Original Research Article

Prevalence of pulmonary tuberculosis in patients with diabetes mellitus and lower respiratory tract infection

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ABSTRACT

Background: Pulmonary Tuberculosis (PTB) still remains a global public health problem. Diabetes Mellitus (DM), is a metabolic disorder characterized by hyperglycaemia. Diabetes along with poor glycaemic control leads to an immune compromised state. As prevalence of both TB and DM is increasing in India, this association of PTB and DM may prove a threat to TB control program. Aims and objectives of the study was to detect prevalence of pulmonary tuberculosis in patients with DM and Lower Respiratory Tract Infection (LRTI).

Methods: Sputum specimen from consecutive 250 known diabetic adult patients with type 2 diabetes and clinical evidence of LRTI were processed for microscopy, solid culture and Xpert MTB/RIF assay. Clinical findings, duration of DM, regularity of treatment and recent fasting blood glucose level were noted.

Results: TB was detected in 31(12.8%) patients. Microscopy, culture and Xpert assay were positive in 14(5.6%), 29(11.6%) and 24(9.5%) cases respectively. Culture detected seven cases more than Xpert assay. Two additional cases were detected by Xpert assay than culture. Rifampicin resistance was detected in seven (29.17%) cases by Xpert assay. TB detection rate was higher in patients with more than two weeks of cough (14.38%), history of tuberculosis (15.9%), hyperglycemia (13.9%) and significantly higher in those with irregular anti-diabetic treatment (35.7%).

Conclusions: Irregular anti-diabetic treatment, hyperglycaemia and history of tuberculosis were strongly associated with pulmonary TB. Xpert assay should be used as the initial diagnostic test for detection of tuberculosis as well as rifampicin resistance in diabetic patients by TB control programme.

Keywords: Diabetes mellitus, Lower respiratory tract infection, Pulmonary tuberculosis

INTRODUCTION

Pulmonary Tuberculosis (PTB) caused by Mycobacterium Tuberculosis (MTB) still remains a considerable global public health concern, mainly affecting vulnerable population.[1] India accounts for 25% of the global burden of Tuberculosis (TB) and 29% of global TB mortality.[2] The risk of developing TB is higher in persons with chronic debilitating diseases with immunocompromised state. Diabetes Mellitus (DM), which is increasing globally, is a metabolic disorder characterized by hyperglycemia due to absolute or relative insulin deficiency. Diabetes along with poor glycemic control leads to an immune compromised state with defective functioning of cell mediated immunity.[3] In addition, reduced oxygen supply to tissues as a result of micro vascular changes predisposes them to infections.[4]

An association between HIV and TB is already known.[5] But similar association may exist between DM and TB. Diabetic patients are at a higher risk of developing new as well as reactivation of old TB disease.[6] Patients with this

dual disease may be more contagious at diagnosis, may remain infectious for longer period and uncontrolled and undiagnosed diabetes may lead to poor TB treatment outcome.[7,8] As prevalence of both TB and DM is increasing in India, this association of PTB and DM may prove a threat to TB control program. Various studies have been carried out to determine the prevalence of DM in TB patients, but only limited studies are available on prevalence of TB in DM cases. Hence this study was undertaken to detect prevalence of pulmonary tuberculosis in patients with DM and Lower Respiratory Tract Infection (LRTI).

METHODS

This cross-sectional study was carried out over a period of one year (June 2015-May 2016) in a tertiary care hospital after obtaining Institutional Ethics Committee permission (IEC-II reference No EC/194/2014). 250 known diabetic adult patients of any gender, with type 2 diabetes, of any duration, irrespective of their anti-diabetic drug management, with clinical evidence of LRTI, referred by clinicians for microbiological investigations and willing to give written informed consent were included in this study. Patients already on anti-TB treatment or HIV seropositive or those on prolonged steroids were excluded from study. Sample size was calculated by using following standard statistical formula –

$$n = \frac{Z^2 \, P \, (1 - P)}{d^2}$$

Where,

n = sample size,
95% of Z=1.96
P = Prevalence.
d =

Prevalence	Precision
10-90%	d = 5%
<10%	d = P/2
>90%	d = 0.5 (1-P)

In this study, Prevalence is 8%.

Thus, using above formula; sample size = 176 equivalent to 200. Here, sample size for this study was taken as 250. For qualitative data chi-square test and for quantitative data student T-test was used. p<0.05 was considered as significant.

All processing was carried out in Bio Safety Cabinet (BSC) class 2 and level 2 bio safety practices were followed. Two sputum specimens of minimum 2 ml quantity were collected in sterile container from each patient of which one was early morning specimen. Direct microscopy by Ziehl Neelsen (ZN) staining was carried out on both the specimens.

Early morning specimen was tested by Xpert MTB/RIF assay (Xpert assay).[9] Spot specimen was processed by digestion and decontamination with N-Acetyl-L-Cysteine (NALC)-NaOH method and concentrated by centrifugation at 3000 g for 15 minutes. Pellet obtained after centrifugation was inoculated on Lowenstein-Jensen (LJ) medium and incubated aerobically at 37°C.[10] Any growth observed on LJ medium was identified as MTB or Mycobacteria Other Than Tuberculosis [MOTT] using phenotypic characteristics. Any acid-fast isolate which was a slow grower, forming buff coloured colony on LJ but did not grow on LJ containing Para-Nitrobenzoic Acid (PNBA) and MPT64 positive was characterized as MTB.

For each patient, clinical signs and symptoms, duration of DM, regularity of treatment for DM, recent fasting Blood Glucose Level (BSL) and radiological findings were noted. A patient was confirmed as a case of TB if either culture or Xpert assay was positive.

RESULTS

Here, 250 patients satisfying the inclusion criteria were enrolled in this study of which eight patients were excluded due to contamination in culture or invalid result in Xpert assay. The results of 242 patients were analyzed further. Mean age of enrolled patients was 57 years (range is 39-91 years). Maximum number of MTB positive cases were detected in age group 51-60 years (33.88%) followed by 61-70 years (25.62%). 137(56.61%) patients were men and 105(43.39%) were women. MTB was detected in 31(12.8%) patients of which 26(18.97%) were men and 5(4.76%) were women. Positivity in men was significantly higher than in women with p value <0.05 (p value = 0.001039).

Table 1: Comparison of mycobacterial culture with microscopy and xpert MTB/RIF assay.

Test result	LJ culture positive	LJ culture negative
Smear positive Xpert positive	13	1
Smear negative Xpert positive	9	1
Smear positive Xpert negative	0	0
Smear negative Xpert negative	7	211
Total	29	213

Acid fast stained microscopy, LJ culture and Xpert assay were positive in 14(5.6%), 29(11.6%) and 24(9.5%) cases respectively. Culture as a gold standard detected 16 cases more than smear and seven cases more than Xpert assay. These seven specimens had very low bacillary load by Xpert assay and were negative by microscopy. Xpert assay detected two cases more than culture. Rifampicin

resistance was detected in seven (29.17%) cases by Xpert assay. All cases positive by microscopy were also positive by culture and Xpert assay. Average duration of culture positivity was 39 days. Microscopy had 44.82% sensitivity and 99.53% specificity and Xpert assay had 75.86% sensitivity and 99.06% specificity in comparison with culture (Table 1).

TB detection rate was higher in patients with more than two weeks of cough (14.38% vs. 10.42%), with history of tuberculosis (15.9% vs. 12.12%), hyperglycemia (13.9% vs. 9.09%) and significantly higher in those with irregular

anti-diabetic treatment at p value <0.05 (p value is 0.02552) (35.7% vs. 11.4%) (Table 2, 3).

Table 2: Comparison of TB cases with fasting plasma sugar.

Fasting plasma sugar (mg/dl)	Total 242	TB positive (total - 31)	TB negative (total - 211)
≤ 126	55	5(9.09%)	50(90.91%)
>126	187	26(13.90%)	161(86.10%)

Table 3: TB cases (%) based on duration of cough, history of TB and DM treatment.

	Duration of cough		History of TB		DM treatment	
	≤ 2 weeks	>2 weeks	Present	Absent	Regular	Not regular
Total	96	146	44	198	228	14
Positive (TB)	10	21	7	24	26	5
Percent (%)	10.42%	14.38%	15.9%	12.12%	11.4%	35.7%

DISCUSSION

Diabetes mellitus has emerged as a growing worldwide chronic health condition, as a consequence of obesity, changing patterns of diet, physical inactivity and aging populations.[11] The epidemic growth of DM has occurred in developing countries where TB is highly endemic. Diabetes can worsen the clinical course of TB, and TB can worsen glycemic control in people with diabetes.[11]

Individuals with both conditions thus require careful clinical management. Strategies are needed to ensure that optimal care is provided to patients with both diseases: TB must be diagnosed early in people with diabetes for eliminating disease and move to "End TB".[8]

In the present study, 242 consecutive known diabetic patients were included and tested for presence of MTB in sputum specimen by microscopy, culture and Xpert assay. MTB was detected in total 31(12.8%) patients either by microscopy or culture or Xpert assay. As a standalone test, microscopy, culture and Xpert assay detected MTB in 5.6%, 11.6% and 9.5% of the cases respectively. Other studies done on diabetic patients detected 9.5% to 11.8% cases.[12-14]

In the present study, culture detected seven cases more than Xpert assay. Culture is a gold standard test for diagnosis of TB. It detects only viable bacilli and has better sensitivity (10-100 bacilli / ml) as compared to Xpert assay which detects 131 bacilli / ml. Culture takes 2-8 weeks for MTB to grow due to the slow replication rate whereas Xpert assay provides result within two hours. Xpert assay result in these seven specimens were

valid as Sample Processing Control (SPC) was amplified and Probe Check Control (PCC) also worked.

Xpert assay detected seven (29.17%) cases with rifampicin resistance. Of these one (14.28%) was previously treated and six (85.71%) were newly diagnosed cases. Previous studies showed that there is greater risk of MDR-TB among patients with diabetes, compared to those without diabetes and diabetes is associated with three fold higher risk of rifampicin resistance.[11,15,16] The present study was conducted in the city of Mumbai and previous reports have consistently shown higher levels of MDR-TB in Mumbai than in other parts of India.[17] Rifampicin resistance reported in other parts of India ranges from 1%-13% and 12%-40% in new and previously treated cases respectively.[17]

The rifampicin resistance rate detected in the present study is higher than the national average but similar to Mumbai data. Because of the low number of confirmed PTB cases in this study, conclusion regarding rifampicin resistance rate in diabetics compared to general population cannot be drawn. Considering the significant number of the specimens showing higher grade in microscopy and in Xpert assay and the associate rifampicin resistance, diabetic patients should be screened for TB whenever they complain of cough more than a week duration with Xpert assay being the initial diagnostic test.

Two specimens were Xpert positive and culture negative, which may be either true positive or false positive. Considering the closed system of amplification in Xpert assay, there is minimal chance of cross contamination resulting in false positive assay. Positive Xpert assay in

these two specimens may be due to better digestion of specimen by sample reagent provided in the assay which release the bacilli. One of these two specimens was negative by microscopy and had very low bacillary load by Xpert assay. Culture may be negative because of harsher decontamination procedure killed very few bacilli present in the specimen. Use of solid LJ medium instead of the more superior option liquid culture (MGIT 960) may be another reason for culture negative results.

To study the probable predisposing factors for development of clinical PTB infection, information was collected about duration of DM, regularity of anti-diabetic treatment, Fasting Plasma Sugar (FPS) level, and history of TB in past with details of present illness. With the advancement of age and duration of DM, there is gradual increase in blood sugar level, HbA1c and fasting insulin level which directly correlates with increasing insulin resistance. In the present study, 41.94% of detected TB cases had a duration of diabetes ≤2 years. Regular check-up for early detection of diabetes and proper control of blood sugar level may control the physiological changes resulting in TB infection. In some studies, reported in literature, maximum cases were detected when diabetes duration >10 years. In the present study, only 5/31 TB cases had a duration of >10 years.[18,19]

Hyperglycemia can lead to multiple complications such as vascular disease, neuropathy and increased susceptibility to infections including MTB. It has indirect effects on macrophages and lymphocyte function leading to diminished ability to contain the organism.[11] It also affects chemotaxis, phagocytosis, activation and antigen presentation by phagocytes in response to MTB.[11] BSL >200 mg/dl can significantly reduce macrophage respiratory burst and >250 mg/dl impairs white cell function.[20] Chemotaxis of monocytes is also impaired and this defect does not improve with insulin.[21] Thus, depressed immunological functions in DM might predispose a patient to infections, the control of which requires cell mediated immunity, such as tuberculosis. Thus, increased duration of diabetes leads to increased hyperglycemia. In the present study, 26/ 187(13.90%) TB positive cases were detected in patients when FPS >126 mg/dl and 5/55(9.09%) than cases with controlled FPS <126 mg/dl (Table 2). This finding is statistically not significant. More studies are required to comment on effect of hyperglycemia on association with TB as compared to the duration of DM and controlled diabetes is not a risk factor for TB.

Prediabetes and diabetes may affect the risk of latent, primary and reactivation tuberculosis.[21] Reactivation of TB may occur when a person suffered from TB in the past develop immunocompromised condition. Such situation may occur in diabetes cases due to poor glycemic control. In the present study, TB was detected in 15.9% of patients with previous history of TB as against 12.12% without it (Table 3).

Another important finding in the present study was that those who were not taking regular DM treatment had higher TB positivity (35.7%) as compared to those on regular treatment (11.4%). The difference was statistically significant with p value <0.05 (p value is 0.02552) (Table 3). Insulin is the medical treatment for type 1 DM and Oral Hypoglycemic Agents (OHA) for type 2 DM. The main function of insulin or OHA is to utilize glucose or to decrease insulin resistance. Thus, irregular diabetes treatment causes uncontrolled and deranged blood sugar levels and poor glycemic control.

In the present study, 14.38% of patients with cough duration >2 weeks and 10.42% patients with cough duration ≤2 weeks had PTB (Table 3). The difference is not statistically significant at p< 0.05 (p value is 0.3663). Since TB was detected even in patients with a ≤2 weeks cough, a strong suspicion of tuberculosis in symptomatic diabetic patients with cough duration even less than two weeks is important.

In the present study, 137 men (56.61%) and 105 women (43.39%) were included. Male to female ratio was 1.3: 1. MTB detection rate was more in men (18.97%) as compared to women (4.76%). This result was statistically significant at p value <0.05 (p value is 0.001039). As per the global prevalence of DM, diabetes is higher in men than women (215.2 million Men vs. 199.5 million Women).[22] As diabetes mellitus is more common in men than in women, chances of acquiring TB are higher in males than in females. Male predominance may be due to more exposure to risk factors such as smoking, outdoor activity, HIV-TB co-infection and airborne transmissibility of the pathogen. In case of females, there is under notification due to socioeconomic and cultural factors which become barriers in accessing health care for women.[23] Being a tertiary care center, there is a possibility of referral bias while selecting diabetes patients with lower respiratory tract infections. It is also possible that the use of a liquid culture system could have improved detection rate.

CONCLUSION

Irregular anti-diabetic treatment, hyperglycemia and history of tuberculosis were strongly associated with pulmonary TB. Xpert MTB/RIF assay should be used as the initial diagnostic test for detection of tuberculosis as well as rifampicin resistance in diabetic patients by TB control programme. At the same time, all suspected TB-DM patients should also undergo TB culture for confirmation.

Ethical approval: The study was approved by the Institutional Ethics Committee (IEC-II reference No EC/194/2014)

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