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Cytokines levels as biomarkers for the diagnosis of latent tuberculosis among Sudanese patients with poorly controlled diabetic mellitus type Ⅱ, SUDAN 2019

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

Objective of the study:- To evaluate cytokines IL6,IL10 and TNFα among study groups (diabetic and tuberculosis patients , non diabetic tuberculosis patients, diabetic and cardio vascular disease patients and control group of healthy individuals).

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. Estimation of the cytokines(IL6,IL10 and TNFα) levels from all participate were done by using direct ELISA technique.

Results:- The mean levels of IL6 was recorded in 161 healthy persons 94.80pg/ml. while recorded 196.27pg/ml in 41 tuberculosis non diabetic patients, 210.27pg/ml in 40 tuberculosis diabetic patients and 7.90pg/ml in 160 patients tuberculosis diabetic with CVD 199.33pg/ml.

The mean levels of IL10 was recorded in 161 healthy persons mean 4.21pg/ml, while mean 8.84pg/ml in 41 TB non diabetic, mean 8.61pg/ml in 40 TB diabetic patients and 7.90pg/ml in 160 TB diabetic with CVD patients.

The mean levels of TNFα was 81.59pg/ml in 161 healthy persons, while it was 232.29pg/ml in 40 TB non diabetic patients, 261.78pg/ml in 40 TB diabetic patients and 297.42pg/ml in 160 TB diabetic with CVD.

Conclusion:- Serum levels of IL6,IL10 and TNF alpha increased in patients with no signs and symptoms of tuberculosis (latent phase). The cut off values 68pg/ml ;5.58pg/ml;279.42pg/ml for IL6, IL10 and TNF alpha respectively are diagnostic for latent tuberculosis

Where was no relationship between gender and IL6, IL10 and TNF alpha levels.

Recommendation:- All patients with complicated diabetic should be test for the latent tuberculosis in poorly controlled diabetic mellitus type Ⅱ.

Keywords: **Pulmonary tuberculosis, Sudanese diabetic patients, healthy control.**

1. INTRODUCTION

The burden of tuberculosis (TB) especially in developing countries continues to remain high despite efforts to improve preventive strategies (Emest *et al*., 2017). Known traditional risk factors for TB include poverty, malnutrition, overcrowding, and HIV/AIDS; however, diabetes,

which causes immunosuppression, is increasingly being recognized as an independent risk factor for tuberculosis, and the two often coexist and impact each other. Diabetes may also lead to severe disease, reactivation of dormant tuberculosis foci, and poor treatment outcomes(Emest *et al*., 2017).

Tuberculosis as a disease entity on the other hand and some commonly used antituberculous medications separately may cause impaired glucose tolerance. This review seeks to highlight the impact of comorbid TB and diabetic on each other(Emest *et al*., 2017). it is our hope that this review will increase the awareness of clinicians and managers of TB and diabetes program of the effect of the interaction between these two disease entites and how to better screen and manage patintes (Emest *et al*., 2017).

Clinical presentation of TB in diabetic patients:-

Active TB disease may present atypically with altered symptoms and signs in those with DM.

Among persons with DM , TB may progress faster , present with more chest and systemic symptoms and more frequent and higher grade smear and culture positivity. Severity at presentation seems to be the degree of uncontrolled hyperglycemia(WHO, 2006).

The effects of DM on chest radiograph findings are inconsistent. Some studies have described more frequent isolated lower lung field lesions and an increase in consolidation and cavities in PTB in patients with DM, sometimes mimicking the pattern of radiographic TB seen in people living with HIV. No studies have yet reported on whether there are differences in presentation in patients with EPTB (WHO, 2006).

Cytokines assay:-

Introduction:-

Cytokines are soluble, small proteins that are produce by cells and act in largely paracrine manner to influence the activity of other cells. Currently, the term “cytokines” describes proteins such as the tumor necrosis factor family, the interleukin, and chemokine’s. virtually every nucleated cell can produce and respond to cytokines placing these molecules at the center of most the body’s homeostatic mechanisms. Much of our knowledge of the function of cytokines has been derived from studies where in homeostasis has been disrupted by infection and the absence of specific cytokines results in a failure to control the disease process(Racquel *et al*.,2016). 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 chemokine’s that have been studies 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(Racquel *et al*., 2016).

Why is TB such as 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).

Cytokines:-

Tumor Necrosis Factor alpha(TNFα):-

TNFα is a cytokines that is released following activation of the immune system. Although it is primarily produced by macrophages, TNF alpha 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 interferon’s(IFNs) are classified in to two types(Racquel *et al*., 2016). IFNγ is the only type Ⅱ interferon and while structurally related to the type1 interferon’s

IFNα and IFNβ, these cytokines use different receptors and have distinct chromosome locations. Unlike type1 IFNs that bind to common heterodimer receptor comprised of IFNR1 and IFNR2 chains(Racquel *et al*.,2016). IFNγ binds to the IFNγ receptor (IFNGR) which is comprised of two ligand is essential for survival following Mtb infection the type 1 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 heterodimeric cytokines and this unique feature bestows diverse and pleiotropic functions due to promiscuous chain pairing (Racquel *et al*.,2016). 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);(Racquel *et al*.,2016). 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 co-ordinating the organized and sequential recruitment and activation of cell 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 cytokines/chemokine interplay in light of the conditional role these molecules play throughout infection and disease development. Much of the data highlighted in this chapter appears at first glance to be contradictory but it is the balance between the cytokines and chemokines 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 chemokines/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 immune-sorbent 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 describe 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 assay 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(Chiswick *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. 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) (chiswick *et al*., 2012).

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

Objective:-

General objective:-

To evaluate the different cytokines among study groups(diabetic tuberculosis patients, non-diabetic tuberculosis patients, diabetic tuberculosis with CVD and healthy individuals).

Specific objective:-

1- To compare IL6,IL10 and TNFα between the following groups:-

a/ diabetic and healthy controls.

b/ diabetic with and without tuberculosis.

c/ patients with pulmonary and healthy controls.

d/ diabetic patients with pulmonary tuberculosis and diabetic without.

e/diabetic with CVD and those without .

f/ poorly controlled and good controlled diabetics.

All compaition were done by using ELISA assay.

2- To correlate the results of IL6,IL10 and TNFα with duration of T2DM,by using SSPS, ONE way and TWO way ANOVA.

3- To study the association of IL6,IL10 and TNFα with age and gender.

2. MATERIALS AND METHODS

This study was designed as a descriptive prospective, analytical case-control study .cross sectional hospital based study , which concerned to detect the cytokines(IL6,IL10 and TNFα) by ELISA and detection of pulmonary tuberculosis (AAFB) mycobacterium tuberculosis , the tests of ELISA were performed for all study groups.

This study was conducted in different hospitals (alribat hospital university) were 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 2019.

This study was started in September 2014 and ended in 2020.

This study was enrolled 402 individuals 40 were diabetic and tuberculosis patients, 41 tuberculosis and non-diabetic, tuberculosis diabetic with cardio vascular disease were 160 and healthy control.

Five ml 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.

Cytokines Assay:-

IL6,IL10 and TNFα was measured by ELISA

BioLegend's ELISA MAX™ Deluxe Set is a sandwich Enzyme-Linked Immunosorbent Assay (ELISA). A human IL-6 specific monoclonal antibody is first coated on a 96-well plate. Standards and samples are added to the wells, and IL-6 binds to the immobilized capture antibody. Next, a biotinylated anti-human IL-6 detection antibody is added, producing an antibody-antigen-antibody "sandwich". Avidin-horseradish peroxidase is subsequently added, followed by TMB Substrate Solution, producing a blue color in proportion to the concentration of IL-6 present in the sample. Finally, the Stop Solution changes the reaction color from blue to yellow, and the microwell absorbance is read at 450 nm with a microplate reader.

HbA1c:-

HbA1c was measured using chromatography methods.

The diabetic patients was divided into two groups according to the HbA1C levels into well controlled group (HbA1c<6.5%) and insufficiently controlled group (HbA1c>6.5%).

Data analysis:-

The statistical analysis of the results was performed by using the statistical package for social sciences (SPSS) version 15.0 (SPSS Inc, Chicago, IL, USA) for windows version 10 using T-test for testing difference significance and Pearson correlation test (r value as the coefficient). A P value <0.05 was considered statistically significant.

The results were formulated into figures and tables using the Microsoft Excel computer program.

3. RESULTS

The study was run on 402 patients, 161 of them were healthy as control group. 41 were TB non diabetic, 40 tuberculous and diabetic patients. The group of 160 categorized as CVD, TB and Diabetes. The different laboratory investigation were performed to diagnosed of this cases, including detection of cytokines such as IL-6, IL10 .TNFα, HBA1C, R.TPCR.

The distribution of frequency on study groups:

Show the distribution of study groups which categorized 160 as healthy individuals, TB non diabetic patients 41, TB diabetic 40 and CVD+ TB+ DM 160.

Table 1: The distribution of frequency on study groups

study population		Frequency	Percent
	Healthy	161	40.0
	TB non diabetic	41	10.2
	tuberculosis + diabetes	40	10.0
	CVD+ TB+ Diabstus	160	39.8
	Total	402	100.0

The distribution of study groups according gender:-

Table 2: The distribution of study groups according gender

Sex		Frequency	Percent
	F	193	47.8
	M	209	52.2
	Total	402	100.0

The mean level of cytokins IL6 in different groups:-

IL6 regarding the healthy group they represent 40% of total patients, the mean = 94.80, Std. Deviation = 84.786 , and std errors was = 6.682.

IL6 regarding the TB non-diabetic group they represent 10.2% of total patients, the mean =196.27, Std. Deviation = 109.559, and std errors was = 17.110.

IL6 regarding the TB + diabetic group they represent 10% of total patients, the mean = 210.13, Std. Deviation = 89.481, and std errors was = 14.148.

IL6 regarding CVD +TB + diabetic group they represent 39.8% of total patients, the mean 199.33 =, Std. Deviation = 133.084, and std errors was = 10.521.

Totally 100% of patients had mean = 158.23, std deviation = 120.719, and the std error was = 6.02.

IL6 regarding the healthy group they represent 40% of total patients, the mean = 94.80, Std. Deviation = 84.786 , and std errors was = 6.682.

Table 3: The mean level of cytokines IL6 in different groups

		N	Mean Pg/ml	Std. Deviation	Std. Error
IL6	Healthy	161	94.80	84.786	6.682
	TB non diabetic	41	196.27	109.559	17.110
	tuberculosis + diabetes	40	210.13	89.481	14.148
	CVD+ TB+ Diabstus	160	199.33	133.084	10.521
	Total	402	158.23	120.719	6.021

One way anova test:- p.value =0.000 which was highly a significant

The mean level of cytokins IL10 in different groups:-

IL10 regarding the healthy group they represent 40% of total patients, the mean = 4.21, Std. Deviation = 5.713, and std errors was = .450.

IL10 regarding the TB non-diabetic group they represent 10.2% of total patients, the mean =8.84, Std. Deviation =4.705, and std errors was = .735.

IL10 regarding the TB + diabetic group they represent 10% of total patients, the mean = 8.61, Std. Deviation = 4.265, and std errors was = .674.

IL10 regarding CVD +TB + diabetic group they represent 39.8% of total patients, the mean = 7.90, Std. Deviation = 5.085, and std errors was = .402.

Totally 100% of patients had mean =6.59, std deviation =5.579, and the std error was = .278.

IL10 regarding the healthy group they represent 40% of total patients, the mean = 4.21, Std. Deviation = 5.713, and std errors was = .450.

Table 4: The mean level of cytokines IL10 in different groups

		N	Mean Pg/ml	Std.Devition	Std.Error
IL10	Healthy	161	4.21	5.713	.450
	TB non diabetic	41	8.84	4.705	.735
	tuberculosis + diabetes	40	8.61	4.265	.674
	CVD+ TB+ Diabstus	160	7.90	5.085	.402
	Total	402	6.59	5.579	.278

One way anova test:- p.value =0.000 which is a significant

The mean level of cytokines TNFα in different groups:-

TNF regarding the healthy group they represent 40% of total patients, the mean = 81.59, Std. Deviation = 91.466, std errors = 7.209, and the maximum reading of cytokines was 608.

TNF regarding the TB non-diabetic group they represent 10.2% of total patients, the mean = 232.29, Std. Deviation =139.118, std errors = 21.727, and the maximum reading of cytokines was = 612.

TNF regarding the TB + diabetic group they represent 10% of total patients, the mean =261.78, Std. Deviation =129.382, std errors = 20.457, and the maximum reading of cytokines was 612.

TNF regarding the CVD +TB + diabetic group they represent 39.8% of total patients, the mean =297.42, Std. Deviation =198.423, std errors =15.687, and the maximum reading of cytokines was = 902.

Totally 100% of patients had mean = 200.79, std deviation = 180.029, std error = 8.979, and maximum reading of cytokines was = 902. This is one-way anova test result: - value = 0.000 which is significant. TNF regarding the healthy group they represent 40% of total patients, the mean = 81.59, Std. Deviation = 91.466, std errors = 7.209, and the maximum reading of cytokines was 608.

Table 5: The mean level of cytokines TNFα in different groups

	N	Mean Pg/ml	Std. Deviation	Std. Error	Maximum
Healthy	161	81.59	91.466	7.209	608
TB non diabetic	41	232.29	139.118	21.727	612
tuberculosis + diabetes	40	261.78	129.382	20.457	612
CVD+ TB+ Diabstus	160	297.42	198.423	15.687	902
Total	402	200.79	180.029	8.979	902

One way anova test:- p.value =0.000 which was highly a significant.difference

The HBA1C levels in different groups:-

HBA1C regarding the healthy group they represent 40% of total patients, the mean =5.00, Std. Deviation = .000, and std errors was = .000.

HBA1C regarding the TB non-diabetic group they represent 10.2% of total patients, the mean = 5.00, Std. Deviation=.000, and std errors was =.000.

HBA1C regarding the TB + diabetic group they represent 10% of total patients, the mean = 9.34, Std. Deviation =1.928, and std errors was = .152. .

Totally 100% of patients had mean = 7.21, std deviation =2.602, and the std error was = .130.

HBA1C regarding the healthy group they represent 40% of total patients, the mean =5.00, Std. Deviation = .000, and std errors was = .000.

Table 6: The HBA1C level in different groups

		N	Mean pg/ml	Std deviation	Maximum
HBA1C	Healthy	161	5.00	.000	.000
	TB non diabetic	41	5.00	.000	.000
	tuberculosis + diabetes	40	9.80	1.896	.300
	CVD+ TB+ Diabstus	160	9.34	1.928	.152
	Total	402	7.21	2.602	.130

One way anova test:- p.value =0.000 which was a significant

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.

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

Table7: TNF, IL6, IL10 and HBA1C distribution of study groups

Group Statistics						
	Sex	N	Mean Pg/ml	Std. Deviation	Std. Error Mean	P.value
TNF	F	193	199.91	180.716	13.076	
	M	209	202.53	180.400	12.479	0.885
IL6	F	193	165.81	125.108	9.053	
	M	209	151.99	116.699	8.072	0.254
IL10	F	193	6.52	4.684	.339	
	M	209	6.64	6.321	.437	0.833
HBA1C	F	193	7.25	2.632	.190	
	M	209	7.19	2.582	.179	0.833

Two way anova

4. 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.

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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 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.

5. CONCLUSION

1- Serum levels of IL6 ,IL10 and TNFα increased in patients with no signs and symptoms of tuberculosis (latent phase).

2- The cut off values 68pg/ml ; 5.85pg/ml; 297.42pg/ml for IL6; IL10 and TNFα respectively are diagnostic for latent tuberculosis.

3- There was no relationship between Gender and IL6, IL10 and TNFα levels.

6. RECOMMENDATION

1- All patients with complicated diabetic should be test for the latent tuberculosis in poorly controlled diabetic mellitus type Ⅱ,

2- 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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