INTRODUCTION

The "captain of all these men of death", tuberculosis (TB) has been a menace to the humankind since the ancient time. TB—a serious and highly infectious disease caused by Mycobacterium tuberculosis is the second leading cause for the high incidence of mortality rates worldwide. TB affects the lungs (Pulmonary tuberculosis—PTB) more commonly than the other parts including pleura, central nervous system, lymphatic system, bone and joints (extra-pulmonary tuberculosis). Hemoptysis, night sweats, loss of appetite and weight loss are the common symptoms of PTB [1, 2].

India is the country of highest TB and diabetes mellitus (DM) burden [3, 4]. According to the world health organization (WHO) 2014 estimates, incidence of 2.5 million cases of active TB was reported to be in India out of 9.6 million cases globally in India. It also adds that "about 40% of the Indian population and one-third of the world's population are infected with the Mycobacterium tuberculosis, where most of them have a latent infection rather than the active disease" [5, 6].

TB is referred as the top infectious killer disease worldwide [7]. In India, therapeutic success is achieved only in 80% of the treated TB patients, with 4% death, 2% treatment failure and 6% treatment default in patients on anti-tubercular therapy (ATT) [8]. TB is extremely associated with other comorbid conditions like diabetes mellitus, HIV/AIDS, multi-drug and extreme drug resistance [8].

The WHO recommends combination therapies with isoniazid, rifampicin, ethambutol, pyrazinamide, streptomycin and other second line agents in intensive and continuous phases for the treatment of pulmonary tuberculosis [9]. Being highly bactericidal, rifampicin forms the nucleus of anti-tubercular therapy. However, the drug is associated with high inter-individual variability, and various clinical, environmental and genetic factors tend to alter the pharmacokinetic profile of rifampicin [10].

Diabetes mellitus has been reported to influence oral rifampicin pharmacokinetics by causing variability in absorption leading to decrease the systemic bioavailability of the drug [11]. Decreased systemic exposure to the drug leads to the emergence of multi-drug resistant bacilli and/or treatment failure [12]. Although several studies have been reported on the pharmacokinetics of rifampicin in tuberculosis patients, the impact of diabetes as a comorbid disease, on the peak serum rifampicin concentration is not addressed in India. Hence this study was carried out with an objective to determine the effect of diabetes mellitus on the peak serum concentration of rifampicin.

MATERIALS AND METHODS

Method

The study was carried out as a cross-sectional observational study in the chest and TB department of a tertiary care hospital. The study protocol was approved by the institutional ethics committee, Sri Ramachandra University [IEC/12/MAR/94/09]. The study participants were recruited after obtaining written informed consent. All PTB patients between ages eighteen and sixty-five of either gender were included in the study. Pregnant females with tuberculosis, tuberculosis patients of both genders who were retrospective were excluded from the study.

Serum collection and storage

Five ml of blood was withdrawn by venipuncture from each patient at a time point of 2 h post dose administration at steady state (C ss). The samples were collected in serum separator vacutainers containing clot activator and allowed to stand for 30 min. The vacutainers were centrifuged at a rate of 3500 rotations per minute (rpm) for a period of fifteen minutes, and the resultant serum was stored at -70 °C until analysis.
Estimation of rifampicin concentration [Srivastava et al., 2012] [13]

The frozen samples were thawed at room temperature, and an aliquot of 200 µl sample was transferred to pre-labeled Ria vials. 50 µl of internal standard (Roxithromycin 1.000 µg/ml) was added and vortexed well using cyclomixer. 0.400 ml of 100% acetonitrile was added, and the capped vials were re-vortexed in vibramax at 2000 rpm for 10 min followed by centrifugation at 4500 rpm for 10 min at 4 °C. 0.300 ml of supernatant was transferred into pre-labeled injector vials and loaded into LC-MS autosampler. Estimation of rifampicin concentration was carried out in Thermo TSQ Ultra (MS/MS) With Shimadzu 20 AD UFLC LC-MS. ZORBAX Eclipse Plus C18 column of dimensions 4.6 mm x 150 mm, 5 µm and acetonitrile 10 mmol, ammonium acetate (80:20% v/v) were used as stationary and mobile phases respectively at a flow rate of 1 ml/minute.

Statistical analysis

All statistical analyses were performed using SPSS 17.0 and Graph pad prism 7.0. Pearson’s correlation was used to determine the linear dependency of Cmax on individual covariates. Chi-square analysis was used to determine the effect of dichotomous categorical variables. Unpaired student t-test was used to compare two groups. A p-value less than 0.05 were considered statistically significant throughout the study (95% CI).

RESULTS

Forty-five patients who fulfilled the inclusion criterion were recruited in the study. Twenty-five patients were non-diabetic whereas 20 patients were diabetic. The mean (SD) age of the study population was 46.8 (14.2) years. The study participants constituted of 68.88% male and 31.12% female (fig. 1). The distribution of study population based on their age group is depicted in table 1.

The mean (SD) age of males was comparatively higher than that of females with values of 48.7 (10.6) and 43.1 (11.2) respectively (P=0.532). The mean age of non-diabetic TB patients was comparatively higher than that of diabetic patients with values of 46.6 (8.4) and 42.4 (9.2) respectively.

Table 1: Age-wise distribution

| Age  | No. of patients N = 45 | Percentage (%) |
|------|-----------------------|----------------|
| 21-30| 16                    | 35.5           |
| 31-40| 11                    | 26.5           |
| 41-50| 7                     | 15.5           |
| 51-60| 9                     | 20             |
| >61  | 2                     | 4.5            |

The body weight of about 86.6% of the patients was less than 50 kg and 13.4% of patients was greater than 50 kg. No statistically significant difference in body weight was found between diabetic and non-diabetic patients (P=0.5440). About 51.12% of the patients were smokers and 48.88% were non-smokers. The most commonly prescribed concomitant medications were ranitidine and aluminum hydroxide (36%). The other co-medication prescribed were insulin (34%), paracetamol (18%), theophylline (4%) and supplements (8%). The distribution of study participants based on the RNTCP (revised national tuberculosis control program) treatment (CAT I and CAT II) they received is shown in fig. 2.

Around 55.55% were found to be alcoholic and 44.45% were non-alcoholic. Nearly 68.8% patients had a prior history of TB whereas 31.2% patients had no history of TB. Twenty-five (58%) patients of the study population were non-diabetic, and 20 (42%) were diabetic. Almost 83% of the diabetic patients were on a combination of insulin and metformin. Very few patients received the other oral hypoglycemic medications.
The mean serum $C_{\text{max}}$ of rifampicin was significantly less in diabetic patients with pulmonary tuberculosis ($P=0.0305$) as shown in fig. 3. Statistically, a significant difference in the incidence of a decrease in $C_{\text{max}}$ was found between diabetic and non-diabetic patients ($P=0.0335$) as depicted by fig. 4.

**DISCUSSION**

This study has examined the effect of diabetes on the peak serum concentration of rifampicin in pulmonary tuberculosis patients, comparing diabetic and non-diabetic patients. The correlation between RBS and rifampicin $C_{\text{max}}$ in pulmonary tuberculosis patients without diabetes is shown in fig. 5. The fig. 6 depicts the correlation between RBS and rifampicin $C_{\text{max}}$ in pulmonary tuberculosis patients with diabetes.

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