Technical Report, 15 February 2016

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Title of Project: **Assessment of diabetes amongst tuberculosis patients presenting at a tertiary care facility in Pakistan**

SUMMARY OF WORK DONE

Pakistan ranks 6th amongst high tuberculosis burden countries worldwide. It also ranks 5th amongst countries with a high burden of diabetes. WHO has recommended bi-directional screening of tuberculosis and diabetes. Currently, the rate of diabetes amongst patients with tuberculosis in Pakistan is not well understood. This study aimed to determine the prevalence of diabetes amongst patients with TB who were presenting at tertiary care health centers in Karachi, Pakistan.
A total of 216 patients with tuberculosis were recruited. Diabetes was diagnosed with an HbA1c of > 6.5 % and random blood sugar > 180 mg/dl. Pre-diabetes was diagnosed as HbA1c of 5.7-6.4.
Laboratory data for 211 TB patients was analyzed. Out of 211 patients, 24 (11.4%) had diabetes. Out of 24 diabetic patients, diabetes was newly detected in 17 of TB patients and 7 were known cases of diabetes. Pre-diabetes was identified amongst 45 (21.3%) of TB patients.
Of the total number of patients recruited, 165 were newly diagnosed whilst 46 were re-treatment cases. The large majority of patients (60%) were underweight with a BMI less than 18.5.

Follow up information on 171 patients was available. Of the 24 diabetic patients identified, we have guided 21 to appropriate medical management of their condition. Out of 45 Pre-diabetic TB patients, 29 were contacted and given appropriate advice regarding food choices and exercise to manage their condition.
The work done has been appreciated by the patients and the TB clinic which has been very cooperative. The patients have been eager to have their blood sugar levels checked and to know their results.
Analysis of circulating serum cytokines in TB patients demonstrated that IFN-gamma (p=0.028) and IL-13 (p=0.003) levels were significantly different between individuals with normal and those with Pre-diabetes and diabetes. As IL-13 has been shown to affect glucogenesis, it is possible that this could be a potential biomarker for identifying patients with dysregulated blood sugars.

Objectives of the study:

1.To determine the rate of diabetes amongst TB patients
2.To identify pre-diabetics amongst TB patients
3.To identify cytokine biomarkers associated with diabetes risk in patients with TB

Introduction

Tuberculosis (TB) results in approximately 1. 7 million deaths each year [1]. Diabetes mellitus (DM), or diabetes, is a group of metabolic diseases in which there are high blood sugar levels over a prolonged period. As of 2014, an estimated 387 million people have diabetes worldwide [2], with type 2 diabetes making up about 90% of the cases. Type 2 diabetes begins with insulin resistance, but as the disease progresses a lack of insulin may also develop. Diabetes occurs equally in both high and low income countries [3] however, 80% of diabetes related deaths occur in low and middle income countries. Pakistan ranks 5th amongst high TB burden countries worldwide, with an incidence of 231/100,000 annually[1]. The incidence of non-communicable diseases such as diabetes and cardiovascular diseases is growing in Pakistan. Pakistan has a diabetes prevalence of 6.8%, ranks 7th amongst high diabetes burden countries and there are estimated to be 3.5 million undiagnosed diabetics in the country [2]. Diabetes weakens the immune system and makes individuals more prone to infectious diseases such as, tuberculosis. Unfortunately, as both TB and diabetes are often not detected early enough, their public health burden can be quite high. In TB with diabetes, there is an increased risk of death due to TB treatment and of TB relapse after treatment [4]. A recent study from Lahore, Pakistan identified 6% of newly diagnosed diabetics amongst TB patients [5]. A study of diabetic patients in Karachi indicated that they were 10 times more likely to have TB [6].

WHO recommendations indicate bidirectional screening of TB in patients with diabetes, and for diabetes in TB patients [7]. A number of tests have been recommended for testing of glycaemic control in individuals. These include random blood sugar, fasting blood sugar (FBS), glycosylated hemoglobin (HbA1c), oral glucose tolerance test (OGTT) and fasting insulin levels. The utility of these tests varies according to availability, financial feasibility and accessibility for the patients.

Identification of diabetes or 'pre-diabetes' defined as 'at risk of diabetes' in patients with TB is important as uncontrolled blood glucose levels result in unfavourable treatment outcomes. The risk of treatment failure and of relapse TB treatment completion is higher in patients with diabetes [7].

The immunopathology of TB, its impact on diabetes and *vice versa* has been the focus of recent scientific studies. TB is caused by infection with *Mycobacterium tuberculosis (Mtb)* which resides within macrophages. Control of *Mtb* infection requires effective activation of T cells and macrophages through the production of cytokines and chemokines, which recruit immune effector cells to the site of infection [8;9]. In *Mtb* infected individuals with type 2 diabetes mellitus, an impaired T cell mediated immune response has been demonstrated [10]. Recently it has been shown that in type 2 diabetics, the production of reactive oxygen species in the *Mtb*-induced inflammasone is reduced [11]. Macrophages from diabetic individuals have a reduced effector response inflammatory response in the presence of the pathogen. Bacterial loads in macrophages from diabetic individuals with TB are higher than in non-diabetics [12]. Production of host protective responses such as interferon(IFN)-gamma and interleukin (IL)-12 is reduced in TB patients with diabetes, as are levels of downregulatory IL-10 levels [13]. Thus, the homeostatic balance between pro-inflammatory and anti-inflammatory cytokines is affected [12].

Whilst there is an increasing awareness of diabetes in the general population in Pakistan, the health systems to facilitate screening for it are not as yet in place. TB remains common but TB patients may be unaware of their having diabetes.

In this study, we surveyed patients with TB to determine what proportion of patients had diabetes or were at 'risk of diabetes'. Patients were recruited and subsequently tested for diabetes by screening FBS and HbA1c levels. Diabetes will be defined by HbA1c ≥ 6.5% and/or FBS ≥ 126 mg/dl; pre-diabetes will be defined by HbA1c 5.7-6.4 % and/or FBS 100-125 mg/dl. Those identified as having diabetes were advised treatment accordingly. TB treatment outcomes were followed in all cases.

Pro-inflammatory markers such as, tumor necrosis factor alpha, interleukins-2,-6, -10 and -17 are found to be raised in serum of patients with TB [14-16]. In diabetes, the cytokine profile is thought to be shifted[17] and recently it has been shown that pre-diabetics a hyper-responsive cytokine profile [17]. We measured serum cytokines in all patients to correlate the immune profiles in TB patients with and without diabetes as compared with those 'at risk for diabetes'.

This study provided important information regarding the occurrence of diabetes co-incident with TB in this Pakistani cohort. Such information will form the basis of treatment and management algorithms for TB. It will also contribute important information for public health in Pakistan, leading to information required for health policy making. Importantly, information regarding immune biomarkers associated with TB and diabetes will be important to new diagnostic tools for early diagnosis of TB.

Materials and Methods

SUBJECT SELECTION
We recruited 216 patients with TB patients from the AKUH and Indus Hospital, Karachi; with written and verbal informed consent from each participant. Ethical approval was obtained from The Aga Khan University and also the Indus Hospital – Institutional Review Board.

Study population and period of observation

Inclusion criteria: male or female, ages 18 years and older
In each case, patients were recruited either after being newly diagnosed or on their first follow up visit after start of anti-tuberculous therapy (ATT). Therefore, all patients had been given less than one month of ATT prior to recruitment in the study.

Diagnostic criteria for patient selection

Pulmonary TB. Patients had a positive chest Xray coincident with TB with characteristic clinical features and a positive sputum smear/MTB culture/Gene Xpert TB and diagnosed according to National TB Program guidelines.
Extrapulmonary TB. Patients had Pleural TB, tuberculous lymphadenopathy (LNTB), abdominal TB or other extrapulmonary site involvement. Diagnosis was made on a positive GeneXpert of site specific specimen or histopathology confirming granulomatous inflammation, co-incident with clinical history.

Sampling Method

Demographic and clinical data, medical history, physical exam findings, and information regarding chest radiography were entered in the Enrollment form (Appendix 1) in each case.
Cases have been recruited from Indus Hospital TB Clinic on Monday, Tuesday and Wednesday morning of each week. Thus, the Research Medical officer visited Indus Hospital on each of these days from 9 am to 1 pm.

During the week, any TB patient identified through the Department of Medicine clinics at AKUH has also been recruited.

At Indus Hospital, we have worked with the TB Clinic. Patients who have been screened for TB and diagnosed with a positive mycobacterial smear or GeneXpert TB result were informed about the study by doctors at the TB clinic, who then referred the patient to the TB counselor and subsequently, to the study team for recruitment. In the case of patients who have already been diagnosed with TB and started on ATT but treated less than 1 month, the TB clinic doctors inform the patient about the study and directly refer them to the study team/medical officer.

At AKUH, the consulting physicians Dr. Irfan, Dr. Jamil and Dr. Salahuddin have been responsible for clinical evaluation, initial screening tests (chest X-ray, sputum culture/microscopy or other mycobacterial culture) for confirmation of TB, after which the patient was recruited into the study.
The study team in each case discussed the project with the patient and took written informed consent from them. In the case that the patient was illiterate, a thumb impression was taken on the consent form and the name of the individual written by the study team.
Detailed demographic information and information regarding clinical algorithm was logged by an attending medical officer.

Sampling Process
Once recruited into the study, 9 ml of blood was taken from each patient for testing RBS, HbA1c and for measurement of serum cytokines / chemokines.
Normal levels of HbA1c were 5.7 % of less with RBS of 80-160 mg/dl. Diabetes was defined by HbA1c $\geq$ 6.5% and/or RBS $\geq$ 200 mg/dl; Pre-diabetes was defined by HbA1c 5.7-6.4 %.

Results

A total of 216 patients were recruited in the study from the two sites, The Aga Khan University Hospital and The Indus Hospital, Karachi (see Fig. 1). Four patients refused to give a blood sample. Therefore, blood samples taken from 212 patients and tested for random blood sugar (RBS) and glycosylated hemoglobin (HbA1c). One sample was hemolysed and could not be tested. Data from results of 211 TB patients have been analysed and are presented in this report.

General Characteristics of the Study Subjects

Of the 211 TB patients, 142 had normal HbA1c levels, whilst 45 were pre-diabetic and 24 were diabetic as per HbA1c (Table 1). Of the diabetic patients, 17 were newly diagnosed whilst 7 already knew that they had diabetes.

TB patients with diabetes were comparatively older than the subjects with pre-diabetes and normoglycaemia (Median 50 vs. 40 vs. 23 years), p<0.001 (Table 2)..

There were an equivalent number of males and females amongst the study subjects 101 (47.9%) vs 110 (52.1%), Table 3. The median household size of participants was 7 individuals (Male-3, Female-3). The median age of the study subjects was 26 years. One hundred (47.4%) subjects were aged less than 25 years; seventy-six (36%) of them were aged between 26–50 years, and thirty-five (16.6%) were aged above 50 years. Women were comparatively younger than men (25y vs 33y), Table 4.

The Median BMI of the TB subjects was 17.78. One hundred patients (47.39%) were underweight with BMI (kg/m2) <18.5, 55 (26.06%) had BMI 18.5–24.9 (normal BMI) and 12 (5.68%) had BMI >24.9 (overweight), Table 4.

Tuberculosis Profile of the Study Patients

TB had been diagnosed in a household member amongst 76 (36%) of cases. One hundred and thirty-four (63.5%) were newly diagnosed (prior to treatment) and 77 (36.5%) of cases were recruited at their first follow up and had received about one month of anti-tuberculous therapy. Of the total number of cases recruited, 165 (78.2%) were on Category I treatment, or diagnosed for the first time, whilst 46 (21.8%) had Category II treatment, and were previously treated or relapsed cases.

Characteristics of TB Patients as per Stages of Glucose Intolerance

Among the newly diagnosed TB cases (n=134), 16 patients had diabetes and another 33 had pre-diabetes vs 8 diabetics and 12 pre-diabetics among those recruited on their first follow-up (n=77). AFB smear was done in 150 patients out of them, 80 were smear positive. A higher proportion of patients with TB DM were smear positive (n= 55) compared to pre-diabetes 15 or those with normoglycaemia n=9, Table 4 .

Among 211 study subjects, 172 (81.5%) were diagnosed with pulmonary TB and 39 (18.5%) with Extra-pulmonary TB. Both pulmonary and Extra-pulmonary TB were more common among TB patients with normoglycaemia (109 pulmonary TB and 33 extra pulmonary TB) compared to patients with pre-diabetes (39-pulmonary TB and 6 extra pulmonary TB) and diabetes (24 pulmonary and 0 extra pulmonary) as shown in Table 4. All of the 24 diabetic patients diagnosed had pulmonary TB.

Approximately 80% in all the three groups were receiving Category-I treatment. A small proportion of patients were receiving Category-II treatment due to relapse, treatment defaulters, or failure cases in all categories of glucose intolerance. (165 on Cat-I and 46 on Cat-II). The majority of TB patients were underweight (Table 4).

Microbiological and radiological findings

TB patients had been identified as AFB smear positive (71%) and MTB GeneXpert positive (68%). Of the 121 patients who had positive GeneXpert results, 118 were found to be susceptible to rifampicin, 2 were indeterminate whilst one showed a positive rifampicin resistance (Table 5).
Amongst extrapulmonary TB patients, pleural TB was most common, followed by abdominal and lymphnode sitest (Table 5).

Co-morbids of TB patients

We also assessed co-morbids of the TB patients, of the 211 patients, 2 were HIV positive, three were IV drug users, 3 had hepatitis virus infections and one had an autoimmune disease (Table 6).

Management of study subjects and coordination with diabetes clinic

Results of diagnostic tests have been provided to the primary physician and the patient was telephoned with his/her result in each case. Of the 24 patients diagnosed with diabetes, 21 have been successfully contacted. When the patient was contacted, they were informed of their results of HbA1c and random blood sugar test (RBS). If patients are identified as being diabetic, at Indus Hospital, they have been referred to the Diabetes clinic at Indus Hospital. The diabetes clinic will provide medication for management of diabetes to the TB patients.
At the Aga Khan University Hospital, the patients were facilitated with an appointment in consultation with Dr. Qamar Masood, Endocrinologist, AKUH.
There were 3 patients whom it has not as yet been possible to contact regarding their diabetes status, this is because they had either provided an incorrect contact number or their telephone was switched off.
In the case of pre-diabetics, 29 out of 45 TB patients have been advised regarding dietary modifications and adoption of a healthy lifestyle (Appendix II).

Circulating serum levels of TB patient indicate raised IL-13 in diabetics
To investigate whether any cytokine biomarkers were associated with diabetes risk, we determined circulating levels of cytokines in the sera of all patients tested. Cytokines were measured using a BIORAD Multiplex assay run on the Luminex equipment.

We observed measurable levels of IFN-γ, IL-10, IL-12p70, IL-13, IL-2, IL-5 and IP-10 (CXCL-10). With regarding to diabetes status, it was observed that IFN-gamma levels were significantly different between diabetics and normal ($p=0.028$), with raised levels observed in pre-diabetics and diabetics as compared with individuals with normal blood sugar levels (Table 8.) Additionally, IL-13 levels were raised in patients with pre-diabetes and diabetes as compared with TB patients who had normal blood sugars, $p=0.003$ (Table 7).

Conclusions

This study has now been completed. In 211 patients we have identified 11.4% cases of diabetes and 21.3% cases of pre-diabetes. The work done has been appreciated by the patients and the TB clinic which has been very cooperative. The patients have been eager to have their blood sugar levels checked and to know their results. The TB clinics have been made aware the importance of screening for diabetes amongst identified TB patients. The cytokine testing for TB patients has shown and trend of raised IL-13 levels in patients with raised blood sugar levels. This suggests that IL-13 can potentially be a biomarker for diagnosis of diabetes in patients with TB. We hope to write up this work and publish it in an international journal.

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Figure 1. Flow chart of study. Assessment for diabetes in patients with TB presenting at a tertiary care facility in Pakistan

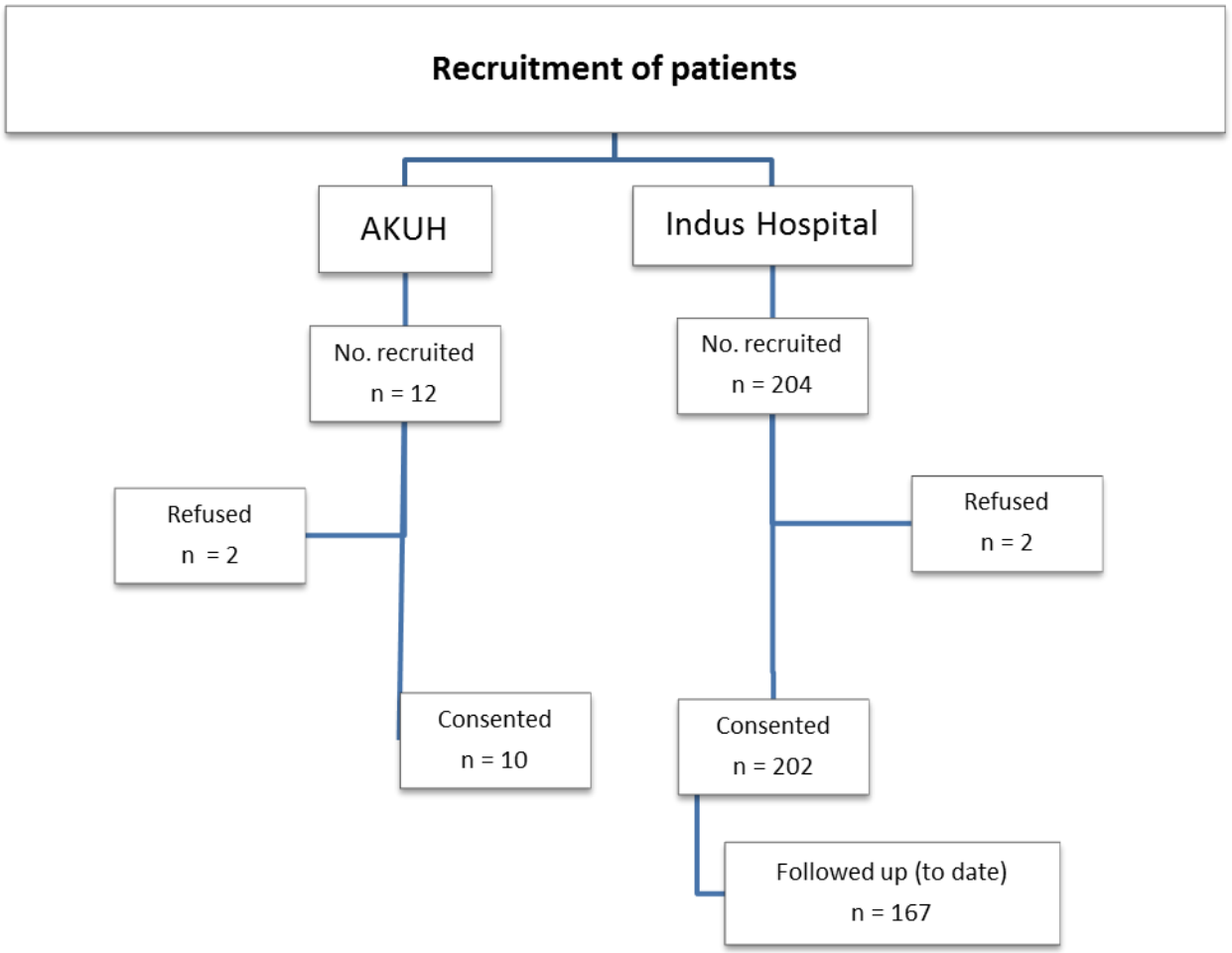

Table 1: TB Patient with Diabetes, Pre-Diabetes and No Diabetes on the basis of HbA1c Results (n=211)

TB Patient Groups	HbA1c Levels Number (% of total)		
Non-Diabetics (<5.7)	142 (67.3%)		
Pre-diabetic (5.7-6.4)	45 (21.3%)		
Diabetic (>6.4)	24 (11.4%)	Newly Diagnosed	17
		Known Diabetics	7

Table 2: Median Ages of TB Patient that are Diabetics, Pre-Diabetics and Non Diabetics with Inter Quartile Ranges of HbA1c and RBS (n=211)

	Total N (%)	**Median Age (Years)**	**HbA1c IQR=Median(Q_3 – Q_1)**	**RBS IQR=Median(Q_3 – Q_1)**
Non-Diabetics	142 (67.30%)	23	5.30(5.50-5.00)	95(108-89)
Pre-Diabetics	45 (21.30%)	40	5.80(6.00-5.70)	100(122-93)
Diabetics	24 (11.40%)	50	10.6(12.00-9.55)	281(390-191)
Total	211 (100%)	26	5.50(5.80-5.10)	98(118-90)
P-value	----	P<0.0001*	P<0.0001*	P<0.0001*

Table 3: Median Age, BMI, RBS, HbA1c and Household size with Male and Female Comparison (n=211)

Variables	**Median**	**Gender**	**Median**
Age	26 (Years)	Male	33 (Years)
		Female	25 (Years)
BMI = Weight(kg)/Height(M2)	17.78	Male	17.96
		Female	17.76
RBS	98 (mg/dl)	Male	98
		Female	98
HbA1c	5.50 (%)	Male	5.60
		Female	5.30
No. Of Household		Male	3

Contacts	7	Female	3

Table 4: Comparison of study characteristics among TB patients with Normoglycemia, Pre-diabetes and Diabetes (n=211)

		Non-Diabetics N (%)	Pre-Diabetics N (%)	Diabetics N (%)	Total N (%)
Age	<= 25	89(89%)	9(9.0%)	2(2.0%)	100(47.4%)
	26-50	42(55.26%)	22(30.5%)	12(15.7%)	76(36.0%)
	>50	11(31.42%)	14(40.0%)	10(28.57%)	35(16.6%)
Gender	Male	58 (57.42%)	29 (28.71%)	14 (13.86%)	101(47.9%)
	Female	84 (76.36%)	16 (15.54%)	10 (9.09%)	110(52.1%)
Category of TB	Pulmonary TB	109 (63.37%)	39 (22.67%)	24 (13.95%)	172(81.5%)
	Extra Pulmonary TB	33 (84.61%)	6 (15.38%)	0 (0.0%)	39(18.5%)
Family History of TB	Positive Family History	57 (75.0%)	16 (21.05%)	3 (3.9%)	76(36.0%)
	No Family History	85 (62.96%)	29 (21.48%)	21 (15.5%)	135(64.0%)
Category of Treatment	New treatment	108 (65.45%)	36 (21.81%)	21 (12.72%)	165(78.2%)
	Retreatment	34 (73.91%)	9 (19.56%)	3 (6.52%)	46(21.8%)
Status of TB treatment	Newly diagnosed (Day 1 of ATT)	85 (59.70%)	33 (24.62%)	16 (11.94%)	134(63.5%)
	First follow-up (After 30 Days of ATT)	57 (74.02%)	12 (15.58%)	8 (10.38%)	77(36.5%)
BMI Categories	Underweight (<18.49)	77 (77%)	18 (18%)	5 (5.0%)	100(59.9%)
	Normal (18.5-24.9)	34 (61.81%)	11 (20%)	10 (18.18%)	55(32.9%)
	Overweight (>25)	4 (33.33%)	6 (50.0%)	2 (16.66%)	12(7.2%)
AFB Smear	Positive	*56 (70%)*	*15 (18.75%)*	*9 (11.25%)*	*80 (38%)*
	Negative	45 (64.28%)	15 (21.42%)	10 (14.28%)	70 (33.2%)
Patient information	Informed	98 (66.21%)	29 (19.59%)	21 (14.189%)	148 (70.14%)
status	Not Informed	44 (69.84%)	16 (25.39%)	3 (04.76%)	63 (29.85%)
PTB severity					
Minimal		7 (10)	1 (8.3)	0	11 (9.8)
Moderate		35 (50)	8 (66.7)	5 (71.4)	60 (53.6)

Advanced	28 (40)	3 (25)	2 (28.6)	41 (36.6)

Note: All values represent the absolute number followed by the percentage in parentheses unless otherwise indicated. Due to missing information, totals may not always add up to the sample size. Percentages have been rounded. Severity of PTB disease was determined based on the extent of lung involvement based on radiological assessment of the case.

Table 5: Microbiological and Radiological Findings of TB patients (n=211)

AFB Smear	150 (71.1%)	Positive	70 (33.2%)
		Negative	80 (38%)
AFB Culture	16 (7.6%)	Positive	16 (7.6%)
		Negative	0 (0.0%)
Genexpert (MTB)	144 (68.2%)	Positive	121 (57.34%)
		Negative	23 (10.9%)
RIF Resistance		Not Detected	118
		Indeterminate	2
		Detected	1
Chest X-Ray	189 (89.6%)	Minimal	*18(9.5%)*
		Moderate	*85(44.9%)*
		Advanced	*54(28.57%)*
		Normal	*32 (16.93%)*
Category Of Extra Pulmonary TB	39 (18.48%)	Pleural	16 (7.50%)
		Abdominal	9 (4.20%)
		Lymph node	7 (3.30%)
		Others	7 (3.30%)

Table 6: Past Medical History of TB Patients

HIV	***2 (.9%)***
IV Drug Addict	***3 (1.4%)***
Hepatitis B or C	***3 (1.4%)***
DM	***7 (3.3%)***
Autoimmune diseases	***1 (0.5%)***
Cancer	***0 (0.0%)***
History of the corticosteroid	***1 (0.5%)***
Any Diabetic Medications	***7 (3.3%)***

Table 7. Serum cytokine levels in TB patients with and without diabetes

	N	**IFN-gamma** Mean +/- SD (pg/ml)	**IL-10** Mean +/- SD (pg/ml)	**IL-12p70** Mean +/- SD (pg/ml)	**IL-13** Mean +/- SD (pg/ml)	**IL-2** Mean +/- SD Mean (pg/ml)	**IL-5** Mean +/- SD (pg/ml)	**TNF-alpha** Mean +/- SD (pg/ml)
Normal	38	27.58 +/- 89.65	155.56 +/- 490.18	117.66 +/- 437.49	2.90 +/- 17.85	0.00	25.24 +/-155.60	112.07 +/- 416.08
Pre-diabetic	24	444.11 +/- 1298.58	261.47 +/- 747.79	362.00 +/- 835.35	72.23 +/- 210.24	63.20 +/- 220.39	102.63 +/- 263.72	730.74 +/- 2448.77
Diabetic	13	257.30 +/- 746.37	0.00	128.16 +/- 462.07	68.00 +/-245.19	64.34 +/- 231.98	76.84 +/- 277.05	1700.78 +/- 5881.97
P value		*0.028***	*NS*	*NS*	*0.003***	*NS*	*NS*	*NS*

Values in each group were compared using the Kruskal-Wallis test with $p<0.05$ regarding as significant.