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Original Research Article

Extensiveness and homogeneity of diabetic/non-diabetic patients and their co-relation with CBNAAT confirmed tuberculosis patients in a tertiary care hospital of India

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ABSTRACT

Background: Many studies reported association between TB susceptibility and diabetes mellitus (DM). Some studies were retrospective, did not assess other co morbidities related with tuberculosis. The effects of diabetes on tuberculosis severity (EDOTS) can be hypothesizing that burden of cases India is leading in TB and runner-up in diabetic. We report interim findings after enrolling 732 of a planned 212 subjects.
Methods: This study conducted on patients with TB in west India with DM and normoglycemia defined by glucose tolerance test (GTT) and glucose fasting. Glycocylated hemoglobin (HbA1c), lipids profile and 25-hydroxyvitamin D were measured at the time of enrollment of patients. All patients were monitored monthly while they visited in TB and chest clinic for TB treatment.
Results: Of 212 eligible patients, 117 (55.18%) were classified as diabetic, 49 (23.11%) with pre diabetic history (PDM), and 46 (21.70%) as normoglycemic (NG). DM patients were more likely to have a family history of diabetes in comparison to NG patients. Low density lipoprotein (LDH)) was higher in KDM as compared with NDM and NG patients. More patients (32) found diabetic through OGTT as compared with HbA1c (29).
Conclusions: Early EDOTS, glycemic control and improve lifestyle can reduce the heterogeneity and implications for the TB-DM. Early diagnosis of TB and DM plays an important role in the management and treatment of TBDM.

Keywords: CBNAAT, Diabetes mellitus, Diabetic/non-diabetic, GeneXpert, Pulmonary impairment, Tuberculosis

INTRODUCTION

Tuberculosis (TB) is one of the major public health problems worldwide and it is a major cause of morbidity and mortality. India has the highest number of TB cases; in 2015, of total global annual incidence of 9.6 million TB cases, 2.2 million were estimated from India in which the prevalence of TB was 195 per lacs and incidence rate was 167 per lacs population.[1] As a global disease, tuberculosis is one of the most dangerous infectious diseases in the world, causing the death of millions of people every year.[1-3] TB is caused by the bacillus *Mycobacterium tuberculosis*, the single infectious agent.[4]

With an annual TB incidence of 2.2 million cases (range 2.0-2.5 million) and an estimated 63 million people living

with diabetes mellitus (DM), India has the highest TB burden and second highest DM burden in the world. Nearly half of DM patients do not know their status, and a further 77 million people are estimated to have impaired glucose tolerance and are at higher risk of becoming diabetic.[5]

In 2015, India alone was estimated to have a staggering 69 million people aged 20-79 years affected with DM. Furthermore, with an estimated over two million new cases annually, India accounts for 18% of the world's estimated TB burden. Therefore, the public health implications of the convergence of these two epidemics in India are paramount, and screening for TB among people with DM may be an important strategy. While several studies have evaluated the burden of DM among TB patients, data on the burden of TB among people with DM in India are limited.[6] Worldwide TB takes more lives than any other infectious diseases. In the South East Asia Region, around 4.7 million people become infected with TB in 2015 and 710,000 people died due to the disease.[7] Globally, 10 million cases of tuberculosis (TB) are anticipated in 2020. there are 1.1 million kids, 3.3 million women, and 5.6 million males. TB exists in all nations and among all age groups. However, TB can be treated and avoided.[8]

Currently, non-communicable diseases (NCDs) accounting a major role in worldwide epidemiology that disproportionately affects low- and middle-income countries (LMIC) where, concomitantly, the burden of infectious diseases is high.[9] NCD Countdown 2030 is an independent collaboration to inform policies that aim to reduce the worldwide burden of NCDs, and to ensure accountability towards this aim.[10]

The World Health Organization (WHO) as well as the Revised National Tuberculosis Program (RNTCP) in India, has recommended routine testing of diabetes amongst TB patients, particularly in high TB burden settings.[11] However, a study conducted by Restrepo BI in the year 2016 elucidates the need to further explore of co-morbidities with TB, such as diabetes. Further, they have been unable to describe the prevalence of TB-DM in India due to insufficient data and limitation of the studies.[5,12,13]

It recommends screening of all registered TB patients for diabetes and ensures comprehensive diabetes care and management among diagnosed TB cases.[12,14] Such integrated efforts can augment early diagnosis and enhance good treatment outcomes.[6,15]

Aim and objectives

The aim of the present study was to assess the prevalence of DM and pre-DM, other TB co-morbidities and to know the risk of pulmonary impairment in patient of TB. The efficacy of smear and liquid TB culture positive samples with cartridge based nucleic acid amplification test (CBNAAT) in suspected diabetic patients.

METHODS

The study was conducted in the department of microbiology at Index Medical College Hospital and Research Centre, Indore (Madhya Pradesh).

Study design and duration

It was a descriptive cross sectional study conducted for a period of two years (July 2019 to July 2021) including 2 years of data analysis.

Sample size

A total number of 732 samples were collected which includes sputum, bronchoalveolar lavage and gastric aspirate.

Study population

Patients visited in TB and chest clinic and diagnosed for pulmonary tuberculosis (PTB). We included all the age groups and gender after taking written informed consent in our study.

Inclusion criteria

Patients qualified for screening if they were 25 to 60 years of age and both gender with signs and symptoms associated with PTB such as cough more than 2 weeks, weight loss, fever, chest pain and abnormal chest x-ray findings results, CBNAAT positive and patients with single drug resistance.

Exclusion criteria

Patients dis-qualified for screening if they were below than 25 years and more than 60 years of age. Patients with pre diabetic history were also excluded from the study.

Ethical approval

This study was approved by Institutional Ethics Committees (IEC), Index Medical College Hospital and Research Center (Malwanchal University) vide-MU/Research/EC/Ph.D/2019/51.

Specimen collection (PTB)

Two consecutive morning spot sputum samples were collected from suspected cases of PTB as per RNTCP protocol in a sterile, wide mouthed, leak-proof plastic container. All the patients were directed to cough deeply to produce sputum specimen and to collect without contaminating the sample collection container. If the

patient was unable to produce sputum as in the case of children or elderly patients, gastric aspirate and bronchoalveolar lavage fluid (BAL) were accepted for further processing.

Transport

The specimens transported from concern departments to central laboratory by maintaining cold chain with triple layer packaging.

Sample processing

The specimens processed in BSL-II laboratory with taking proper aseptic precautions and personal protective equipment (PPE).

Visually the quality of sputum sample was judged for viscosity; if it contains more saliva then the sample was rejected and requested for a fresh sample.

Z-N staining

Direct smears were prepared from the samples and stained using Z-N staining. Samples with two negative smears were recorded. These patients were approached. Study performa was filled up for those with clinico-radiological suspicion of pulmonary tuberculosis and were willing to give consent for participation in the study. Enrolled patients samples were processed further.

Middlebrook 7H9 broth culture

Decontamination procedure

2-3 times volume of 4% NaOH solution was added to an acceptable sample and left at 37ºC for 30 minutes until the sample was completely liquefied. One part of liquefied sample was separated into 1.5 ml MCT tube for further processing for liquid culture. 900 µl liquefied sample along with 500 µl negative control and 500µl positive control were pipetted into a separate 1.5 ml MCT tubes. All MCT tubes were centrifuged at 13,000 rpm for 10 minutes. The upper phase liquid was discarded. 1 ml sterile physiological saline was added to the precipitate and vortex to resuspends. The tube was centrifuged at 13,000 rpm for 10 minutes. The upper phase liquid was discarded.

Procedure

The pallet from one MCT tube was cultured into Middlebrook 7H9 broth. One smear was checked by Z-N staining. Result was recorded.

Middlebrook 7H9 broth supplemented with 0.8 ml OADC and PANTA was used for liquid culture. It was prepared as per manufacturer's instruction Hi Media (Hi Media Pvt. Ltd, Mumbai, India). 0.5 ml of processed sample was inoculated and tubes was incubated at 37±1°C. Readings were taken visually twice weekly up to 6 weeks. Positive culture with granular appearance without significant turbidity was noted. If growth was observed, Z-N staining was done to confirm the presence of AFB.

CBNAAT

It is a novel rapid automated machine for the rapid diagnosis of TB. This is the cartridge-based nucleic acid amplification test (CBNAAT) that can detect TB within 2 hours of collection along with RIF's resistance directly from the pulmonary samples. Detection based on the target sequences and nucleic acid amplification by RT-PCR and reverse transcriptase. In conical tube containing 1 ml of a sample (sputum, BAL, and gastric aspirate), 2 ml of sample reagent added and mixed vigorously. This mixture incubated at room temperature for 10 to 15 minutes and treated sample transferred into the sample cartridge chamber by using a sterile graduated/ ungraduated pipette and then cartridge loaded into the GeneXpert machine. Result interpretation done by using GeneXpert Dx System software, which measured fluorescent signals algorithm.[16]

Sample collection and processing to assess the blood sugar level

Blood samples (5 ml) were collected aseptically from each of the study participants in a dry, clean, plane tube from patients. Serum lipid profile levels were measured using an automated biochemical analyzer. Serum levels of vitamin B-12 were measured by electrochemi-luminescence immunoassay. Levels of 25-hydroxy vitamin D3 [25(OH) D3] were measured by radioimmunoassay (RIA). Levels of hemoglobin A1C (HbA1c) were measured by high-performance liquid chromatography (HPLC) through central pathology of medical college.

Process

All patients diagnosed as having active TB were screened for diabetes mellitus and measurement of fasting blood glucose, in which all TB patients were assessed for DM and vice versa. Fasting blood glucose values of ≥126 mg/dl and 110-125 mg/dl were considered as DM and pre-diabetes, respectively.

RESULTS

The total 937 patients were registered in TB and chest clinic which were as suspected of having TB but only 732 patients were enrolled on the basis of age criterion and out of 732 only 212 were eligible and found true positive in our study after confirmed through CBNAAT as shown in Table 1. The rest (520 patients) were found negative. In 212 eligible patients, 117 had DM (diabetes mellitus) and 49 had PDM (pre-diabetes mellitus) while 46 were named as NG (normoglycemic). 117 patients with

diabetes in which 80 were KDM (known diabetes mellitus) and 37 named as NDM (new diabetes mellitus).

Table 1: Comparison of result of geneXpert with AFB smear and culture.

Variables (n=732)	Smear	%	Culture	%	CBNAAT	%
Positive	159	21.72	245	33.46	212	28.96
Negative	573	78.27	453	61.94	509	69.53
Contamination/invalid result	0	0	34	4.6	11	1.51

The present analysis was conducted only on 163 participants those who completed 3 months of treatment out of 212 because 49 have pre diabetic mellitus (PDM) were excluded. Among 163 cases, 14 patients which include DM (10) and NG (4) were also not considered due to not completion of 3 months treatment. Sputum was collected at enrollment and monthly during TB treatment at which HbA1c ascertainment was replicate are shown in Table 2. DM patients were more likely to have a family history of diabetes in comparison to NG patients as they old too, but there was no differentiation in terms to history of TB.

Table 2: Summary of patients screened, enrolled, and monitored in the study.

Subjects	n=163
OGTT and FPG	142
KDM enroll	80
NG enroll	42
NDM enroll	35
KDM negative culture	11
KDM positive radiograph	2
KDM negative radiograph	3
NDM negative culture	5
NDM positive radiograph	3
NDM negative radiograph	2
NG negative culture	13
NG positive radiograph	8
NG negative radiograph	5

Patients demography, TB, family history of diabetes and body mass index. Patients accordingly to KDM, NDM and NG respectively as shown in Table 3. Average HbA1c level was notably dissimilarity in all patients group. The level of hemoglobin remains constant in glycemic condition and relapse adapting for hemoglobin don't cause any change in HbA1c group, as we wind up that anemia didn't disconcert relativeness of HbA1c levels.

Total cholesterol, low density lipoprotein (LDH) were higher in KDM as compared with NDM and NG patients while NDMs had lower high density lipoprotein cholesterol (HDL) than the other two groups. Serum triglycerides were higher in KDM and NDM than in NG patients. All three groups were vitamin D insufficient with lower values in patients with diabetes versus those who were non-diabetic as shown Table 4.

Table 3: Patient demographic, lifestyle, and anthropometric details at enrollment (in %).

Variables	KDM (n=73)	NDM (n=34)	NG (n=42)
Age (years)	46.6±10.6	42.2±8.7	38.7±11.3
Male	76	84.5	86
Female	24	15.5	14
Educated	74	54	65
Uneducated	26	42	35
Rural	45	41	44
Urban	55	59	46
Sedantary	34	26	16
Non- sedentary	66	74	84
Smoking current	18	27	29
Smoking former	24	29	32
Smoking never	58	44	39
Alcohol yes	32	29	54
Alcohol no	68	71	46
Family history of TB	30.13	17.64	23.8
Family history of DM (yes)	42	21	16.6
Family history of DM (no)	58	79	83.4
BMI, kg/m^2	23.2 T 4.0	21.8 T 3.6	19.2 T 2.0

The KDMs did not undergo OGTT; their classification was based on history and HbA1c level. NDMs and NG patients were classified on the basis of OGTT and FPG test results, in accordance with WHO guidelines. There was only moderate agreement between oral glucose tolerance test (OGTT) and hemoglobin A1c, or glycohemoglobin (HbA1c) for DM diagnosis.

Out of 76 patients who go through both tests i.e. 32 were diabetic by OGTT and 29 were diabetic by HbA1c as shown in Table 5. The lower sensitivity of HbA1c for DM diagnosis in our cohort matches results from a study conducted in a similar population and a trend identified in most studies comparing these methods for DM diagnosis.

Table 4: Laboratory data at enrollment (findings in average).

Variables	KDM (n=73)	NDM (n=34)	NG (n=42)
HB g/dl	13.4	11.9	12.4
HbA1c%	11.2 (9.3-12)	7.0 (5.7-9.2)	5.6 (4.2 -5.6)
Creatinine, mg/dl, mg/dl	0.78	0.82	0.8
Total cholesterol, mg/dl	188	165	152
LDH cholesterol, mg/dl	102	93	84
HDL	40	35	38
Triglycerides, mg/dl	127	114	92
25-hydroxyvitamin D, mg/dl	9	14	19

Table 5: Agreements between HbA1c and OGTT results for diabetes mellitus classification.

DM status	Total	OGTT		HbA1C	
		<200 mg/dl	≥200 mg/dl	<6.5%	≥6.5%
NDM	34	3	31	10	24
NG	42	41	1	39	3
Total	76	44	32	49	29

DISCUSSION

Our study has provided valuable information on the burden and related risk factor of DM (65.64%) in TB patients but it was 83% in the study conducted by Workneh et al in 2017.[17] The ratio between male and female was 4.3:1, the male participants were high in our study comparatively with the studies conducted by Thapa et al and Thorny et al because male sex was identified as a risk/associated factor for TBDM co-morbidity. Men usually practice smoking cigarettes and alcohol drinking which can predispose them to both disease conditions.[18,19] In the coming decade, TB-DM interaction will assume increase rapidly the analytic.

This study was based on the impact of DM on outcomes of TB treatment determines that DM increases the risk of the combined outcome of failure and death, death, and relapse. Notably, the pooled effect estimates for death among studies that adjusted for age and other confounding factors was found to be higher than the pooled effect estimates among the unacclimated studies. This finding not only suggests that patients with dm receiving tb therapy are at risk for poor outcomes, but that outcome studies that do not control for appropriate confounders may underestimate the negative impact of dm in tb patients.[20] We analyse socio-demographic, behavioral, clinical and other factors associated with TBDM co-morbidity.[17,21]

Most clinical evidence on TB risk and outcomes in DM comes from recollective studies, many conducted in countries less affected by the dual burden than India. Discovery from screening, conscription, and look into with roughly planned standard thought.

Similarly, being women was found to be risk factor for TBDM co-morbidity. The reason may be linked to poor health service utilization, care taking role of women for the sick, and influence of estrogen on cytokine production during TB infection that increases the vulnerability of women to TB and consequently to DM Studies conducted around the world show wide variations in the burden of DM among TB patients ranging from 1.9% to 45%.[17]

Systematic review on burden of DM among active TB patients showed the median prevalence in Asia and Indian subcontinent to be 17% and 19%, respectively, which were considerably slightly high than our finding which shows only 11.4% of DM among total 932 cases of TB.[22] However, other studies conducted in South India (Puducherry: 29%; Tamil Nadu: 25.3%; Andhra Pradesh: 31%; Kerala: 32%; Karnataka: 35%) and neighbouring countries (Pakistan: 39%; China: 30%) showed findings very high.[22-25]

Behavioral attributes such as tobacco smoking and alcohol drinking are associated with TBDM co-morbidity.[26,27] There is a bidirectional relationship between TB and diabetes; the presence of long-term diabetes may trigger TB disease and affect the prognosis, while TB worsens the glycaemic control in TB-diabetes population.[28,29]

Diabetes has been associated with delayed sputum conversion/radiographic severity in TB and TB treatment failure which were also very difficult challenge to overcome in our study.[20,30-32]

CONCLUSION

Our data suggest that DM might increase the risk for pulmonary impairment after TB, with its attendant negative impact on quality of life and capacity to work.

We expect that the trends in DM and pre-DM prevalence was confirmed when the planned accrual is achieved. The varied prevalence of other TB co-morbidities (smoking, alcohol consumption, under nutrition, and vitamin D insufficiency) and metformin treatment may also have influenced TB severity and treatment response. This was comprehensively analysed when full cohort data are available.

Finally, our data suggest that the population of people at risk for TB because of glucose metabolic disorders is larger than reflected by DM prevalence alone If pre-DM hampers immunity to TB to a degree approaching that of DM, then the estimation of the population- attributable fraction of TB cases among people with disorders of glucose metabolism would need to be revised upward.

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