Association between Pulmonary Tuberculosis and Type 2 Diabetes in Sudanese Patients

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Abstract

Introduction: Tuberculosis (TB) and diabetes mellitus (DM) are both important health issues, and the association between DM and TB may be the next challenge for global TB control worldwide, type 2 DM (T2DM) responsible for 90% of DM cases. Persons with diabetes have a significantly increased risk of active TB, which is two to three times higher than in persons without diabetes. The aim of this study was to determine the association between pulmonary tuberculosis (PTB) and T2DM among Sudanese patients and also to determine the association between hemoglobin A1c (HbA1c) percentage in diabetic patients and development of PTB and effect of duration of T2DM in developing PTB.

Materials and Methods: A total of 120 sputum samples were collected from patients during 6 months in Ribat University Hospital, Khartoum, Sudan. Sixty of them were known type 2 diabetic patients categorized as study group and sixty were nondiabetic patients categorized as control group. Ziehl–Neelsen smear preparation and DNA were extracted from sputum for detection of Mycobacterium tuberculosis by polymerase chain reaction (PCR). Results: Among the 120 sputum specimens, 72 (60%) were males and 48 (40%) were females. Fourteen (19.4%) males and 6 (12.5%) females had PTB, the difference was not statistically significant according to gender P = 0.229. According to treatment modalities, diabetic patients were treated with injectable insulin (36.7%), PCR positive was 4(33.3%) P value (0.853), oral hypoglycemic drugs (51.7%) PCR positive 7 (58.3%) P value (0.849) and dietary control (11.7%) PCR positive (1 (8.3%) P value (1.000) Were insignificant differences. The frequency of HbA1c of 58 patients with diabetes was 24 (41.4%) who had controlled DM (HbA1c level ≤6.5%) and 34 (58.6%) had uncontrolled DM. Of the 60 patients with diabetes, 12 had PTB with uncontrolled DM, with significant difference (P=0.000). The mean duration of diabetes mellitus was (6.92 years ± Std 6.801) and the frequency of diabetes mellitus in first 10 years was 47 (78.3%), in (11-20) years was 10 (16.7%) and in (21-30) years was 3 (5%), the PCR positive PTB showed 10(21.3%) for the first 10 years, (11-20) years was 2 (20%) and zero (0.0%) for (21-30) years, P-value (0.480) insignificant different. Conclusions: In summary, we found consistent evidence for an increased risk of TB among patients with uncontrolled DM (high-level HbA1c).

Keywords: Diabetes mellitus, polymerase chain reaction, pulmonary tuberculosis, Sudan, Ziehl–Neelsen stain

INTRODUCTION

Tuberculosis (TB) remains a major source of morbidity and mortality throughout the world. One-third of the world’s population is estimated to be infected with Mycobacterium tuberculosis (MTB), whereby approximately 9 million people develop the disease each year, and almost 2 million of populations die annually as a result of TB. According to the World Health Organization, there are over 347 million people in the world suffering from diabetes mellitus (DM). Projections indicate that by 2030 DM will become the seventh leading cause of death worldwide, and type 2 DM (T2DM) will be responsible for 90% of DM cases. Persons with diabetes have a significantly increased risk of active TB, which is two to three times higher than in persons without diabetes. People with diabetes also suffer from a number of pulmonary physiologic abnormalities including diminished bronchial reactivity. Abnormalities in the pulmonary physiologic functions lead to delayed clearance of microorganisms from the respiratory system and facilitate the spread of infections in the host which is also due to their immunocompromised status with reactivation of older foci of TB rather than through fresh contact and often exhibit lower lobe involvement more commonly than in persons without diabetes.

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The aim of the present study was to determine the association between PTB and T2DM among Sudanese patients and also to determine the association between hemoglobin A1c (HbA1c) percentage in patients with diabetes and developing pulmonary TB (PTB) and effect of duration of T2DM in developing PTB.

**Materials and Methods**

This is a case–control study conducted in a total of 120 sputum samples collected from patients during 6 months in Ribat University Hospital, Khartoum, Sudan. Sixty of them were known type 2 diabetic patients categorized as study group and sixty were nondiabetic patients categorized as control group.

Basic information of the patients was collected by a questionnaire including age, sex, socioeconomic status, duration of diabetes, type of treatment, and HbA1c%.

Ziehl–Neelsen (ZN) smear preparation, fixation, and staining were done according to Monica Cheesbrough[12]

**Molecular biology processing**

**Sputum preparation for DNA extraction**

Equal volumes of specimen and NaOH 4% (decontamination and homogenization reagent) were added to the sample. Finally, the solution was homogenized by vortexing the tube.

**DNA extraction**

Genomic DNA was extracted from sputum by using Cinna Pure™ DNA kit, according to manufacturer’s instructions. MTB gene was detected by polymerase chain reaction (PCR) using two primers from published data[13] as follows: F: CCTCCGAGCTAGGCGTCGG, R: CTCGTCCAGCGCCGCTTCGG. The primers amplify and detect an Is6110 gene (123 bp) with 25 μl of reaction mixture containing 5 μl DNA sample, 5 μl Maxime PCR PreMix Kit (i-Taq) (iNtRON Biotechnology Inc., Korea), 1 μl of each forward and reverse primer, and 13 μl nuclease-free water.

The conditions for PCR analyses were as follows: Incubation at 94°C for 5 min, followed by 30 cycles at 94°C for 1 min, 67.5°C for 1 min, 72°C for 1 min, and end (1 cycle) at 72°C for 7 min. After the last cycle, the samples were incubated at 72°C for 1 min, 72°C for 1 min, and end (1 cycle) at 72°C for 10 min. PCR products were analyzed by electrophoresis in a 2% agarose gel and observed under UV transillumination.

**Data analysis**

Data were analyzed using computerized program Statistical Package for (SPSS) software (version 16, SPSS, Inc, Chicago, IL, USA.), namely Chi-square and cross tabulation.

**Results**

A total of 120 sputum samples were collected from patients during 6 months, sixty of them were known type 2 diabetic patients categorized as study group and sixty were nondiabetic patients categorized as control group. Seventy-two (60%) were males and 48 (40%) were females. Fourteen (19.4%) males and 6 (12.5%) females had PTB, the difference was not statistically significant according to gender ($P = 0.229$).

The results of sputum smears of ZN staining were positive in 15 (12.5%) in comparison with 20 (16.7%) positive by PCR and 100 (83.3%) negative by PCR [Figure 1], which showed statistically significant difference ($P = 0.000$) [Table 1].

In the study group (diabetic group), PCR positive was 12 (20%) higher than control group (nondiabetic group) which was 8 (13.3%), which was insignificantly different ($P = 0.232$) [Table 2].

The frequency of HbA1c of 58 diabetic patients was 24 (41.4%) with controlled DM (HbA1c level ≤ 6.5%) and 34 (58.6%) with uncontrolled DM. Of the 60 patients with diabetes, 12 had PTB with uncontrolled DM, which shows statistically significant difference ($P = 0.000$) [Table 2].

The mean duration of diabetes mellitus was (6.92 years ± Std 6.801) and the frequency of diabetes mellitus in first 10 years was 47 (78.3%), in (11-20) years was 10 (16.7%) and in (21-30) years was 3 (5%) the PCR positive PTB showed 10 (21.3%) for the first 10 years, (11-20) years was 2 (20%) and zero (0.0%) for (21-30) years, $P$-value (0.480) insignificant different [Table 2].

According to treatment modalities, in the study group (diabetic patients), 22/60 (36.7%) of them were treated with injectable insulin, PCR positive was 4 (33.3%) $P$ value (0.853). 31/60 (51.7%) received oral hypoglycemic drugs, PCR positive 7 (58.3%) $P$ value (0.849) and 7/60 (11.7%) had dietary control, PCR positive 1 (8.3%) $P$ value (1.000) was insignificant differences [Table 2].

| Table 1: Comparison between polymerase chain reaction and Ziehl-Neelsen stain |
|-----------------|---------|---------|--------|-----|
| Factor          | PCR positive | PCR negative | Percentage | $P$ |
| ZN smear positive | 15       | 0       | 100     | 0.000 |
| ZN smear negative | 5       | 100     | 25      |     |
| Total            | 20       | 100     | -       |     |

**Figure 1:** IS6110 gene results for tuberculosis samples; lane 3 and 4 represent the positive sample for IS6110 with band 123bp. Lane 1 is a positive control (123bp) and lane 2 is a negative control and lane M is 100 bp molecular ladder.
When the frequency according to socioeconomic status was concerned, PTB was found in low socioeconomic class (19.2%), moderate (16.9%), and high (25%), which showed insignificant difference ($P = 0.956$) [Table 2].

The mean age of patients in this study was 46 years ± 16, and the most frequent age group was (50–59) years’ (26.7%), the distribution of PTB among age group in non diabetic was higher in age group (20–29 years) which was 4 (50%) PCR positive, while diabetic group showed higher PTB in age group (50–59 years) where was 4 (33.3%) PCR positive [Table 3].

**DISCUSSION**

More than 8 million of people develop active PTB all over the world, and 2 million die because of this highly contagious infection. It is said that about one third of the world’s population is infected with MTB, but not all of those infected develop active TB because the immune system controls the infection. However, in some people, the bacteria can remain dormant for years and could become active disease at a later stage, especially in those with risk factors such as old age, diabetes, those on immunosuppressive treatments, and patients with HIV.

Despite the control strategies, TB remains a major health problem in Sudan. While DM is a known risk factor for TB, no studies have been conducted in Sudan on TB in type 2 diabetic patients, and the exact prevalence of TB in this group of patients is still unknown.

PCR is an alternative method for diagnosis of PTB besides the culture and ZN stain, with the advantage of a rapid and simultaneous identification of MTB, but with the disadvantage of a higher cost while ZN is cheap and easy to perform but its low sensitivity is a major drawback.

PCR technique can still be advantageous when compared with conventional methods for the rapid diagnosis of TB. This technique can reduce the diagnosis time and may increase the detection of mycobacteria in smear negative TB, and that is approved in our study whereas PCR positive result of PTB showed 16.7% which was higher than 12.5% of positive result by ZN smear.

This study showed that PTB among DM group was higher than in nondiabetic patients; 12 (20%) and 8 (13.3%) respectively, among the total population, 12 (60%) patients who got PTB infection belong to DM group. This result indicates that DM is

| Table 2: Pulmonary tuberculosis and different variables |
| --- |
| **Factors** | **Frequency** | **PCR** | **P** |
| Gender | | | |
| Male | 72 | 14 | 58 | 0.229 |
| Female | 48 | 6 | 42 | 0.232 |
| Diabetic | 60 | 12 | 48 | 0.232 |
| Nondiabetic | 60 | 8 | 52 | 0.232 |
| Treatment of DM and PTB | | | |
| Injection | 22 | 4 (33.3) | 18 (37.5) | 0.853 |
| Tablets | 31 | 7 (58.3) | 24 (50) | 0.849 |
| Diet regulation | 7 | 1 (8.3) | 6 (12.5) | 1.000 |
| PTB and level of HbA1c among DM | | | |
| Controlled HbA1c | 24 | 0 | 24 | 0.000 |
| Uncontrolled HbA1c | 34 | 12 | 22 | 0.000 |
| Association of diabetic duration with PTB (years) | | | |
| 1-10 | 47 | 10 | 37 | 0.480 |
| 11-20 | 10 | 2 | 8 | 0.849 |
| 21-30 | 3 | 0 | 3 | 0.853 |
| PTB in different socioeconomic classes | | | |
| Low | 26 | 5 (25) | 21 (22.6) | 0.956 |
| Moderate | 83 | 14 (70) | 69 (74.2) | 0.956 |
| High | 4 | 1 (5) | 3 (3.2) | 0.956 |
| Missed | 7 | - | - | 0.956 |

PCR: Polymerase chain reaction, DM: Diabetes mellitus, PTB: Pulmonary tuberculosis, HbA1c: Hemoglobin A1c

| Table 3: Distribution of pulmonary tuberculosis among different age groups of the study population |
| --- |
| **Factor** | **Result of PCR positive** |
| **Age group (years)** | **Frequency (%)** | **Non diabetic, frequency (%)** | **Diabetic, frequency (%)** |
| <20 | 5 (4.2) | 0 | 0 |
| 20-29 | 20 (16.7) | 4 (50) | 0 |
| 30-39 | 14 (11.7) | 1 (12.5) | 2 (16.7) |
| 40-49 | 20 (16.7) | 0 | 3 (25) |
| 50-59 | 32 (26.7) | 2 (25) | 4 (33.3) |
| >60 | 29 (24.7) | 1 (12.5) | 3 (25) |
| Total | 120 (100) | 8 (100) | 12 (100) |

PCR: Polymerase chain reaction
associated with PTB infection although the statistical analysis showed insignificant relation ($P = 0.232$). However, several other studies showed a significant association.$^{[9,20,21]}$ These variations may attribute to the sample size, type of DM, or duration of DM and study duration.

This study showed insignificant difference with diabetes treatment and PTB, while in another study in Ethiopia, it was found that the risk of developing TB among insulin-dependent patients with diabetes and noninsulin-dependent diabetic patients was 26 times and seven times, respectively, that of the general population.$^{[22]}$

This study showed an association between PTB and uncontrolled diabetic patients with high level of HbA1c, which is statistically significant ($P = 0.000$), similar result was found in other studies.$^{[23,24]}$

Patients with DM duration of 1–10 years showed higher incidence of PTB, other studies showed increase of duration significant with higher prevalence of PTB.$^{[25]}$ According to Bokam and Pujitha Thota,$^{[26]}$ increase of HbA1c with increase in duration of diabetes may be due to that the patients do not know they have diabetes and the infection itself may be the cause for higher HbA1c. In addition, some investigators suggested that the association reflects the effect of DM on TB, some controversy over the directionality of the association remains due to observations that TB disease induces temporary hyperglycemia, which resolves with treatment.$^{[27]}$

Our study has the following limitation; our database does not include differences in the outcome of TB treatment in patients with and without DM.

Data from human studies are consistent with emerging information on the biological mechanisms by which hyperglycemia may affect the host immune response to TB. Our findings suggest that TB control programs should consider targeting patients with diabetes for interventions such as active case finding and the treatment of latent TB; diagnosing, detecting, and treating DM may have a beneficial impact on TB control.

**CONCLUSION**

We found consistent evidence for an increased risk of TB among patients with uncontrolled DM (high-level HbA1c) in Sudanese population.

**Recommendation**

Based on our results, we recommend that:

- Negative results of ZN smears should be confirmed by PCR
- Patients with diabetes should follow up the level of blood glucose to avoid the complication
- Screening of TB among patients with diabetes and screening of diabetes among patients with TB
- Surveillance is needed in our country to demonstrate the prevalence of DM in patients with TB
- Differentiation between Type I diabetic patients versus Type II diabetic patients in their association with TB
- The outcome of TB treatment in patients with and without DM
- We also recommend further studies investigating how TB risk varies by type, duration, and severity of DM, for a more thorough understanding of the association that could be translated into a clear public health message.

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**Conflicts of interest**

There are no conflicts of interest.

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