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INCREASED GLYCEMIC LEVEL FAVORS THE GROWTH OF MICOBACTERIUM TUBERCULOSIS MICRO- BACILLI

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ABSTRACT

Pulmonary tuberculosis and diabetes mellitus are coexisting frequently. Diabetic patients have an increased tendency to acquire tuberculosis. The frequency of tuberculosis is 4-5 times more aggressive in poorly controlled diabetics and is significantly associated with the development of pleural effusion. Increased reactivation of tuberculosis has also been recorded in diabetics. One hundred and five patients of Diabetes Mellitus (Type-1=3, Type-2=102) with Pulmonary Tuberculosis were enrolled as case group, two hundred and ten patients of only Pulmonary Tuberculosis were enrolled as control group. Assess the clinical, bacteriological radiological images of all enrolled patients were assessed. Sex distribution (male 64.76% vs. 59.05% , female 35.23% vs. 40.95%). 55.28% of case group were positive for tuberculin skin test and 60.52% of control group were positive for tuberculin skin test. 69.67% patients of case group showed atypical pattern with lower and lower middle lung field involvement. Level of HbA1c 8.99 ±1.07 & AFB 1.51 ± 0.69 in case & level of HbA1c 5.43 ± 0.76 & AFB 1.15 ± 0.50 in control.. Hyperglycemia is significantly correlated with load of micro bacilli (Correlation Coefficient(r)= 0.3321 vs. 0.1294 & p=.0478 vs. p=0.5375). Hyperglycemia is strongly associated with severity of fever and haemoptysis. Hyperglycemia is significantly correlated with the load of acid fast micro-bacilli. So theDiabetic patients are more prone for the severity of Pulmonary Tuberculosis. Diabetic patients showed worst condition of lungs and increase the frequency of complications.

KEY WORDS: **Diabetes Mellitus, Pulmonary Tuberculosis, Atypical Pattern, Acid Fast Bacilli (AFB), HbA1c.**

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INTRODUCTION

INDIA accounts for nearly one third of the global burden of tuberculosis (TB)[1]. Although it has the second largest and fastest growing DOTS programme in the world[2], there has not been a discernible reduction in the incidence of TB in this country[3]. In 2010, WHO estimated that 285 million people were living with diabetes, of whom 7 million people developed the disease during that year and 3.9 million deaths were attributed to diabetes [4,5,]. Current predictions estimate that the prevalence of diabetes will reach 438 million by 2030 and that 80% of prevalent cases will occur in the developing world [5]. The increase is mainly driven by changes in diet and levels of physical activity [6]. In the poorest countries, diabetes is more common among the better-off, but economic development quickly reverses this trend so that people from lower socioeconomic groups are more affected by diabetes; sequelae are worse among the poor in all countries [7]. People from lower socio economic groups are therefore, more vulnerable to both diseases [8.] Diabetes mellitus (DM) increases the risk and odds of developing tuberculosis, especially in young people and in developing countries with a high background incidence of Tuberculosis. Many studies have shown that the prevalence of tuberculosis among diabetics is 2–5 times higher than in the non-diabetic population [9,10]. Experts have raised concern about the twin epidemics of DM and TB. Especially in low to middle income countries like India and China that the experiencing the fastest increase in DM prevalence and have the highest burden of TB in the world[11].

METHODS

STUDY DESIGN

This was a case control study conducted at Chhatrapati Shahuji Maharaj Medical University, Lucknow, Uttar Pradesh. Subjects were included in two group, cases (n=105) of Diabetes Mellitus with Pulmonary Tuberculosis and controls (n=210) of Pulmonary Tuberculosis without Diabetes mellitus

PATIENT SAMPLE

We included new patients of pulmonary TB patients with Diabetes Mellitus and without Diabetes Mellitus, attended outdoor or admitted in different units of Pulmonary Medicine and Medicine Department, CSMMU Lucknow from March 2009 to July 2011. The both group consisted of newly diagnosed, Pulmonary TB patients, Age 20-60 years of both sexes and there were no history of previous Anti tuberculosis treatment (ATT). Diagnosis of TB was made on the basis of clinical presentation and chest radiograph findings and was confirmed by microscopic detection of acid-fast bacilli. All patients with confirmed TB were screened for DM, if they had previous history of DM and were receiving antidiabetic treatment or were later found to have fasting blood glucose level greater than 126mg/dl , post Prandial blood glucose level more than 200 mg/dl and random blood glucose level more than 200mg/dl in accordance with WHO (World Health Organization) guidelines.

ETHICAL CONSIDERATION

The whole protocol was reviewed by Institutional Ethics Committee. The patients were provided with both verbal and written information about the whole study and related procedures. Written informed Consent was obtained from each patient willing to participate in the study, (Approval Code No-XXXV ECM-B/P5) . Each patient of this study had passed through by detailed history and through clinical examinations. This was recorded in proforma; special emphasis was on following such as Fever ,Cough, Expectoration, Haemoptysis, Chest pain, Dyspnoea, Loss of appetite,Weight loss. Diagnosis of Pulmonary Tuberculosis was done as per WHO criteria . All patients were subjected for sputum smear examination.

Three days morning sputum examination for Acid Fast Bacilli was done by Direct Smear Ziehl Neelson Method. Each patient was subjected for their Purified Protein Derivative Test (PPD) had done by Montoux Method.

ZIEHL NEELSON METHOD

REQUIREMENT

1. Carbol fuchsine
   - Basic fuchsine
   - Absolute alcohol

Solution of phenol (5 % in water)- 1000 ml. the dye is dissolved in alcohol and phenol is added to it.

2. 20 % sulphuric acid
3. Methylene blue (0.1 %).

PROCEDURE

A smear is prepared, initially, and then dried later fixed by flaming, the smear is covered with carbol fuchsine and heated until steam rises. The smear is allowed to stain for 5-10 minutes, heat being applied at intervals to keep the stain hot. Further it is washed with water and then decolorized with 20 % sulphuric acid till the real color and counter stain with methylene blue for 15-30 seconds.

Now the slide is scanned for acid fast bacilli using oil immersion lens. On microscopic examination each slide is searched for acid fast bacilli for at least 100 fields before being declared negative.

Grading according to a standard classification of WHO (0, +1, +2, +3). [*WHO, 1994*][12]

All patients were passed through their biochemical examination. Blood sample was collected from all the patients after overnight fast taken between 9.00 A.M. to 12A.M. and after two hours sub meal. Blood sugar level was estimated by GOD-POD (Glucose Oxidase –Peroxiase Method) Method. All the patients were subjected for their Glycosylated Haemoglobin Test. It was estimated with the help of ERBA Kit by Column Chromatography with Cation-Exchange Resin Method.

The glycohaemoglobin percentage is determined by measuring the absorbance at 415 nm of glycohaemoglobin fraction and the total haemoglobin fraction. The ratio of the two absorbance gave the percentage glycohaemoglobin[13]. There was no patient with risk or suspicion of AIDS or immunodeficiency virus.

STATISTICAL ANALYSIS

Data are expressed as mean and standard deviation (SD). Statistical analysis was done using GraphPad inStat Version 3.05 software Inc year 2000. A P-value of less than 0.05 was considered statistically significant. Independent t – test was used to compare continuous variable and chi-square or Fisher's exact test for categorical variable and correlation (Pearson r) assume Gaussian distribution.

RESULTS

There were 315 (case group=105, control group=210) patients enrolled in this case control study. The characteristics of socio-demographics of all participants are shown in Table 1. In this study there was no female patient as a pregnant woman in both groups. 43 patients (27 male, 16 female) had family history of diabetes mellitus in case group. Family history of Pulmonary Tuberculosis (19.05% vs. 27.14%) was found, results are shown in Table 1. Table-2 shows Patients distribution according to their HbA1c level. Table-3 Shows the Patients distribution According to their Age group and HbA1c Level. Occurrence of Fever (75.23% vs. 88.57%) and cough (87.61% vs. 91.42%) were the commonest presenting symptoms of Pulmonary Tuberculosis in both group, table-4 and figure-1 shows clinical characteristic of both group. According to clinical characteristic severity, we found that severity of fever was significantly higher in case group p=<.0001, odds ratio =6.313, 95% CI= 2.68-14.81. But there is no significant association was found in severity of cough. Mostly patients of Diabetes Mellitus with Pulmonary Tuberculosis had come with the complaint of haemoptysis as

compare to only Pulmonary Tuberculosis patients (51.42% vs. 27.14). So the interesting point in our study was that haemoptysis was significantly higher in case group (p=<.0001, odds ratio=2.84, 95% CI= 1.74-4.63) of the sample. Severity of haemoptysis was significantly higher in case group as compare to control group, p=.0095, odds ratio=3.74, 95% CI=1.42-9.813. Table-5 shows the Patients Clinical Characteristic According to their Severity. In this study we found that acid fast bacilli were less frequently sputum positive in case group as compare to control group (68.34% vs.88.41%). But the number of acid fast bacilli in per field area was found higher in case group (AFB 1.51 ± 0.69 vs. AFB 1.51 ± 0.69). Increased HbA1C is significantly correlated with load of micro bacilli (Correlation Coefficient(r)= 0.3321 vs. 0.1294 & p=.0478 vs. p=0.5375). Table-6 shows the Patients distribution according to their AFB Positivity for three days sputum Examination.

TABLE -1

SOCIOECONOMIC DEMOGRAPHIC PROFILE

Parameters	Mean ±S.D.	
	Patients of DM with PTB	Patients of only PTB
	N=105 (%)	N=210 (%)
Age	47.05±10.89	36.50±12.54
Male	68 (64.76)	124 (59.05)
Female	37 (35.23)	86 (40.95)
Area of Residence		
Rural	48 (45.71)	90 (42.85)
Urban	57 (54.28)	120 (57.14)
SES		
Upper	04 (3.81)	00 (0)
Upper Middle	24 (22.85)	10 (4.76)
Lower Middle	42 (40)	57 (27.14)
Upper Lower	26 (24.76)	106 (50.47)
Lower	09 (8.57)	37 (17.62)
Marital Status		
Married	98 (93.33)	169 (80.47)
Unmarried	07 (6.67)	41 (19.53)
Family History		
Pulmonary Tuberculosis	20 (19.05)	57 (27.14)
Diabetes Mellitus	43 (40.95)	36 (17.14)

TABLE-2

PATIENTS DISTRIBUTION ACCORDING TO THEIR AGE GROUP AND HBA1C LEVEL

Age Group	Case (105)			Control (210)		
	No.	Mean ±S.D.	HbA1c Mean ±S.D.	No.	Mean ±S.D.	HbA1c Mean ±S.D.
20-29	(7)	24.28±3.59	8.63±1.24	(79)	23.63±2.9	5.49±.81
30-39	(21)	34.71±2.34	8.91±1.01	(39)	33.41±3.20	5.34±.66
40-49	(24)	44.70±3.02	8.99±1.21	(50)	44.32±2.76	5.54±.77
50-59	(40)	54.72±3.38	9.06±1.04	(23)	53.47±2.79	5.32±.73
60	(13)	60.00±00	9.13±1.02	(14)	60.00±00	5.33±.70
Total	105	47.05±10.89	8.99±1.07	210	36.50±12.54	5.43±.76

TABLE-3
PATIENTS DISTRIBUTION ACCORDING TO THEIR HBA1C LEVEL

HbA1c	Case	Control
>4-5	0	67
>5-6	2	101
>6-7	8	33
>7-8	19	6
>8-9	29	0
>9-10	33	0
>10	14	0
Mean/SD	**8.99±1.20**	**5.43±.76**

TABLE- 4
SHOWS THE CLINICAL CHARACTERISTIC OF BOTH GROUP

Characteristic	Mean ±S.D.	
	Case, N=105 (%)	Control, N=210(%)
Fever	79 (75.23)	186 (88.57)
Cough	92 (87.61)	192 (91.42)
Haemoptysis	54 (51.42)	57 (27.14)
Night Sweat	66 (62.85)	155 (73.81)
Chest Pain	67 (63.81)	151 (71.90)
Breathlessness	80 (76.19)	162 (77.14)
Weight loss	7.02 ±6.20	6.85±4.12

Table-5
Patients Clinical Characteristic According to their Severity

Characteristics	Case (n=105)	Control (n=210)	p value
Fever absent	24 (11.42%)	26 (24.76%)	
Fever severity			
Mild	86 (40.95%)	21 (20.00%)	.0001
Moderate	92 (43.81%)	37 (35.23%)	
Severe	08 (03.81%)	21 (20.00%)	
Cough absent	18 (08.57%)	13 (12.38%)	
Type of cough			0.42
Dry	54 (25.71%)	21 (20.00%)	
Productive	138 (65.72%)	71 ((67.61%)	
Severity of sputum:			
Purulent	55 (26.19%)	16 (15.23%)	0.97
Mucopurulent	53 (25.23%)	39 (37.14%)	
Mucoid	30 (14.28%)	15 (14.28%)	
Haemoptysis Absent	153 (72.85%)	51 (48.57%)	
Severity of haemoptysis			
Streaking	31 (14.76%)	18 (17.14%)	.009
Moderate	19 (09.04%)	24 (22.85%)	
Severe	07 (03.33%)	12 (11.42%)	

TABLE-6
PATIENTS DISTRIBUTION ACCORDING TO THEIR AFB POSITIVITY FOR THREE DAYS SPUTUM EXAMINATION

AFB Positivity	Case n=105(%)	Control n=210 (%	OR (95% CI) *p* value	
AFB Positive 1st Day	47 (44.76)	114 (55.28)	.68 (.42-1.09)	.14
AFB Positive 2end Day	60 (57.14)	121 (57.61)	.98 (.61-1.57)	.98
AFB Positive 3rd Day	56 (53.33)	117 (55.71)	.90 (.56-1.45)	.77

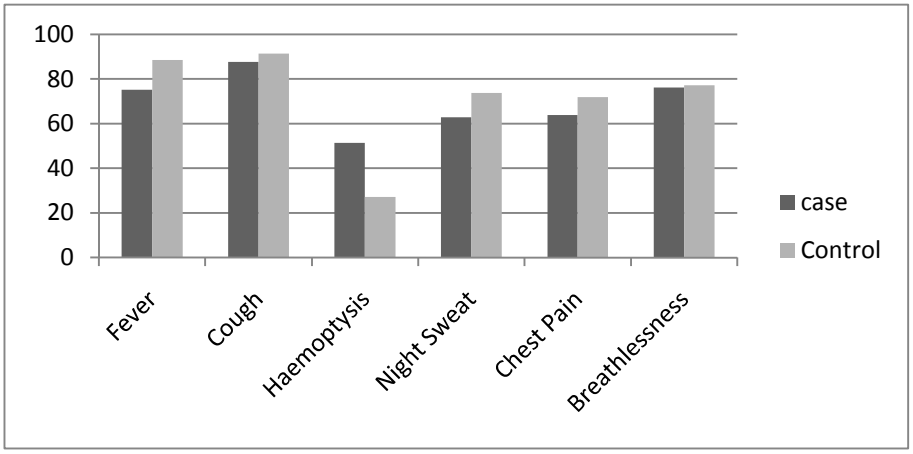

FIGURE-1
SHOWS CLINICAL CHARACTERISTIC

DISCUSSION

There were no study that directly assessed the role of glycemic level and growth of *M. Tuberculosis* (micro-bacilli). Tuberculosis will infect someone with low immune system. They are vulnerable to infection compare to those who have high immune system. Diabetic patients are very vulnerable to tuberculosis infection because they have very low immune system and has two to five times risk towards the tuberculosis compare to the others who did not has diabetes[10,11]. Poorly controlled diabetes lead to multiple complications including vascular disease neuropathy and increased susceptibility to infection[14] Diabetes also lead to increased susceptibility to disused by *M. Tuberculosis* . The mechanisms include those directly related to hyperglycemia and cellular insulinopenia, as well as indirect effects on macrophage and lymphocytes function leading to diminished ability to contain the organism. *M. Tuberculosis* is a strictly aerobic bacteria, it therefore multiplies better in pulmonary tissues (in particular at the apex where oxygen concentration is higher) than in the deeper situated organs. Increased glycemic level provides all the favorable conditions that is required for the multiplication of *M. Tuberculosis*. Increased glycemic level is impaired chemotaxis of monocytes and this defect does not improve with insulin[15]. Increased glycemic level adversely affect T-cell production of interferon γ, and T-cell growth function and proliferation. Interferon γ potentiates the nitric-oxide dependent intracellular killing activity of microphages. In experiment involving mice with streptozotocin-

induced diabetes that were challenged with *M. Tuberculosis,* concentrations of interferon γ were diminished, and production of inducible nitric-oxide synthase by macrophages was low[16]; bacterial burden was also higher than in control mice[17]. Interferon-γ production was further impaired in high glucose conditions[18]. In addition, concentrations of interleukin 12, a T-cell-stimulating factor produced by macrophages, were lower in the lungs and spleen of diabetic animals. Similarly, in the Goto Kakizaki rat model of NIDDM, interferon-γ, interleukin- 12, and nitric-oxide production were diminished in response to *M tuberculosis*[19]. Lymphocyte proliferation in response to phytohaemagglutinin is weak in patients with poorly controlled IDDM.[20]. The results shows that load of *acid fast bacilli is significantly higher in case group.*

CONCLUSION

Lung is the most commonly affected organ in tuberculosis. In the 2004 CDC surveillance reports 79.5% of the newly diagnosed cases of tuberculosis had lung involvement[21]. Hyperglycemia favors the growth, viability and propagation of tubercle bacilli. Disturbance in electrolyte balance and local tissue acidosis favor infection. Impaired phagocytosis and impaired cellular immunity in person with diabetes allow for the spread of the disease over neutralizing antibodies in bronchial secretion. Lower resistance due to vascular damage to lung tissue and Disordered nutritional balance all are the also responsible for the growth of bacilli.

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REFERENCES

1. Khatri G R, Frieden T R. Controlling tuberculosis in India. N Engl J Med 2002; 347: 1420–1425.
2. Narayanan P R, Garg R, Santha T, Kumaran P P. Shifting the focus of tuberculosis research in India. Tuberculosis 2003; 83: 135–142.
3. Khatri G R, Frieden T R. Rapid DOTS expansion in India. Bull World Health Organ 2002; 80: 457– 463.
4. Shaw J, Sicree R, Zimmet P. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Research and Clinical Practice, 2010, 87:4–14.
5. Roglic G, Unwin N. Mortality attributable to diabetes: estimates for the year 2010. Diabetes Research and Clinical Practice, 2010, 87:15–19.
6. Wild S et al. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care, 2004, 27:1047–1053.
7. Whiting D, Unwin N, Roglic G. Diabetes: equity and social determinants. In: Blas E, Sivasankara Kurup A, eds. Priority public health conditions: from learning to action on social determinants of health. Geneva, World Health Organization, 2010 (pp77-94).
8. Blas E, Sivasankara Kurup A, eds. Priority public health conditions: from learning to action on social determinants of health. Geneva, World Health Organization, 2010.
9. Turner WM: Pulmonary tuberculosis and diabetes mellitus. Q J Med 1951;1:26-31.
10. Opsald R, Riddervald HO, Aas TW: Pulmonary tuberculosis in mitral stenosis and diabetes. Acta Tuberc Scand 1961;4:290-294.

11. WHO (2007) Global tuberculosis control: surveillance, planning, financing. WHO report 2007. WHO/HTM/TB/ 2007.376. Geneva: WHO. Available: http://www.who.int/ tb/publications/global_
12. World Health Organization, Treatment of Tuberculosis: Guidelines for national programmes, 2nd ed. WHO/TB/ 97.220, Geneva, 1997
13. Bloomgarden ZT. Infl ammation and insulin resistance. Diabetes Care 2003; 26: 1922–1926.
14. Moutschen MP, Scheen AJ, Lefebvre PJ. Impaired immune responses in diabetes mellitus: analysis of the factors and mechanisms involved. Relevance to the increased susceptibility of diabetic patients to specifi c infections. Diabetes Metab 1992; 18: 187–201.
15. Saiki O, Negoro S, Tsuyuguchi I, Yamamura Y. Depressed immunological defence mechanisms in mice with experimentally induced diabetes. Infect Immun 1980; 28: 127–131.
16. Wang CH, Yu CT, Lin HC, Liu CY, Kuo HP. Hypodense alveolar macrophages in patients with diabetes mellitus and active pulmonary tuberculosis. Tuber Lung Dis 1999; 79: 235–242.
17. Abbras CK. Fc receptor-mediated phagocytosis: abnormalities associated with diabetesmellitus. Clin Immunol Immunopathol 1991; 58: 1–17.
18. Chang FY, Shaio MF. Decreased cell-mediated immunity in patients with non-insulin-dependent diabetes mellitus. Diabetes Res Clin Pract 1995; 28: 137–146.
19. Yamashiro S, Kawakami K, Uezu K, et al. Lower expression of Th1-related cytokines and inducible nitric oxide synthase in mice with streptozotocin-induced diabetes mellitus infected with Mycobacterium tuberculosis. Clin Exp Immunol 2005; 139: 57–64.
20. Martens GW, Arikan MC, Lee J, Ren F, Greiner D, Kornfeld H. Tuberculosis susceptibility of diabetic mice. Am J Respir Cell Mol Biol 2007; 37: 518–524.
21. Sugawara I, Yamada H, Mizuno S. Pulmonary tuberculosis in spontaneously diabetic goto kakizaki rats. Tohoku J Exp Med 2004; 204: 135–145.