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DOES DIABETES MELLITUS AFFECT THE PLASMA CONCENTRATION OF ANTITUBERCULOSIS AGENTS?

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ABSTRACT

Plasma concentrations of isoniazid, rifampicin, and pyrazinamide were measured in 22 diabetic and 22 non-diabetic pulmonary tuberculosis patients by high performance liquid chromatographic methods. From every patient, five blood samples were taken at 0.5, 1, 2, 3, and 4 hours after witnessed ingestion of antituberculosis agents. The maximum plasma concentration (C_{max}) and the time to reach the C_{max} (T_{max}) for each drug were determined directly from the concentration-time profile. Area under the curve (AUC) was calculated from isoniazid and rifampicin only. The presence of diabetes mellitus did not significantly affect any pharmacokinetic parameters of the three agents. AUC_{0-24h} of isoniazid was significantly higher in female patients. The AUC_{0-24h} of rifampicin was significantly correlated with patients' age. Weight-adjusted dose of each drug positively and significantly correlated with the corresponding drug C_{max}.

Keywords: antituberculosis, pharmacokinetic, diabetic, tuberculosis

INTRODUCTION

Tuberculosis (TB) continues to be a major global health problem. Each year, tuberculosis seriously affects the health, and kills millions of people worldwide [1]. In 2014, world health organization (WHO) estimated the incident of 9.6 million new TB cases. Most of TB patients are resident in Asia and Africa which account for 58% and 28% of the estimated cases respectively [1]. Although the disease is curable, especially with the availability of the short course chemotherapy, it still ranks as a second killer infectious disease alongside HIV [1]. The prevalence of diabetes mellitus (DM) is increasing due to increasing the prevalence of obesity, and changing the life style. It has been estimated that the number of diabetic patients will rise from 171 million in 2000 to 366 million by 2030 [2]. About 75% of diabetic patients will be living in low income countries where TB is also prevalent [2]. Diabetes mellitus is a known risk factor for development of active TB; the risk of active TB is three times higher in diabetic patients [3, 4]. Among the diabetic patients, the risk of developing active TB is higher in Insulin-dependent DM (IDDM) and in patients with poor glycemic control [5, 6]. Diabetes mellitus was found to be associated with increased treatment failure and death during the treatment of tuberculosis [7-10]. Diabetes mellitus may also affect the absorption of antituberculosis drugs; this issue is of a special importance since unfavorable TB treatment outcomes such as treatment failure, relapse and death was associated with low area and the curve (AUC) or maximum concentration (C_{max}) of some of the first line antituberculosis agent [11, 12].

Plasma concentrations of rifampicin and isoniazid after two hours of drug administration were significantly lower in diabetic patients [13, 14]. In one study, the AUC of rifampicin was significantly reduced in diabetic patients [15]. However, other studies did not found a significant effect of DM on the AUC or C_{max} of rifampicin and other first line antituberculosis agents [16–18]. In face of the contradictory of the available data regarding the effect of diabetes mellitus on the blood concentration of antituberculosis agents, we perform a case control study to clarify this issue.

The main objectives of this study are; 1) to find out the effect of diabetes mellitus on the pharmacokinetics of isoniazid, rifampicin, and pyrazinamide. 2) To explore the effect of other patients' clinical and social variables on the pharmacokinetic parameters of isoniazid rifampicin and pyrazinamide.

METHOD

Ethical approval: Ethical approval was obtained from the Ministry of Health and Ethic Committee (MREC). Every enrolled patient was given an informed consent form. The patients have to sign the informed consent form, in order to be accepted in the study. Verbal clarification was given to any patient according to their wishes.

Study design and inclusion of patients: A case control study was conducted in the out-patients clinic in a referral hospital (Pulau Pinang hospital) in Penang, Malaysia. Patients were included in the study if they meet the following inclusion criteria

1. Confirmed diagnosis of pulmonary tuberculosis
2. Being treated with the standard 6-months short course regimen under direct observation therapy (DOT)
3. Still in the intensive phase of tuberculosis regimen.
4. Age of 18 years old or more

Patients were excluded if they were infected with human immunodeficiency virus (HIV), were having cancer, or hepatic impairment. Patients who are infected with multidrug resistance (MDR) *M. tuberculosis*, pregnant women and lactating women were also excluded. Diagnosis of pulmonary TB (PTB) was based on the clinical symptoms consistent with the disease such as cough with or without sputum, loss of appetite and weight, fever, night sweating and dyspnea or chest pain. The diagnosis of PTB was confirmed by chest radiographic finding consistent with PTB and/or positive sputum smear microscopy for acid fast bacilli (AFB).

Hepatic impairment was defined as the elevation of Alanine transaminase (ALT), or Alkaline Phosphatase (ALP), to ≥ 2 times the upper limit of normal (ULN), or the elevation of total bilirubin level to more than 25 µmol/L. Patients who meet the inclusion and exclusion criteria and had previously established diagnosis of DM and were on anti-diabetic medication were considered as (cases), and referred to as (TB-DM patients). Each case was matched with a control non-diabetic TB patient according to the gender, body weight (± 5kg) and smoking status, these control patients are referred to as (TB patients)

Blood collection: The day of collecting the blood samples was during the intensive phase, after at least 15 days of starting the therapy to ensure that all the antituberculosis agents, special rifampicin are in the steady state concentration [19]. On the day of blood samples collection, the patients were asked to refrain from food intakes eight hours before the administration of antituberculosis agents until the completion of blood sampling. Five venous blood samples (2 mL each) were collected at 0.5, 1, 2, 3, and 4 hours after the administration of antituberculosis agents. Each blood sample was collected in 2 mL BD vacutainer tube containing 3.6 mg K2 EDTA. Each blood sample was centrifuged within 10 minutes of collection. Separated plasma was stored at -20 C°.

Determination of the plasma concentration of isoniazid and pyrazinamide: Plasma concentration of INH and PZA was determined simultaneously by a validated high-performance liquid chromatographic (HPLC) method with ultra-violet (UV) detection. One hundred and fifty µL of plasma was placed in 1.5 mL micro-centrifuge tube, and 75 µL of 10% trichloroacetic acid (TCA) containing 100 µg/mL of acetanilide as the internal standard (IS) was added. After mixing on a vortex shaker for 20 seconds, the mixture was centrifuged at 3000 rpm for 7 minutes. To a100-µL aliquot of the supernatant, 20 µL of 0.1% *trans*-cinnamaldehyde in methanol was added and left for 10 minutes. To neutralize the PH, 40 µL of 1M ammonium acetate was added. Finally, 20 µL of the solution was injected into HPLC system. HPLC analysis was performed on Zorbax Eclipse Plus C_{18} column (150 × 4.6 mm. particle size 5µm), equipped with Zorbax C_{18} guard column. The mobile phase consists of HPLC grade Acetonitrile as solvent A and 20mM 1-hexane sulfonic acid sodium salt (PH 2.7) as solvent B.

The mobile phase was delivered as gradient elution, started as 4% solvent A at flow rate of 1 mL/minute for the first 4.5 minutes. Then, the percentage of acetonitrile and the flow rate were increased linearly to reach 50% and 1.5 mL/minute respectively by 9 minutes. Finally, the percentage of acetonitrile and the flow rate were decreased linearly to reach the initial setting by 10-minute and kept stable to end of the run at 13 minutes. The volume of injection was 20 µL. The signals were monitored at 269 nm, 254 nm and 340 nm for pyrazinamide, acetanilide and isoniazid-*trans* cinnamaldehyde derivative respectively. Pyrazinamide was eluted firstly with a retention time of 4 minutes, followed by acetanilide at 7.5 minutes and finally the isoniazid at 8.5 minutes.

The method was validated for pyrazinamide in the range of 3 to 75 mg/L and for isoniazid in the range of 0.6 to 15 mg/L. All calibration curves were linear (r^2 > 0.998). The method was accurate as the percentage of relative error (RE, %) was < 4.5% for each drug. Intra- and Inter-day precision were good for both drugs, with the highest relative standard deviation (RSD, %) was 8.51%. Recoveries of isoniazid, pyrazinamide, and Acetanilide were in the range of 86.5 to 101.5%. The lower limit of detection (LOD) was 0.25 and 1.00 mg/L for isoniazid and pyrazinamide respectively. The lower limit of quantification (LLOQ) was 0.60 mg/L for isoniazid and 3.00 mg/L for pyrazinamide

Determination of the plasma concentration of rifampicin: One hundred µL of plasma was mixed with 100 µL of acetonitrile containing 4 mg/L of acetanilide as the internal standard (IS). After mixing on a vortex shaker for 20 seconds, the mixture was centrifuged at 3000 rpm for 7 minutes. From the supernatant, a 20 µL was taken and injected directly into HPLC system. HPLC analysis was performed on same instruments used for the determination of pyrazinamide and isoniazid.

The mobile phase consists of HPLC grade acetonitrile as solvent A, and 20mM ammonium acetate (pH 4.7) as solvent B. The mobile phase was delivered as 30% solvent A for the first 2 minutes, then percentage of acetonitrile was increased linearly to reach 50% at minute 6. This composition was maintained for a further 1 minute. At minute 7, the percentage of acetonitrile was decreased linearly to the original composition by minute 7.50. This later composition was maintained until the end of the run at minute 10. The flow rate was set at 1mL/minute. The volume of injection was 20 µL, and signals were monitored at 254 nm, and 334 nm for IS and RIF respectively. Calibration curve was linear in the range of 0.75 to 40 mg/L, (r^2 = 0.997 ± 0.002, n =3). Accuracy was good since all quality control standards had < 7% deviation from their nominal concentrations. Intra-day and inter day precision was good, with the highest (RSD, %) was 8.1%. Mean recovery of rifampicin and IS from plasma was 108.8%, and 99.6% respectively. Lowe limit of detection was 0.5 mg/L, and the lower limit of quantification was 0.75 mg/L.

Pharmacokinetic parameters: For every patient the maximum drug concentration (C_{max}) was the highest concentration among the five samples. The T_{max} was the corresponding time at which the C_{max} was observed. Areas under the curve were estimated for RIF and INH only according to the equations developed by Magis-Escurra et al. [20] as the following;

For isoniazid $AUC_{0\text{-}24h}$ = - 1.15 + 7.97 (H C_{3h}), where HC_{3h} is the plasma concentration of isoniazid in the three hours post dose sample

For rifampicin $AUC_{0\text{-}24h}$ = - 0.86 + 7.16 (R C_{4h}), where RC_{4h} is the plasma concentration of rifampicin in the four hours post dose sample

Statistical analysis: All the statistical analyses were carried out by IBM SPSS version 22, P value of less than 0.05 was considered as statistically significant

The different concentrations of INH, RIF, and PZA in the different plasma samples as well as the C_{maxs} and $AUC_{0\text{-}24h}$ were represented as Mean with (minimum – maximum) range if the data exhibit normal distribution or as a median with (minimum – maximum) range if the normality of the distribution was significantly violated. Normality of data distribution was assessed by the normality tests provided in the SPSS package (Shapiro-Wilk, *P* value of 0.05 or less indicate the violation of normality). C_{maxs} of INH, RIF and PZA were also categorized into 3 groups (low, normal and high) according to their proposed reference range of 3 – 6, 8 – 24 and 20 – 60 mg/L for INH, RIF, and PZA respectively [21]. Comparison of continuous variables between diabetic and diabetic patients was made by independent sample T-test or Mann-Whitney U test. The effect of other factors on the different pharmacokinetic parameters of INH, RIF, and PZA was tested by bivariate correlation if the two variables were presented as continuous data or by independent samples T-test or Mann-Whitney U test if one variable was presented as continuous data and the other variable was presented as categorical variable. If both variables were categorical, Pearson's μ^2 test or Fisher's exact test was used.

RESULTS

Patients' characteristics: Twenty two diabetic patients with confirmed diagnosis of PTB (TB-DM) was recruited and matched with the same number of non-diabetic PTB patients (TB) according to the gender, smoking status and body weight. All the diabetic patients were known cases of diabetes and were using oral anti-diabetic and/or insulin. Twenty six (59.1%) of patients were males. The age of the patients was between 18 and 74 years with a mean of 47.6 ± 14.90 (SD), diabetic patients were older, but the difference between the two groups was not statistically significant. During the intensive phase, all the patients were prescribed isoniazid, rifampicin, pyrazinamide, and ethambutol in a fixed dose combination (FDC), known commercial as Akurit4® which contains 75 mg isoniazid, 150 mg rifampicin, 400 mg pyrazinamide, and 275 mg ethambutol per tablet. Drug dosing was according to the patients' body weight band recommended by the world health organization (WHO). Patients who weight 30 - 37 kg received 2 tablets daily, patients who weight 38 – 54 kg received 3 tablets daily, patients who weight 55 kg or above received 4 tablets daily. There was no different in mean weight-adjusted dose of any drug between diabetic and non-diabetic patients. Baseline laboratory investigations were not significant different between TB-DM and TB patients. Table 1 shows the basic social and clinical characteristic of the diabetic and non-diabetic patients included in the study

Pharmacokinetic parameters of isoniazid: Plasma concentration of isoniazid in the five blood samples as well as the isoniazid C_{max}, T_{max}, and AUC_{0-24h} are presented separately for diabetic and non-diabetic patients in table 2. In both diabetic and non-diabetic patients there was a considerable interindividual variability in each parameter, for example the isoniazid C_{max} ranges from 1.60 to 11.70 mg/L in diabetic patients and from 1.20 to 8.10 mg/L in non-diabetic patients. In diabetic and non-diabetic patients one hours post dose was the median isoniazid T_{max}.
Both isoniazid C_{max}, and AUC_{0-24h}, were higher in diabetic patients. However, these differences were small and statistically insignificant when tested by independent samples T-test or Mann-Whitney U test. Similarly the T_{max} of isoniazid was not significantly different between diabetic and non-diabetic patients. (table 2). According to the reference range of isoniazid C_{max}, the proportion of patients with low, normal or high isoniazid C_{max} was almost equally distributed between the two groups of patients.
Isoniazid AUC_{0-24h} was significantly affected by the patients' gender. Mann-Whitney U test revealed that median isoniazid AUC_{0-24h}, was significantly higher in female patients (30.17 vs. 16.14 mg ×h /L, U = 151.50, z = - 1.97, P = 0.049, r = 0.300).
Isoniazid C_{max} was significantly and positively correlated with the weight-adjusted dose of isoniazid (Pearson correlation, r = 0.299, P = 0.049).
Isoniazid T_{max} was negatively and significantly correlated with the patients' body weight (Spearman's rank order correlation, rho = - 0.429, P = 0.004).

Pharmacokinetic parameters of rifampicin: Like isoniazid, in both diabetic and non-diabetic patients there was a wide interindividual variation in the rifampicin plasma concentration in each blood sample, as well as in the C_{max} and AUC_{0-24h} of rifampicin (table 3). Both C_{max} and AUC_{0-24h} of rifampicin were higher in diabetic patients, but the difference between the two groups did reach statistical significant levels. Median rifampicin T_{max} was similar in the two groups (2.00 hours). More than half of the patients in each group have rifampicin C_{max} below < 8 mg/L. Low rifampicin C_{max} was more common in non-diabetic patients (72.7% vs. 54.5%), however, this difference did not reach statistical significant level.

Rifampicin AUC_{0-24h} was significantly and positively correlated with the patients' age (r = 0.388, P = 0.009), and the weight-adjusted dose of rifampicin (r = 0.316, P = 0.037). Contrary to isoniazid, the mean rifampicin AUC_{0-24h} was higher in male patients (39.67 vs. 30.22 mg × h/L); however, this difference did not reach statistically significant level as shown by independent samples T-test (P = 0.056).
Rifampicin C_{max} was positively correlated with the weight-adjusted dose of rifampicin (r = 0.338, P = 0.025). Rifampicin T_{max} was negatively correlated with the body weight of the patients (rho = - 0.346, P = 0.021).

Pharmacokinetic parameters of pyrazinamide: In both diabetic and non-diabetic patients, there was a marked interindividual variation in each of the pyrazinamide concentrations (table 4). The C_{max} of pyrazinamide ranges from 20.50 to 71.40 mg/L in diabetic patients and from 34.80 to 63.40 mg/L in non-diabetic patients. Presence of diabetes mellitus did not significantly affect the C_{max} of pyrazinamide. Pyrazinamide C_{max} below 20 mg/L was not encountered in any patients. However, the proportion of patients with pyrazinamide > 60 mg/L was higher in diabetic patients (27.3% vs. 13.6%) but this difference was not statistically significant. Pyrazinamide tend to reach its maximum concentration faster in diabetic patients, as the

median T_{max} was 1.50 hours in diabetic patients and 2.00 hours in non-diabetic patients. Pyrazinamide C_{max} was positively and significantly correlated with the weight-adjusted dose of pyrazinamide (r = 0.394, P = 0.008). Pyrazinamide T_{max} was negatively and significantly correlated with the patients' body weight (rho = - 0.413, P = 0.005)

DISCUSSION

Effect of diabetes mellitus on the pharmacokinetics of antituberculosis agents: In the current study, presence of diabetes mellitus did not significantly affect the maximum plasma concentration or the time to reach the C_{max} of isoniazid, rifampicin, and pyrazinamide. Similarly, the estimated AUC $_{0\text{-}24h}$ of isoniazid and rifampicin were not affected significantly by the presence of DM. In fact each of the three C_{maxs} and the two AUC $_{0\text{-}24h}$ was higher in diabetic patients, specially the C_{max} and $AUC_{0\text{-}24h}$ of rifampicin. The median time to reach the C_{max} of each drug was similar in both groups except for pyrazinamide for which the median T_{max} was slightly shorter but not statistically significant in diabetic patients. Previous studies have shown different results regarding the effect of diabetes on these pharmacokinetic parameters. Nijland et al. [15] found that the C_{max} and the AUC $_{0\text{-}6h}$ of rifampicin was significantly lower in diabetic TB patients. However, it should be emphasized that, in that study the patients were not matched according to the body weight, therefore, the mean body weight was significantly higher in diabetic patients (55.6 vs. 46.2 Kg, P = 0.01). Furthermore all the patients had received the same dose of rifampicin (450 mg three times weekly); consequently, the dose of rifampicin per kilogram of patients' body weigh was much lower in diabetic patients. In our study, although the drugs were administered in weight-adjusted doses, we found a significant positive correlation between the dose per kilogram of patients' body weight of each drug and the C_{max} of that drug. The positive correlation between the drug's doses per Kg of patients' body weight was found in other studies [17, 22]. Therefore, the significant reduction in C_{max} and AUC of rifampicin in diabetic patients observed in Nijland et al. study might be because diabetic patients were receiving lower dose (as mg/kg body weight) than the non-diabetic patients

In another study, where diabetic patients were matched with non-diabetic according to the body weight and gender, Ruslami et al. [16] found no significant effect of DM on the pharmacokinetic parameters of rifampicin, pyrazinamide and ethambutol.

Babalik et al. [13] found that the C_{2h} of isoniazid and rifampicin was significantly lower in diabetic patients. However, in this study the body weight of diabetic patients was significantly higher than non-diabetic patients, and drugs doses per kilogram of patients' body weight were significantly lower in diabetic patients. These two factors might explain the significant negative effect of diabetes on the C_{2h} of isoniazid and rifampicin. This explanation is supported by the fact that in that study there was a significant negative correlation between the patients' body weight and C_{2h} of both isoniazid and rifampicin. In our study the C_{2h} of both isoniazid and rifampicin was slightly but not significantly higher in diabetic patients.

Burhan et al. [14] also found that the isoniazid C_{2h} was significant lower in diabetic patients (1.1 vs. 1.5 mg/L, P = 0.02). However, similar to other studies, the difference in mean body weight between diabetic and non-diabetic patients may contribute to significant difference in drug concentration. Requena-Méndez et al. [18] found that the rifampicin C_{2h} and C_{6h} were not significant affected by the presence of diabetes mellitus

The effect of the patients' social and clinical factors on the pharmacokinetics of isoniazid, rifampicin, and pyrazinamide: The AUC $_{0\text{-}24h}$ of isoniazid was significantly higher in female patients (30.17 vs. 16.14 mg X h/L, P = 0.049). Similar result was reported by McIlleron et al. [17] who found that AUC $_{0\text{-}8h}$ of isoniazid was significantly reduced by 23% in male patients. In our study we did not find any other significant different in the pharmacokinetic parameters of antituberculosis agent between males and females; however, the AUC $_{0\text{-}24h}$ of rifampicin was higher in male patients (39.70 vs. 30.20 mg/L, P = 0.056). Although, this difference did not reach statistical significance, it is against the finding of other studies [15, 17, 18, 23] which all demonstrate that AUC or the concentration of rifampicin was higher in female patients. The discrepancy between our finding and the results of other studies could be due to that in our study, female patients were significantly younger than male patients (42.11 ± 16.57 vs. 51.42 ± 12.56, P = 0.040), and there was a significant positive correlation between rifampicin AUC $_{0\text{-}24h}$ and the patients' age which overcome the negative effect of male gender on the AUC $_{0\text{-}24h}$ of rifampicin. Partial correlation indicated that, positive correlation between age and rifampicin AUC $_{0\text{-}24h}$ remains after the control for the effect of gender (r = 0.327, P = 0.032 "2-tailed").

We found a significant negative correlation between the patients' body weight and the T_{max} of isoniazid, rifampicin, and pyrazinamide. The magnitude of these correlations was higher with the T_{max} of INH. However, all of these correlations are considered as medium strength correlation. No previous study has reported a significant effect of the patients' body weight on the T_{max} of antituberculosis agents. The dose per kilogram patient's body weight of each drug was significantly and positively correlated with the C_{max} of that drug. The strength of association was higher with pyrazinamide followed by rifampicin and isoniazid. The dose per kilogram patient's body weight of rifampicin was also significantly correlated with its AUC_{0-24h}. Our results are in line with the result reported by McIlleron et al. [17] who found a significant positive correlation between doses per kilogram patients' body weight of rifampicin, isoniazid, and pyrazinamide and their AUC. Similarly, Um et al. [22] demonstrated a significant positive correlation between dose per kilogram patients' body weight of rifampicin, isoniazid, and pyrazinamide and their C_{2h}.

CONCLUSION

The presence of diabetes mellitus did not alter the pharmacokinetic of isoniazid, rifampicin, and pyrazinamide significantly. Regardless the presence of diabetes mellitus, the C_{max} of rifampicin was below the reference range in most of the patients. However, the C_{max} of isoniazid was below the reference range in about 20% of the patients only, and the C_{max} of pyrazinamide was not below the reference range in any patients. The C_{max} of isoniazid, rifampicin, and pyrazinamide correlated positively and significantly with the weight-adjusted dose of the corresponding drug. There was a significant and negative correlation between the T_{max} of each drug and the body weight of the patients. The AUC_{0-24h} of isoniazid was significantly higher in female patients. The AUC_{0-24h} of rifampicin positively and significantly correlated with the patients' age, and the rifampicin weight-adjusted dose.

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Tabe1: Base-line patients' social and clinical characteristics

Patients' characters	TB-DM n =22	TB n =22	P value
Gender, No. (%) male	13 (59.1)	13 (59.1)	1.00[$]
Smoking status No. (%) of smoker	7 (31.8)	6 (27.3)	1.00[$]
Body weight in kilogram Mean (Min – Max)	55.8 (39.0 – 76.0)	53.31 (34.0 – 72.0)	0.358[#]
Age in years Mean (Min – Max)	51.59 (23.0 – 74.0)	43.77 (18.0 - 71)	0.079[#]
INH dose in mg/kg Mean (Min – Max)	4.83 (3.70 – 5.60)	4.88 (3.95 – 5.60)	0.769[#]
RIF dose in mg/Kg Mean (Min – Max)	9.66 (7.40 – 11.20)	9.76 (7.90 – 11.20)	0.769[#]
PZA dose in mg/Kg Mean (Min – Max)	25.82 (19.75 – 30.00)	25.96 (21.00 – 30.00)	0.865[#]
Total protein in g/L Mean (Min – Max)	74.97 (59.00 – 88.00)	78.36 (56.00 – 96.00)	0.226[#]
Albumin in g/L Mean (Min – Max)	28.51 (20.00 – 38.00)	31.13 (17.00 – 42.00)	0.621[*]
Bilirubin in µmol/L Median (IQR)	11.50 (3.00 – 29.00)	10.50 (4.00 – 54.00)	0.144[*]
HGB in g/dL Mean (Min – Max)	12.05 (7.20 – 16.30)	11.79 (8.50 – 15.70)	0.694[*]

TB = Tuberculosis, DM = diabetes mellitus, Min = minimum concentration,
Max = maximum concentration, [$] = Fisher's exact test, [#] = independent samples T-test,
[*]= Mann-Whitney U test

Table 2: Pharmacokinetic parameters of isoniazid in diabetic and non-diabetic pulmonary tuberculosis patients

Pharmacokinetic parameters	TB-DM patients, n =22	TB patients , n =22	*P* value
Isoniazid $C_{0.5h}$, mg/L Median (Min - Max)	4.55 (0.00 – 11.70)	2.85 (0.00 – 8.00)	0.256*
Isoniazid C_{1h}, mg/L Mean (Min - Max)	4.85 (0.00 – 10.30)	3.86 (0.00 – 8.10)	0.237#
Isoniazid C_{2h}, mg/L Mean (Min - Max)	4.36 (0.00 -9.90)	3.36 (0.00 -6.90)	0.151#
Isoniazid C_{3h}, mg/L Median (Min - Max)	2.65 (0.60 – 9.60)	2.40 (0.90 – 6.70)	0.944*
Isoniazid C_{4h}, mg/L Median (Min - Max)	1.90 (0.00 – 8.20)	2.15 (0.00 – 6.10)	0.869*
Isoniazid C_{max}, mg/L Mean (Min - Max)	5.87 (1.60 – 11.70)	5.23 (1.20 – 8.10)	0.425#
Isoniazid AUC, mg×h/L Median (Min - Max)	19.80 (2.68 – 75.52)	18.17 (5.94 – 52.41)	0.944*
Isoniazid T_{max}, hours Median (Min - Max)	1.0 (0.50 – 3.00)	1.00 (0.50 – 4.00)	0.300*
Isoniazid C_{max} proportion of patients in each category	< 3 mg/L = 22.7% 3 – 6 mg/L= 31.8% > 6 mg/L = 45.5%	< 3mg/L = 18.2% 3 – 6 mg/L= 31.8% > 6 mg/L = 50.0%	0.924□

TB = Tuberculosis, DM = diabetes mellitus, Min = minimum concentration, Max = Maximum concentration, * = Mann-Whitney U test, # = independent samples T-test, □ = Chi square test of independence

Table 3: Pharmacokinetic parameters of rifampicin in diabetic and non-diabetic pulmonary tuberculosis patients

Pharmacokinetic parameters	TB-DM patients, n=22	TB patients , n=22	statistical test, *P* value
Rifampicin $C_{0.5h}$, mg/L Median (Min - Max)	2.70 (0.00 – 12.90)	2.40 (0.00 – 20.80)	M, 0.162*
Rifampicin C_{1h}, mg/L Median (Min - Max)	6.20 (0.00 – 16.40)	3.45 (0.00 – 15.00)	0.133*
Rifampicin C_{2h}, mg/L Mean (Min –Max)	7.37 (0.00 – 16.80)	5.61 (0.00 – 11.80)	0.138#
Rifampicin C_{3h}, mg/L Mean (Min –Max)	7.03 (2.70 – 12.40)	5.63 (0.00 – 10.40)	0.115#
Rifampicin C_{4h}, mg/L Mean (Min – Max)	5.51 (2.20 – 10.20)	4.64 (1.10 – 8.40	0.198#
Rifampicin C_{max}, mg/L Median (Min - Max)	8.95 (3.70 – 16.80)	6.80 (1.30 – 20.80)	0.091*
Rifampicin AUC $_{0-24h}$, mg×h/L Mean (Min – Max)	38.70 (14.75 – 72.10)	32.91 (7.02 – 59.21)	0.241#
Rifampicin T_{max}, hours Median (Min - Max)	2.0 (0.50 - 3.00)	2.0 (0.50 - 4.00)	0.211*
Rifampicin C_{max} proportion of patients in each category	< 8 mg/L = 54.5% 8 – 24 mg/L= 45.5% > 24 mg/L = 0.0%	< 8 mg/L = 72.7% 8 – 24 mg/L= 27.3% > 24 mg/L = 0.0%	0.347$

TB = Tuberculosis, DM = diabetes mellitus, Min = minimum concentration, Max = Maximum concentration * = Mann-Whitney U test, $ = Fisher's exact test, # = independent samples, T-test,

Table 4: Pharmacokinetic parameters of pyrazinamide in diabetic and non-diabetic pulmonary tuberculosis patients

Pharmacokinetic parameters	TB-DM patients, n=22	TB patients, n=22	*P* value
Pyrazinamide $C_{0.5h}$, mg/L Mean (Min – Max)	34.50 (0.00 – 71.40)	26.57 (0.00 – 57.30)	0.195[#]
Pyrazinamide C_{1h}, mg/L Mean (Min – Max)	42.82 (6.60 – 68.90)	35.43 (0.00 – 63.40)	0.137[#]
Pyrazinamide C_{2h}, mg/L Mean (Min – Max)	42.02 (18.50 – 71.40)	37.29 (11.80 – 50.60)	0.179[#]
Pyrazinamide C_{3h}, mg/L Median (Min – Max)	36.75 (20.50 – 61.80)	37.50 (26.20 – 62.10)	0.814[*]
Pyrazinamide C_{4h}, mg/L Median (Min – Max)	31.15 (18.50 – 59.00)	35.05 (22.20 – 57.50)	0.805[*]
Pyrazinamide C_{max}, mg/L Mean (Min – Max)	48.47 (20.50 – 71.40)	46.15 (34.80 – 63.40)	0.532[#]
Pyrazinamide T_{max}, hours Median (Min - Max)	1.50 (0.50 - 3.00)	2.0 (0.50 - 4.00)	0.300[*]
Pyrazinamide C_{max} proportion of patients in each category	< 20 mg/L = 0.0% 20 – 60 mg/L = 72.7% > 60 mg/L = 27.3%	< 20 mg/L = 0.0% 20 – 60 mg/L = 86.4% > 60 mg/L = 13.6%	0.457[$]

TB = Tuberculosis, DM = diabetes mellitus, Min = minimum concentration, Max = Maximum concentration [*] = Mann-Whitney U test, [$] = Fisher's exact test, [#] = independent samples, T-test,

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