Increased the Level of IFN-γ Production in Sudanese Patients with Tuberculosis

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Abstract-Background: Tuberculosis is a leading cause of death in low-income countries especially in Africa. Clinical and experimental studies have demonstrated the importance of IFN-γ production in the control of tuberculosis.

Objectives: To determine the level of IFN-γ in TB patients and compared with healthy controls.

Method: Demographic and clinical data were collected using questionnaire and written informed consents were obtained from all participants. IFN-γ level was measured for 150 TB patients and 71 healthy controls using ELISA techniques. Results: The 70.6% of the patients are male, and the age of 45% of patients was ranged from 21-30 years. Body mass index (BMI) showed that 23.1% of the patients were under weight, 71.9% were in normal weight, 3.8% were overweight and 1.2% was obese. Signs and symptoms associated with TB were 71.9% fever, 73.8% chronic cough, 71.2% muco-purulent sputum, 24.4% hemoptysis, 70.6% loss of weight, 65% night sweating, 70.6% fatigue and 66.7% chest pain. Chest X-ray showed that 80.6% of patients had pulmonary tuberculosis and 3.8% had extra pulmonary tuberculosis. The level of IFN-γ was significantly higher in TB patients compared with the healthy control (P< 0.001). Within the patients the levels of IFN-γ were significantly elevated in confirmed TB patients by ZN stain compared with those clinically diagnosed (P < 0.019).There was a significant difference (P<0.036) in the level of IFN-γ between patients started treatment andnon-treated patients.

Conclusion: The levels of IFN-γ were significantly associated with tuberculosis.

Keywords: IFN-γ, tuberculosis, BMI, ELISA

I. INTRODUCTION

Tuberculosis is a leading cause of death in low-income countries, especially in Africa where tuberculosis is an epidemic because of the increased susceptibility by HIV infection (1). It estimated that 1% of world's populations are annually infected, and about 1.5 million died in 2014 (2).Currently, Sudan carries an estimated 8% of the total TB burden, with around 119 per 10,000 cases detected in 2010 alone (3).A total of 670 patients were registered at Kassala hospital with clinical, laboratory and radiological evidence of TB in the year 2011 (4).

Tuberculosis is caused mainly by Mycobacterium tuberculosis bacteria (5). Immune mechanisms necessary for TB protection are poorly understood, although previous studies have indicated T cells as an essential component of the immune response to Mycobacterium tuberculosis. Understanding the function of the cytokine network involved in TB it will assist in the development of effective control and prevention (6).

Interferons are proteins that play a major role in the immune response against different antigens (7), It divided into two major types, type I IFNs are induced and act effectively in responses against viruses: IFN-α is secreted mainly by leucocytes, and IFN-β is produced by fibroblasts. Type II interferon (IFN-γ) is synthesized mostly by T lymphocytes and NK cells, and has been shown to be an important mediator of macrophage activation in controlling a number of intracellular pathogens (8), (9). Clinical and experimental studies have demonstrated the importance of IFN-γ production in the control of tuberculosis (10), (11), (12), (13), (14). IFN gamma secreted in response to Mycobacterium stimulation, give a protection against M. tuberculosis in both in-vivo and in-vitro stimulation (15). Interferon gamma level increases in TB patient's blood, and IFN gamma release assay (INGRA) was used to detect the latent TB infection(16).The aim of the present study was to determine IFN-γ level in the blood of TB patients and compared with healthy controls.

II. MATERIALS AND METHODS

Study Population

This is a case control study for 160 patients infected with tuberculosis from Omdurman hospital (Abu-anja) and police hospital, and 220 healthy volunteer students as control from Faculty of Medical Laboratory Sciences, University of Khartoum. Demographic and clinical data were collected using questionnaire and written informed consents were obtained from all participants. Among the patient's 68 were confirmed TB, from which 62 were positive Zn smear (Acid-fast bacilli) stained by the Ziehl-Neelson method and 6 were positive biopsy granuloma and about 92 were clinically diagnosed. All participants were screened for HIV, and HIV-positive individuals were excluded from the analysis. The controls were normal healthy participants with no symptoms of any infection, they were clinically normal at the time of blood collection.

Sample Collection and Processing

Five ml volume of venous blood samples were collected from all participants in sterile vacotainers contains lithium heparin as anticoagulant. Samples were centrifuged and

plasma was separated in new clean plain containers, stored in -80oC until they were assayed. All samples diluted 1 in 5 by assay diluents reagent provided by the Biolegend USA commercial kit.

INF Gamma Assay

The IFN-γ concentration was measured for 150 tuberculosis patients (107 males and 43 females), and 71 healthy volunteers as control (39 males and 32 females). The IFN-γ was determined in plasma samples using a sandwich Enzyme Linked Immunosorbent Assay kits (ELISA) according to the manufacturer's protocols (Biolegend, USA).

Data Analysis

Interferon gamma concentrations were measured using Graph Pad Prism v6 (Graph Pad Inc., California, USA). The level of significance between case and control was determined by t-test. P value less than 0.05 was considered significant. Statistical analysis was performed by SPSS version 19.

III. RESULTS

The baseline characteristic of the study population showed that 70.6% of the patients are male, while 43.2% of healthy controls are male. The age of most infected TB patients 45% was ranged between 21 to 30 years, 45.6% were worker, and 38.8% were illiterate. Most of patients 67.5% reside in urban areas, and 55% are from western Sudan. Body mass index (BMI) showed that 23.1% of the patients were under weight, 71.9% were in normal weight, 3.8% were overweight and 1.2% was obese Table 1.

Table 1: Baseline characteristics of the study population

Parameter	Case (n=160(%))	Control (n= 220(%))
Gender		
Male	113(70.6%)	95(43.2%)
Female	47(29.4%)	125(56.8%)
Age		
11-20	9(5.6%)	47(21.4%)
21-30	72(45%)	89(40.5%)
31-40	36(22.5%)	34(15.5%)
41-50	17(10.6%)	21(9.5%)
51-60	13(8.1%)	15(6.8%)
61-70	13(8.1%)	10(4.5%)
71-80	0(0%)	4(1.8%)
Occupation		
Business men	21(13.3%)	28(12.7%)
Worker	72(45.6%)	65(29.5%)
Teacher	4(2.5%)	3(1.4%)
House wife	19(12%)	17(7.7%)
Student	38(24.1%)	85(38.6%)
Unemployed	3(1.9%)	13(5.9%)
Medical professional	1(0.6%)	9(4.1%)
Education level		
Illiterate	62(38.8%)	17(7.7%)
Khalwa	10(6.3%)	16(7.3%)
Primary	37(23.1%)	32(14.5%)
Secondary	26(16.3%)	62(28.2%)
University	25(15.6%)	93(42%.3%)
Region		
Western Sudan	88(55%)	95(43.1%)
Southern Sudan	5(3.1%)	2(0.9%)
Eastern Sudan	3(1.9%)	3(1.2%)
Northern Sudan	36(22.5%)	92(37.1%)
Central Sudan	28(17.5%)	28(11.3%)
Residence		
Urban	108(67.5%)	176(80%)
Rural	52(32.5%)	44(20%)
City		
Omdurman	57(35.6%)	50(22.7%)
Khartoum	4(2.5%)	43(19.5%)
Bahri	6(3.8%)	33(15%)
Out site Khartoum	93(58.1%)	94(42.7%)
Marital status		
Single	72(45%)	126(57.3%)
Married	86(53.8%)	85(38.6%)
Divorced	1(0.6%)	5(2.3%)
Widow	1(0.6%)	4(1.8%)
BMI		
Under weight	37(23.1%)	40(18.2%)
Normal	115(71.9%)	137(62.3%)
Over weight	6(3.8%)	35(15.9%)
Obese	2(1.2%)	8(3.6%)

The infected TB patients were 64.9% smear positive, 19.4% suspected and 42.5% culture positive. Signs and symptoms associated with TB were 71.9% fever, 73.8% chronic cough, 71.2% muco-purulent sputum, 24.4% hemoptysis, 70.6% loss of weight, 65% night sweating, 70.6% fatigue and 66.7% chest Pain Table 2.

Table 2. Signs and symptoms associated with Tuberculosis

Symptom	Yes	No
Fever	115 (71.9%)	45 (28.1%)
Chronic cough	118 (73.8%)	42 (26.2%)
Muco-purulent Sputum	114(71.2%)	46(28.8%)
Hemoptysis	39(24.4%)	121(75.6

		%)
Loss of Weight	113(70.6%)	47(28.0%)
Night Sweating	104(65.0%)	47(29.4%)
Fatigue	113(70.6%)	47(28.0%)
Chest Pain	106(66.7%)	53(33.3%)

Chest X-ray shows that 80.6% of patients had pulmonary tuberculosis and 3.8% had extra pulmonary tuberculosis. Whereas, acid-fast bacilli shows that 38.8% had tuberculosis. Previous treatment, 27.5% Fixed Dose Combination (FDC), 3.75% Rifampicin (RFM) and 3.75% Rifampicin + isoniazid (RH) Table 3.

Table 3 Investigation tools

Investigation	N (%)
CX-ray	
Pulmonary tuberculosis	129(80.6%)
Extra-pulmonary tuberculosis	6(3.8%)
Not apply	25(15.6%)
Acid-fast bacilli	
Yes	62(38.8%)
No	98(61.2%)
Previous treatment	
FDC	44 (27.5%)
RFM	6 (3.75%)
RH	6 (3.75%)
Not apply	104 (65.0%)

The level of IFN-γ was significantly higher in TB patients compared with the healthy control (p value < 0.001). Within the patients the level of IFN-γ was significantly elevated in TB patients confirmed by ZN stain compared with those clinically diagnosed (p value < 0.019) Fig 1.

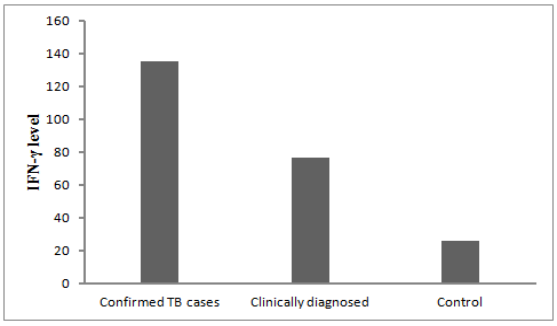

Figure (1): IFN-γ level in confirmed and clinically diagnosed cases and control

The level of IFN-γ showed that there was no significant difference between smokers and non-smoker patients (p value > 0.713). However, there was a significant difference (p value <0.036) between patients started treatment and those non treated patients Fig 2.

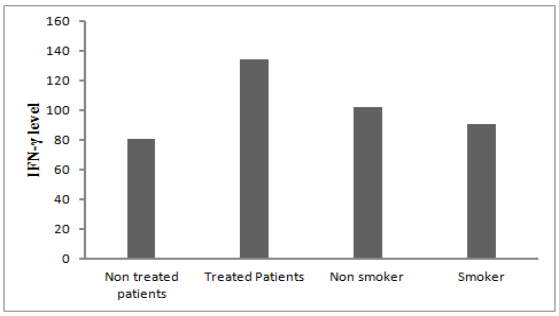

Figure (2): The effect of risk factors on IFN-γ level

IV. DISCUSSION

During the last decade tuberculosis has posed an increasing global threat, for which an effective vaccine is not as yet available, and mycobacterial drug resistance is spreading. In the present study70.6% of the patients are male, and the age of 45% of patients ranged from 21-30 years. In Pakistan among patients 46.30 % were males and 53.70% were females (16). About 80.6% of our patients had pulmonary tuberculosis and 3.8% had extra pulmonary tuberculosis, and 71.9% were in normal weight. Signs and symptoms associated with TB were 71.9% fever, 73.8% chronic cough, 71.2% muco-purulent sputum, 24.4% hemoptysis, 70.6% loss of weight, 65% night sweating, 70.6% fatigue and 66.7% chest pain. Hussain et al. (2010) indicated that 50% of patients had confirmed TB which included 44.44% with Zeil-Nielsen stain positive sputum and 5.56 % were biopsy/granuloma positive cases and 50 % were clinically diagnosed TB patients (16).

Similar to the previous studies the concentration of interferon gamma was significantly (p value< 0.001) higher in tuberculosis patients compared to healthy control (16). In contrast, other studies demonstrated that the mycobacterial antigen induced interferon-gamma by peripheral blood mononuclear cells from tuberculosis patients is usually depressed, compared with that of healthy control subjects, whereas cytokine production at the site of disease is elevated(17),(18). IFN-γ level of confirmed TB patients was higher than that of the clinically diagnosed TB patients (p value <0.019). Similar results were obtained by Hussain et al. who suggest that the difference may be due to the fact that most of clinically diagnosed TB patients were on anti-tuberculosis therapy and therefore the healing effect on granuloma could reduce the number of local and circulating IFN-γ producing activated T cells (16).There was no statistical difference in IFN-γ level according to the age group (P<0.471). While Ch et al. showed that elderly patients are more likely to present the disease (19).

The level of IFN-γ showed that there was no significant difference between smokers and non-smoker patients (P > 0.713). Previous study indicated that smoking is negatively affecting the level of interferon gamma concentration in tuberculosis patients and this is because T cells functions

are greatly reduced in smokers (20).TB therapy significantly increases in interferon gamma concentration ($P < .036$). In contrast, Verbon et al showed that interferon gamma level was elevated during active TB when compared with healthy controls, and declining during and after treatment (21).

V. CONCLUSION

The levels of IFN-γ were significantly associated with tuberculosis.

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