the number of population was 41.
- Total results of IL6 as dependent variable and PCR different results in tuberculosis + diabetes the mean = 210.13, std.deviation = 89.481, the number of population was 40.
- Total results of IL6 as dependent variable and PCR different results in CVD+TB+Diabetes the mean = 199.33, std.deviation = 133.084, the number of population was 160.
- Total results of IL6 as dependent variable and PCR different results the mean = 158.23, St. Deviation = 120.719, the number of population was 402. Table (4.8.2)
- When PCR was positive in TB non-diabetic the mean = 263.20, std.deviation = 91.793, the number of population was 15.

Descriptive Statistics				
Dependent Variable: IL6				
PCR	study population	Mean	Std. Deviation	N
Positive	TB non diabetic	263.20	91.793	15
	tuberculosis + diabetes	266.00	79.428	17
	CVD+ TB+ Diabstus	199.33	133.084	160
	Total	210.22	128.401	192
Negative	Healthy	94.80	84.786	161
	Total	94.80	84.786	161
weak positive	TB non diabetic	157.65	101.232	26
	tuberculosis + diabetes	168.83	73.552	23
	Total	162.90	88.593	49
Total	Healthy	94.80	84.786	161
	TB non diabetic	196.27	109.559	41
	tuberculosis + diabetes	210.13	89.481	40
	CVD+ TB+ Diabstus	199.33	133.084	160
	Total	158.23	120.719	402

Table 1:-IL6 as dependent variable and PCR different results

➢ *IL10 as dependent variable and PCR different results: -*

- When PCR was positive in TB non-Diabetic the mean = 5.85, std.deviation = 2.289, the number of population was 15.
- When PCR was positive in tuberculosis + Diabetes the mean = 8.05, std.deviation = 4.794, the number of population was 17.
- When PCR was positive in CVD+TB+Diabetes the mean = 7.90, std.deviation = 5.085, the number of population was 160.
- Totally in IL10 as dependent variable and PCR when positive the mean = 7.75, std.deviation = 4.913, the number of population was 192.
- When PCR was negative in healthy the mean = 4.21, std.deviation = 5.713, the number of population was 161.
- Totally in IL10 as dependent variable and PCR when positive the mean = 4.21, std.deviation = 5.713, the number of population was 161.
- When PCR was weak positive in TB non-Diabetic, the mean = 10.56, Std. Deviation = 4.902, the number of populations was 26.
- When PCR was weak positive in TB + Diabetic, the mean = 9.02, Std. Deviation =3.887, the number of populations was 23.
- Totally in IL10 as dependent variable and PCR when weak positive the mean =9.84, St. Deviation = 4.477, the number of populations was 49.
- Total results of IL10 as dependent variable and PCR different results in healthy the mean = 4.21, std deviation =5.705, the number of populations was 161.
- Total results of IL10 as dependent variable and PCR different results in TB non-Diabetic the mean = 8.84, std deviation =4.705, the number of populations was 41.
- Total results of IL10 as dependent variable and PCR different results in TB + Diabetic the mean =8.61, std deviation =4.265, the number of populations was 40.
- Total results of IL10 as dependent variable and PCR different results in CVD +TB + Diabetic the mean = 7.90, std deviation =5.085, the number of populations was160.
- Total results of IL10 as dependent variable and PCR different results the mean =6.59, std deviation =5.579, the number of populations was 402.

This is two-way anova test result.

Descriptive Statistics				
Dependent Variable: IL10				
PCR	study population	Mean Pg/ml	Std. Deviation	N
Positive	TB non diabetic	5.85	2.289	15
	tuberculosis + diabetes	8.05	4.794	17
	CVD+ TB+ Diabstus	7.90	5.085	160
	Total	7.75	4.913	192
Negative	Healthy	4.21	5.713	161
	Total	4.21	5.713	161
weak positive	TB non diabetic	10.56	4.902	26
	tuberculosis + diabetes	9.02	3.887	23
	Total	9.84	4.477	49
Total	Healthy	4.21	5.713	161
	TB non diabetic	8.84	4.705	41
	tuberculosis + diabetes	8.61	4.265	40
	CVD+ TB+ Diabstus	7.90	5.085	160
	Total	6.59	5.579	402

Table 2:- IL10 as dependent variable and PCR different results

- *TNF as dependent variable and PCR different results: -*
  When PCR was positive in TB non-diabetic the mean = 389.87, std. deviation = 78.539, the number of populations was 15.

Descriptive Statistics				
Dependent Variable: TNF				
PCR	study population	Mean Pg/ml	Std. Deviation	N
Positive	TB non diabetic	389.87	78.539	15
	tuberculosis + diabetes	386.71	83.851	17
	CVD+ TB+ Diabstus	297.42	198.423	160
	Total	312.55	186.995	192
Negative	Healthy	81.59	91.466	161
	Total	81.59	91.466	161
weak positive	TB non diabetic	141.38	63.397	26
	tuberculosis + diabetes	169.43	59.888	23
	Total	154.55	62.747	49
Total	Healthy	81.59	91.466	161
	TB non diabetic	232.29	139.118	41
	tuberculosis + diabetes	261.78	129.382	40
	CVD+ TB+ Diabstus	297.42	198.423	160
	Total	200.79	180.029	402

Table 3:- TNF as dependent variable and PCR different results

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
IL6	Healthy	161	94.80	84.786	6.682	81.60	107.99	5	457
	TB non diabetic	41	196.27	109.559	17.110	161.69	230.85	24	378
	tuberculosis + diabetes	40	210.13	89.481	14.148	181.51	238.74	39	388
	CVD+ TB+ Diabstus	160	199.33	133.084	10.521	178.55	220.11	9	476
	Total	402	158.23	120.719	6.021	146.39	170.06	5	476
IL10	Healthy	161	4.21	5.713	.450	3.32	5.10	1	63
	TB non diabetic	41	8.84	4.705	.735	7.35	10.32	3	19
	tuberculosis + diabetes	40	8.61	4.265	.674	7.24	9.97	3	20
	CVD+ TB+ Diabstus	160	7.90	5.085	.402	7.10	8.69	1	25
	Total	402	6.59	5.579	.278	6.04	7.13	1	63
	Healthy	161	5.00	.000	.000	5.00	5.00	5	5
	TB non diabetic	41	5.00	.000	.000	5.00	5.00	5	5
	tuberculosis + diabetes	40	9.80	1.896	.300	9.20	10.41	7	15
	CVD+ TB+ Diabstus	160	9.34	1.928	.152	9.04	9.64	6	15
	Total	402	7.21	2.602	.130	6.96	7.47	5	15

Table 4:- Descriptives all groups

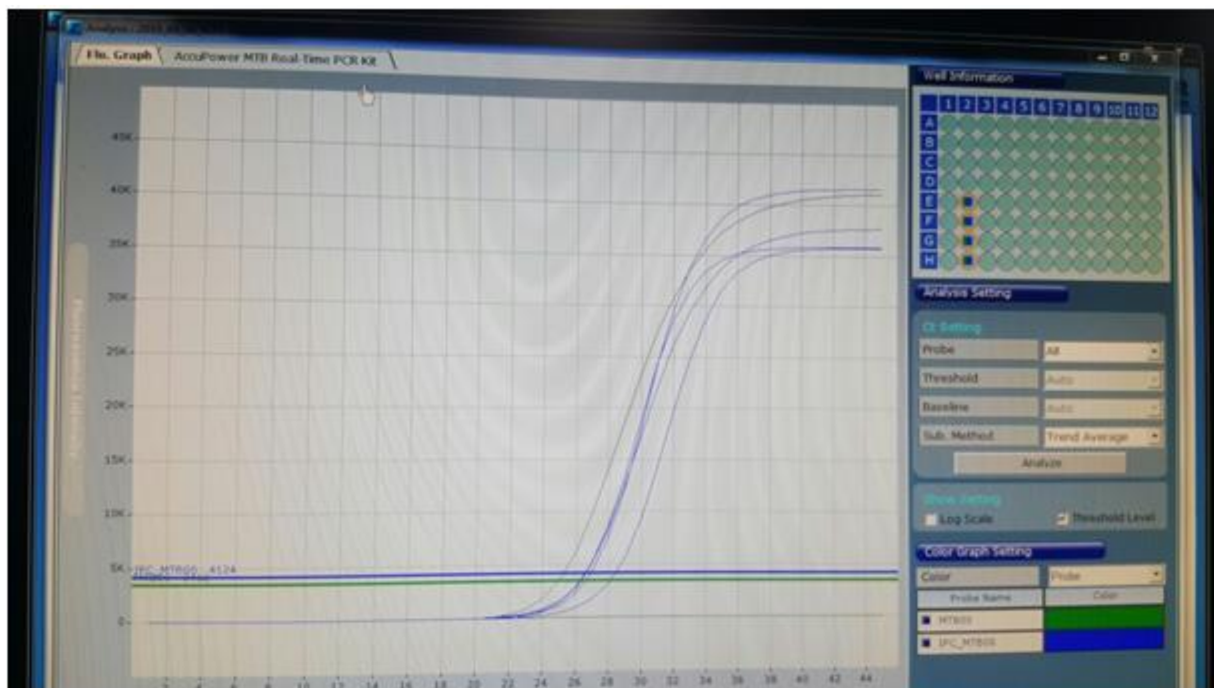

Fig 1:- Real time – PCR

This image showed the positive results above the blue line. The lines in green that rose to the top are strong positive.

V. DISCUSSION

The current study confirms that high levels of some cytokines namely IL6, IL10 and TNFα constitute a diagnostic biomarker for the latent tuberculosis in poorly controlled diabetic. On the other hands, it confirms and extends the findings of previous studies conducted outside Sudan (Pakistan, India, United State, Iran and Sweden).

➢ *IL-6:-*

The finding of this study that increased levels of IL6 in poorly controlled diabetics is associated with positive tuberculosis in these patients which is in agreement with the finding of (Kiran *et al*,. 2016 ; Lavanya *et al*,.2015 ; Blanca *et al* ,. 2008 ; Prati and Amit Goyal ,2013 ; Ponnana et al ., 2017 ; Fakhri et al., 2019 ; Emilie *et al* ,.2019 ;). in the studies conducted in Pakistan, India, United state ,Iran Sweden respectively.

Kiran's and other (2016) study, found that the majority of the study population who had diabetic and tuberculosis have had high levels of IL-6, (study tested of 10 patients with diabetes and 11 healthy endemic controls both with and without MTB infection .The majority of patients tested showed high levels of IL6. Denoting that they are infected of mycobacteria tuberculosis .And this agreement with our study.

The India (Lavanya *et al*,.2015) study tested 150 active pulmonary tuberculosis patients 190 household contacts , and 150 healthy controls. The majority of patients tested showed high levels of IL6. Denoting that they are infected of mycobacteria tuberculosis.

Another study in southern Texas (Blanca *et al* ,. 2008) testet sixty-eight patients with tuberculosis . cytokine responses were significantly higher in patients with tuberculosis who had diabetes than in nondiabetic control subjects. The effect was consistently and significantly more marked in diabetic patients with chronic hyperglycemia

The majority of patients tested showed high levels of IL6.

Another study in India newDelhi (Prati and Amit Goyal ,2013). The majority of patients tested showed high levels of IL6.

A Similar study in Hyderabad in south India (Ponnana *et al* ., 2017) stated cytokine genes associated with disease in the household contact (HHCs) highlight their risk of tendency towards the disease.

In a previous study in Iran (Fakhri et al., 2019) a total of 105 smear-positive, including 78 newly diagnosed (ND) and 27 under treatment (UT) patients with pulmonary TB and 111 age- and sex-matched healthy subjects were recruited. ELISA cytokine assay was used to determine the plasma levels of IL-6 plasma level was higher in the newly diagnosis (ND) patients than healthy subjects and the UT patients.

Another study in Stockholm Sweden (Emilie *et al*,.2019) the study tested of active TB was detected in 54/161 (34%) of the study patients examined for suspected TB. The patients were divided into groups according to clinical and microbiological data; PTB 25, extra pulmonary tuberculosis (EPTB) 18, Clinical TB 11, Previous TB 22, latent tuberculosis infection (LTBI) 62, TB negative 11 and Other causes 12. The majority of patients tested showed high levels of IL6.

In china (pane *et al* .,2019). A study included a total of 227 subjects consisting of active tuberculosis (ATB) patients, latent tuberculosis infection (LTBI) individuals, and healthy controls (HC). The majority of patients tested showed low levels of IL6.This study is not in agreement.

➢ *IL-10:-*

Levels of IL-10 in the current study indicate IL10 is possible diagnostic biomarker for the latent tuberculosis in poorly controlled diabetic .On the other hands it confirms the finding of previous studies conducted outside Sudan (India, United states, Sweden China , and Ghana).

The finding in that increased levels of IL-10 in poorly controlled diabetics is associate with positive tuberculosis in these patients. This results were in agreement with the finding of (Lavanya *et al.,* 2015). Which was conducted in India.

Another study in southern Texas (Blanca *et al.*, 2008) tested sixty-eight patients with tuberculosis. cytokine responses were significantly higher in patients with tuberculosis who had diabetes than in nondiabetic control subjects. The effect was consistently and significantly more marked in diabetic patients with chronic hyperglycemia.The majority of patients tested showed high levels of IL10. Another study in India newDelhi (**Prati** and **Amit, 2013**).Stated the majority of patients tested showed high levels of IL10.

Another study in Stockholm Sweden (Emilie *et al.*, 2019).noted that active TB was detected in 54/161 (34%) of the study patients examined for suspected TB. The patients were divided into groups according to clinical and microbiological data; PTB 25, EPTB 18, Clinical TB 11, Previous TB 22, LTBI 62, TB negative 11 and Other causes 12. The majority of patients tested showed high levels of IL10.

Another study in china (Bai *et al.*, 2014) recruited 364 patients with type 2 diabetes mellitus and 677 healthy controls. Patients carrying the -1082 GG genotype had a significantly increased risk of type 2 diabetes mellitus. The majority of patients tested showed high levels of IL10.

In India (Nathella, 2015) a study tested a group of 88 individuals with PTB, 44 of whom had DM (PTB-DM) and 44 of whom had no diabetes (PTB). They also studied another 88 individuals with LTB, 44 of whom had DM (LTB-DM) and 44 of whom had no diabetes (LTB).plasma Levels of IL-10 were significantly higher in PTB-DM compared to PTB individuals.

Another study in India (Ramesh, 2015) tested 150 cases presenting with diabetic neuropathy and 160 cases of age and sex matched healthy controls were included in the study. The results revealed that the chi- square test for heterogeneity for IL-10 system was found to be significant.

In Ghana (Anthony ,2018) study tasted of eighty-three pulmonary TB cases were used in the study. These included 49 MDR-TB and 34 DS-TB patients. This plasma level of IL-10 was relatively higher than that of the pro-inflammatory cytokines. The India (Lavanya *et al,.*2015) study tested 150 active pulmonary tuberculosis patients, 190 household contacts and 150 healthy controls. The IL-10 levels were low in APTB compared to HHC and HCs and no significant.

In china (pane *et al.*, 2019) a study included a total of 227 subjects consisting of active tuberculosis (ATB) patients, latent tuberculosis infection (LTBI) individuals and healthy controls (HC). The majority of patients tested showed low levels of IL6.this study no agreement.

➢ *TNF alpha :-*

Levels of TNFα in the current study indicates TNFα is a possible diagnostic biomarker for the latent tuberculosis in poorly controlled diabetic patients. On the other hands it confirms the finding of previous studies conducted outside Sudan (Pakistan ,India, United states, Sweden, China , and Ghana). The finding is that increased levels of TNFα in poorly controlled diabetics is associated with positive tuberculosis in these patients.

This results was in agreement with finding of similar (Kiran *et al.*, 2016 ;Lavanya *et al,.*2015). in that studies conducted in Pakistan and India respectively. The Pakistan (Kiran *et al.*, 2016) study tested of 10 patients with diabetes and 11 healthy endemic controls both with and without MTB infection .The majority of patients tested showed high levels of TNFα. Denoting That they are infected of mycobacteria tuberculosis.

The India (Lavanya *et al.,* 2015) study tested 150 active pulmonary tuberculosis patients,190 household contacts and 150 healthy controls The median values of TNF-α cytokine were significantly high among APTP. Another study in southern Texas (Blanca *et al.*, 2008) tested sixty-eight patients with tuberculosis . cytokines responses were significantly higher in patients with tuberculosis who had diabetes than in nondiabetic control subjects. The effect was

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consistently and significantly more marked in diabetic patients with chronic hyperglycemia. The majority of patients tested showed high levels of TNFα this results proved our study.

Another study in Stockholm Sweden (Emilie *et al* ,.2019) noted that active TB was detected in 54/161 (34%) of the study patients examined for suspected TB. The patients were divided into groups according to clinical and microbiological data; PTB 25, EPTB 18, Clinical TB 11, Previous TB 22, LTBI 62, TB negative 11 and Other causes 12). The majority of patients tested showed high levels of TNFα. And this is another agreement with our study.

In china (pane *et al.*, 2018) study included a total of 227 subjects consisting of active tuberculosis (ATB) patients, latent tuberculosis infection (LTBI) individuals and healthy controls (HC). The majority of patients tested showed high levels of TNFα.this study was in agreement.

In Ghana (Anthony ,2018) eighty-three pulmonary TB cases were used in the study. These included forty-nine MDR-TB and thirty-four DS-TB patients. This plasma level of TNFα was relatively higher than that of the pro-inflammatory cytokines. In India (Ramesh ,2015) study tested 150 cases presenting diabetic neuropathy and 160 cases of age and sex matched healthy controls were included in the study. The results revealed that the chi- square test for heterogeneity for TNFα not significantly associated with development of Diabetic Neuropathy.

VI. CONCLUSION

- Serum levels of IL6 ,IL10 and TNFα increased in patients with no signs and symptoms of tuberculosis (latent phase).
- The cut off values 68pg/ml ; 5.85pg/ml; 297.42pg/ml for IL6; IL10 and TNFα respectively are diagnostic for latent tuberculosis.
- There was no relationship between Gender and IL6, IL10 and TNFα levels.

RECOMMENDATION

- All patients with complicated diabetic should be test for the latent tuberculosis in poorly controlled diabetic mellitus type Ⅱ,
- Implementation of cytokines of IL6 , IL10 and TNFα as screening and complementary test with real time PCR for detection of latent tuberculosis in poorly controlled diabetic .

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