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# PLASMA CONCENTRATION OF PYRAZINAMIDE IN NEWLY DIAGNOSED PULMONARY TUBERCULOSIS PATIENTS WITH AND WITHOUT DIABETES MELLITUS.

Pharmacology

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## ABSTRACT

**Aim.** To determine and compare Pyrazinamide 0,2,4 and 6 hours plasma concentration in Pulmonary TB patients with and without DM on Anti-Tubercular therapy. **Method.** A Cross-sectional study was done on 58 newly diagnosed Pulmonary TB patients with and without DM on anti-TB drug therapy. The Sample was collected pre-dose(0hour) and 2,4,6 hours post-dose administration in the intensive phase. Plasma concentrations of Pyrazinamide at 0,2,4 and 6 hours were analyzed by HPLC. Maximum serum concentrations (Cmax) and Areas under the Plasma concentration-time curve (AUC0-6) were also calculated. **Result.** Plasma concentration of Pyrazinamide was significantly lower in Diabetics as compared to Non-Diabetic TB patients. The Mean±SD of Pyrazinamide Cmax were 31.34±4.66 µg/ml and 42.17±8.12 µg/ml in Diabetic TB patients and Non-Diabetic TB patients respectively. Mean AUC0-6 was significantly lower in Diabetic TB patients (146.50 ± 23.05 µg·h/ml) than Non-Diabetic TB patients (197.22 ± 36.71 µg·h/ml). Pyrazinamide plasma levels and HbA1c showed negative correlation in diabetic TB patients with Pearson correlation coefficient r(26) = -0.76,p-value < 0.01.**Conclusion.** Mean Pyrazinamide Cmax level differed significantly by Diabetic Status. Plasma levels of Pyrazinamide were below target (35µg/ml) in most of the diabetic TB patients. There is a need for individualized dosing of Pyrazinamide based on plasma concentration measurements (Therapeutic drug monitoring) in patients of TB with DM.

## KEYWORDS

Diabetes Mellitus, Tuberculosis , Pyrazinamide, Plasma concentration

## INTRODUCTION

Pulmonary Tuberculosis (TB) is one of the most common infectious diseases in developing countries. Among the greatest recognized risk factors for TB include HIV infection, immunosuppressive therapies, smoking, air pollution, alcoholism, malnutrition, chronic kidney disease, and diabetes. Diabetic patients have a three times greater risk of developing TB[1]. The burden of Diabetes has steadily increased over the past quarter-century in India and across the globe, with India contributing a major part of the global burden. The World Health Organization has identified DM as a neglected, important, and re-emerging risk factor for TB. There are now more individuals with TB-DM co-morbidity than TB-HIV co-infection[2].

**Pyrazinamide**, the pyrazine analog of nicotinamide, is a First-line anti-tubercular agent. Pyrazinamide (PZA) has potent sterilizing activity and has the potential to shorten the TB treatment duration[3]. Some studies showed that Diabetes mellitus is associated with low plasma concentration of Pyrazinamide[4,5]. A few studies were done in the Indian population regarding the effect of DM on the concentration of Anti-Tubercular drugs, so the effect of DM and HemoglobinA1c (HbA1c) values on the plasma concentration of Pyrazinamide remains poorly- characterized. The present study aimed to determine and compare plasma concentrations of Pyrazinamide (PZA) in pulmonary TB patients with and without DM.

## MATERIAL AND METHODS

### Study population

The present cross-sectional observational study was performed at the Department of Pharmacology, JLN Medical College and Hospital, Ajmer, India from March 2020 to October 2020 following approval by the institutional Ethics Committee. Patients aged >18 years with a diagnosis of Pulmonary TB With and without Diabetes mellitus were enrolled in the study after providing written informed consent. Patients with any of the following conditions were excluded from the study: HIV infection, chronic alcohol consumption, pregnancy, known drug resistance, end-stage renal or hepatic disease and cystic fibrosis, and patients receiving drugs known to interact with Anti-tubercular drugs.

### Material

The Standard drug was purchased from Indian Pharmacopoeia Commission, Ghaziabad, U.P. HPLC grade reagents methanol, perchloric acid, water, phosphate buffer, and millipore filters of 0.20 µm and 0.45 µm were purchased from a local supplier.

### Instrument

YL9100 HPLC with a binary pump, vacuum degasser, UV/VIS detector, C18 column, and Guard column with YL autosampler was used in the study. HPLC was equipped with YL clarity software for data analysis and processing. For centrifugation microcentrifuge of Chino Scientific instruments, model CSIM-12 was used.

### Method

58 newly diagnosed Pulmonary TB patients with and without DM, registered for the treatment by the Department of Pulmonary Medicine of this college, during the period of 1st March 2020 to 31st October 2020, fulfilling the inclusion criteria were enrolled in the study. Diagnosis of pulmonary TB was based on clinical symptoms, chest x-ray, sputum microscopy and culture. Out of 58 TB Patients, 28 were Diabetics, receiving treatment for DM simultaneously and 30 Patients were Non-Diabetics. Variables studied included age, sex, height, weight, BMI, smoking status, and pre-dose (0 hours),2,4and 6 h post-dose drug plasma concentrations. Cmax and AUC0-6 for Pyrazinamide were also calculated. The area under curve from 0–6 hours (AUC0–6) was calculated by the linear trapezoidal rule. The therapeutic target concentration considered in this analysis was 35 µg/ml for Pyrazinamide[6].

All patients were administered orally first-line anti-TB drugs at DOT center as per NTEP. sampling was performed when patients were on TB treatment for at least 2 weeks, given the expected steady-state (stable pharmacokinetics) at that point. serial venous blood samples (4ml each) were collected just before (0 hours) and at 2, 4, 6 hours after observed TB drug intake. The blood samples were collected in sodium heparin vials and plasma was separated by centrifugation. Collected plasma samples were transferred into cryovials. Ascorbic Acid was added to prevent oxidation of the sample. Plasma was kept at -80º centigrade until analysis. On the day of processing, plasma samples were taken out and thawed at room temperature. A well-validated HPLC-UV method for the determination of Pyrazinamide plasma concentration was adopted for drug concentration analysis by using High Performance Liquid Chromatography (HPLC)[7].

### Plasma drug concentration analysis

1ml of plasma was deproteinated by adding 2 ml of 0.7M perchloric acid. The mixture was vortexed for 10 minutes and centrifuged at 4000 rpm for 5 min. The supernatant was neutralized with 1M NaOH and transferred into pre-labeled HPLC injector vials. The sample was filtered through 0.20 µm syringe filters before loading into the HPLC autosampler. Chromatographic analysis was performed on the C18 column protected by the guard column. The Mobile phase Prepared with methanol and phosphate buffer (pH 7.4) in the ratio of (2:98). The mobile phase was filtered before use through a 0.45µm membrane filter and degassed for 15 minutes. The mobile phase was pumped to the column at the flow rate of 1.5 mL/min. The column temperature was maintained at 30º C and the volume of the injection loop was 20µl. The column was equilibrated for at least 30 minutes with the mobile phase before analysis of drug plasma concentration. The eluent was detected by a UV detector at 268nm for Pyrazinamide. The chromatogram was run for 10 mins. Retention time (Mean ±SD) of Pyrazinamide was 8.1±.09 minutes.

## RESULT

Demographic data of included patients are shown in table no.1. Out of 58 TB patients enrolled in study 28 were Diabetics and 30 were Non-Diabetic. In this study, most of the patients (25.86%) were in the 21-30 years of age group followed by the 41-50 years age group (22.41%). Diabetic TB patients were older than Non-Diabetic TB patients and the Mean age of Diabetics (47.79±13.49 years) was significantly higher than the Non-Diabetics (38.03±15.07 years). There was a significant difference in mean weight (60.71±8.93 vs 51.4±9.66 Kg) of Diabetics and Non-Diabetic TB patients respectively. There was no significant difference in gender-wise distribution among the Diabetics and Non-Diabetics TB patients. A significant difference was found in the Mean BMI of Diabetic patients (22.17±3.39Kg/$m^2$) and Non-Diabetic patients (19.14±3.07 Kg/$m^2$).

No statistical difference was found in smoking habits between the Diabetics and Non-Diabetics TB patients. Mean Cmax of Pyrazinamide was significantly lower in Diabetics (31.34±4.66 µg/ml) as compared to Non-Diabetics (42.17±8.12 µg/ml). Further Time to reach Cmax is also longer in Diabetics (Mean Tmax=2.5±0.44 h) as compared to Non-Diabetics (Mean Tmax=2.06±0.36 h.) Cmax was below therapeutic target in 23/28(82.14%) of Diabetic and 5/30 (16.66%) of Non-Diabetic TB patients. Mean $AUC_{0-6}$ was significantly lower in Diabetic TB patients (146.50±23.05 µg·h/ml) than Non-Diabetic TB patients (197.22±36.71µg·h/ml). Mean RBS in Diabetic TB patients (192.11±40.28mg/dl) was significantly higher than Non-Diabetics (102.93±10.73 mg/dl). A negative correlation was found between RBS and Pyrazinamide plasma concentration in TB patients with and without DM with Pearson correlation coefficient (r) of -0.59, p-value < 0.01 and -0.47, p-value < 0.01 respectively. In TB patients with DM, a negative correlation was found between mean HbA1c (7.37±0.72) and Cmax of Pyrazinamide with Pearson correlation coefficient (r)= -0.75 and p-value <0.01.

**Table 1: Baseline characteristics of 58 TB patients with and without Diabetes**

| Patient Characteristics | Diabetic(n=28)[a] | Non Diabetic(n=30)[a] | P-value[b] |
|---|---|---|---|
| Age (year) | 47.79±13.49 | 38.03±15.07 | 0.04(S) |
| Body weight (Kg) | 60.71±8.93 | 51.4±9.66 | 0.0003(S) |
| Height (m) | 1.66±0.04 | 1.63±.06 | 0.15(N.S) |
| BMI (Kg/$m^2$)[c] | 22.17±3.39 | 19.14±3.07 | 0.0007(S) |
| Males | 22(78.6%) | 21(70%) | |
| Females | 6 (21.4%) | 9 (30%) | |
| Smokers | 15 | 10 | 0.19 (N.S) |
| RBS mg/dl | 192.11±40.28 | 102.93±10.73 | <0.005 (S) |
| HbA1c (gm%) | 7.37±0.72 | - | - |

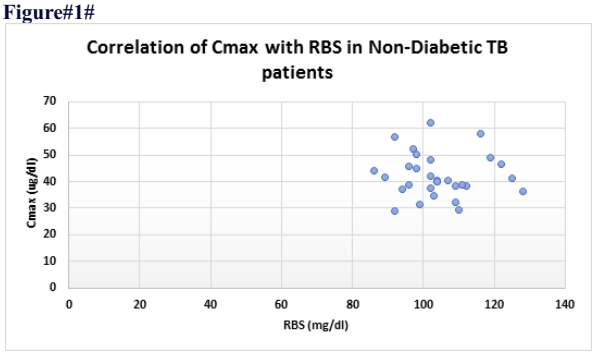
a - Data presented in Mean±SD and percentage
b - P value was considered significant at 95% confidence interval (P-value less than 0.05).
c - Calculated as the weight in kilograms divided by the square of the height in meters.

**Table 2: Plasma concentration of Pyrazinamide among TB patients with and without DM.**

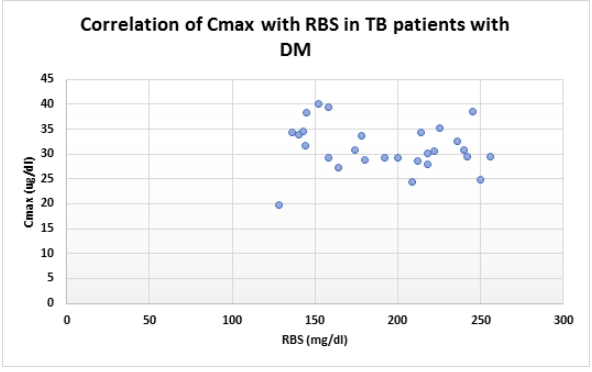
| Pharmacokinetic parameter | Patients with DM[a] | Patient without DM[a] | P-value[b] |
|---|---|---|---|
| Cmax(µg/ml) | 31.34±4.66 | 42.17±8.12 | <0.005 (S) |
| AUC0-6 (g.h/ml) | 146.50±23.05 | 197.22±36.71 | <0.005 (S) |
| Tmax(hours) | 2.5±0.44 | 2.06±0.36 | 0.02(S) |
| Patient below target Cmax(<35µg/ml) | 23(82.14%) | 5 (16.66%) | <0.001(S) |
| Cmax in Male | 31.14±4.89 | 41.64±8.34 | <0.005 (S) |
| Cmax in Female | 32.04±3.99 | 43.41±7.92 | <0.005 (S) |

a - Data presented in Mean±SD and Percentage.
b - P value was considered significant at 95% confidence interval (P-value less than 0.05).

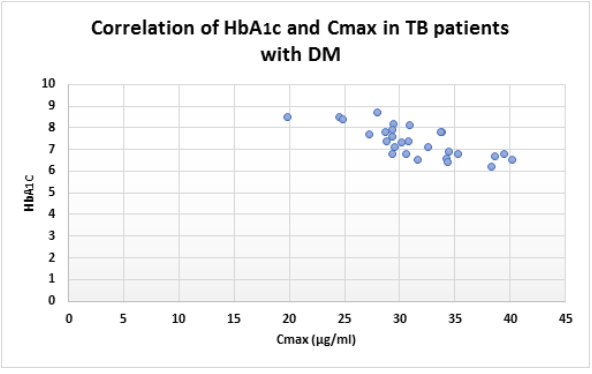
**Figure#1#**



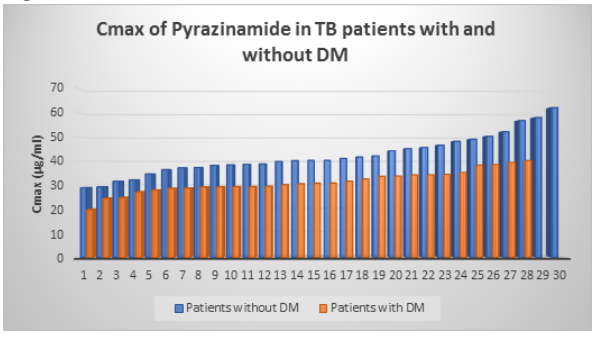

**Figure#2#**




**Figure#3#**




**Figure#4#**




## DISCUSSION

In our study, we found that Pyrazinamide plasma concentrations were significantly affected by the Diabetic status of patients. There was a significant difference in the Mean Cmax of Diabetic and Non-Diabetic

TB patients. Further Cmax of Pyrazinamide was below target in most of the TB patients with DM.

Few studies have been done to look at the effect of DM on the plasma concentration of Pyrazinamide[4,5,6]. Some studies showed a strong association of DM and Pyrazinamide plasma concentration while other studies shown no association at all. In a study by Omamah Alfarisi et al in Indian patients with Pulmonary Tuberculosis, investigators found that there was a significant difference in the Cmax of Pyrazinamide in TB patients with DM. 63.8% of the patients with DM and 34.4% of the patients without DM were below the target concentration(35µg/ml) of Pyrazinamide ($p < 0.001$)[4]. Further, Pyrazinamide concentrations decreased as HbA1c increased. In another study by Rovina Ruslami et al in Jakarta, Indonesia shown no significant differences in the AUC0-24, Cmax, Tmax of Pyrazinamide between Diabetic and non-diabetic TB patients, and no significant correlation was found between fasting blood glucose levels and the Pyrazinamide and Cmax[8]. In our study, a significant negative correlation was found between DM (HbA1c) and plasma concentration of Pyrazinamide. Many studies have shown that low plasma levels of Pyrazinamide in TB patients may be associated with poor treatment outcomes including treatment failure and emergence of MDR TB[9]. There is a need for individualized dosing of Pyrazinamide based on plasma concentration measurements (therapeutic drug monitoring) in patients of TB with DM. Recent pharmacokinetic studies suggest that 25mg/kg Pyrazinamide dose in TB is not sufficient, so higher doses of Pyrazinamide is recommended in TB patients with DM[10,11]. In our study, mean RBS level was significantly higher in diabetic TB patients as compared to non-diabetics. Significant negative correlations were found between plasma concentration of Pyrazinamide and RBS level. The low level of Pyrazinamide in TB patients with DM can be explained by the fact that Pyrazinamide is a prodrug that gets metabolized by deamidase to form the active metabolite Pyrazinoic acid (POA). Xanthine oxidase metabolizes Pyrazinoic acid to 5-hydroxypyrazinoic acid (5-OH-POA). Xanthine oxidase also metabolizes Pyrazinamide to 5-hydroxyPyrazinamide (5-OH-PZA). In some studies increased levels of Xanthine oxidase were found in animals with DM. One study was conducted by Dijana J Miric et al in Malaysia among 650 patients with type 2 DM and 280 healthy participants, the investigators found that patients with type 2 DM had higher levels of xanthine oxidase with a strong association between HbA1c values and oxidative indices[12]. Another study involving six healthy participants to examine the effect of the coadministration of Pyrazinamide with allopurinol, a xanthine oxidase inhibitor, showed that level of pyrazinoic acid and 5 hydroxy pyrazinoic acid were significantly elevated[13]. Elevated Xanthine oxidase may be attributed to low plasma level of Pyrazinamide in Diabetic TB Patients. In this study we did not measure Xanthine oxidase level, this hypothesis has yet to be proven. Another explanation could be given to elucidate low plasma concentration of Pyrazinamide even in Non-Diabetic TB patients is Rifampicin, which induces Hepatic and Intestinal CYP3A subfamily and many other metabolic pathways via activation of the Pregnane X-Receptor (PXR). For this reason, co-administration of Rifampicin results in increased metabolism and clearance of Pyrazinamide, and it could be speculated that Rifampicin may induce microsomal deamidase or some other pathway, thus enhancing Pyrazinamide clearance[14,15]. In this study we found that the time to maximal concentration (Tmax) of Pyrazinamide was much longer in patients with DM, suggesting that lower concentrations may also be associated with DM-induced changes in intestinal motility. Diabetic patients have higher body weight than Non-Diabetic Patients. In our study, the mean weight and BMI of diabetic TB patients were significantly higher than the non-diabetics. An increased volume of distribution due to higher body weight may be responsible for the low plasma concentration of Pyrazinamide[16].

There were also some limitations in our study. The sample size of the pulmonary TB patients was small. Data on the HbA1c levels of Non-diabetic TB patients was not available. To analyse the Drug Plasma levels, blood samples were collected at 0,2,4 and 6 hours post drug administration. AUC0-24 hours has a much better value for comparison of a pharmacokinetic parameter of two groups. Association between plasma concentration of Pyrazinamide and clinical outcome were not assessed. we did not adjust for multiple comparisons in our analyses, so it may be possible that some associations were present by chance. It was a single-center study, the results of which cannot be generalized. However, this provides a window of opportunity for clinicians and researchers to carry out further studies with the involvement of different geographical areas across India.

## CONCLUSION

In TB patients with Diabetes mellitus, current dosing guidelines result in below 'target' concentration of Pyrazinamide. Subtherapeutic concentration may lead to treatment failure and/or development of MDR TB. Mean plasma level of Pyrazinamide was significantly lower in Diabetics as compared to Non-Diabetic TB patients. It concludes that higher doses may be required to achieve therapeutic concentration in TB patients with DM. With increased dose, increasing adverse effects are of important concern. So, there is a need for individualized dosing of Pyrazinamide based on plasma concentration measurements (therapeutic drug monitoring) in patients of TB with DM. Further clinical trials on higher doses of Pyrazinamide in TB patients with DM are warranted to evaluate the safety and efficacy.

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