Effect of patient-specific variables on rifampicin peak serum concentration

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ABSTRACT

Introduction: Tuberculosis (TB) is a common infectious disease with high mortality rate across the globe. In spite of effective strategies and treatment modalities, it remains a global burden due to emergence of drug-resistant bacillary strains. Rifampicin is a potent bactericidal agent used in the antitubercular regimen whose altered pharmacokinetic profile, and decreased systemic bioavailability has been strongly associated with treatment failure and emergence of drug-resistant strains. In our current study, peak serum concentration of rifampicin was studied in pulmonary TB (PTB) patients. Materials and Methods: A total of 60 patients with PTB who volunteered participation were enrolled into the study. Blood samples were collected by venipuncture, and drug concentration was determined using liquid chromatography–mass spectrometry. Logistic regression models were used to determine the effect of patient-specific variables on rifampicin serum concentrations. Results: Interindividual variability was observed in serum rifampicin concentrations. Serum concentrations of rifampicin were found to be decreased in diabetic patients. Body weight, serum albumin, and blood glucose were found to be inversely correlated with rifampicin concentrations. Conclusion: Dosing individualization of rifampicin should be considered in patients with the identified factors so as to decrease the incidence of treatment failures, drug-resistant bacilli emergence, and adverse drug reactions.

KEY WORDS: Individualization, Pharmacokinetics, Rifampicin, Tuberculosis, Variability

INTRODUCTION

Tuberculosis (TB) is the most prevalent infectious disease affecting one-third of the global population. The World Health Organization (WHO) global estimates of 2015 reported 10.4 million people to be newly diagnosed with TB while the disease accounted for 1.8 million worldwide deaths in the year. In addition, TB remains the major cause of death in patients with human immune deficiency virus (HIV).[1,2] India accounts for an estimated 2.2 new cases of TB each year and harbors more than twice as many cases as any other country.[3] In addition, India has the second highest burden of multidrug-resistant TB (MDR-TB) with an estimated 99,000 new cases of MDR-TB per year. The prevalence of MDR-TB in India is 2–3% and 12–17% among new and previously treated cases, respectively.[4] The increased prevalence of MDR-TB and extensively drug-resistant tubercle bacillary strains contributes to the high risk of mortality. Despite the massive efforts of Revised National TB Control Program of India (RNTCP), TB remains a major public health issue owing to emergence of drug-resistant strains. The RNTCP promotes prompt and effective treatment of TB with directly observed treatment short-course using potent first-line drugs which include isoniazid (INH), rifampicin (RIF), ethambutol (EMB), and pyrazinamide (PZA).[5]

Various studies have assessed the importance of RIF in antitubercular therapy (ATT). In combined daily or intermittent chemotherapy, regimens containing RIF where found to be far superior to regimens that lacked RIF in both intensive and continuation phases.[6] Plasma concentrations of RIF have been reported to influence treatment response in TB patients. Strong relationships which exist between emergence of drug-resistant strains and decreased exposure to RIF, and other antitubercular medications suggest a potential irreplaceable role of RIF in ATT.[7,8] However, RIF is associated with high interindividual variability and is an autoinducer. Various factors tend to alter the
pharmacokinetic profile of RIF and thereby decrease its systemic bioavailability. The autoinductive property of the drug, on successive administrations alters the mean pharmacokinetic profile (AUC) and shifts the steady state concentration ($C_{\text{ss}}$) below the minimal effective concentration. RIF activates a ligand-activated transcription factor, the pregnane X receptor (PXR), which in turn activates the transcription of certain CYP450 genes, increasing the rate of metabolism of several drugs and RIF itself. Certain factors that tend to alter the AUC of RIF have already been reported. For instance, comorbid diabetes mellitus, HIV, concomitant administration of certain medication, and other patient-related factors are known to decrease the systemic bioavailability of RIF. Factors that decrease systemic bioavailability of RIF and exposure to RIF either prolong the duration of ATT or potentiate the risk of drug-resistant bacillary strains emergence. Hence, identification of factors and quantifying the magnitude and effect of individual variable on RIF concentrations is crucial since such an approach could aid in individualization RIF dose based on patient-specific parameters. This study was designed to determine the causes of interindividual variability in RIF peak plasma concentration ($C_{\text{max}}$) and assess the effect of identified factors on RIF $C_{\text{max}}$.

**MATERIALS AND METHODS**

**Study Site Approval**

This prospective observational study was conducted in the Government Hospital of Thoracic Medicine (GHTM), Tambaram, for 8 months. The study was permitted to be carried out at the site by the Directorate of Medical Education, Tamil Nadu. The study protocol was approved by the Institutional Review Board of GHTM, Tambaram, and the Institutional Ethics Committee of Vels University (Approval no: IEC/ DOI/2015/21). Consent from the hospital authorities was obtained before accessing the patient data.

Clinical and biochemistry data of the patients who fulfilled the inclusion criterion were documented from the case sheets and recorded in a separately designed case report form.

**Patient Recruitment**

A total of 60 patients with pulmonary TB (PTB) who met the inclusion criterion were enrolled into the study. Detailed information regarding TB, role of RIF and its adverse effects, usefulness, and risks of participating in the study were provided to the patients. Written informed consents were obtained after explaining the study protocol to each individual patient.

**Inclusion Criterion**

Adult patients of both genders tested sputum positive for *Mycobacterium tuberculosis* bacilli and objectively confirmed diagnosis through chest X-ray, both newly diagnosed and relapse TB to be started on ATT with RIF were included in the study.

**Exclusion Criterion**

Pregnant and lactating females, HIV diagnosis, unwillingness to participate in the study, and patients with underlying medical conditions that compromise their safety and warrant study participation.

**Blood Sampling**

About 5 mL of blood sample was collected by venipuncture from each patient after 2 h of RIF administration as it was the expected time taken for RIF to attain $C_{\text{max}}$ ($t_{\text{max}}$).

**Serum Separation and Storage**

The blood samples were collected in serum separator vacutainers containing clot activator and allowed to stand for 30 min. The vacutainers were shifted to laboratory for centrifugation after packing with ice bags containing silica gel. The separated serum was centrifuged at a rate of 3500 rotations per minute (RPM) for 15 min, and the resultant serum was stored at −70°C until analysis.

**Estimation of RIF Concentration**

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 revortexed 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 liquid chromatography–mass spectrometry (LC–MS) autosampler. Estimation of RIF 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 × 150 mm, 5 µm and acetonitrile 10 mM, ammonium acetate (80:20% v/v) were used as stationary and mobile phases respectively. The flow rate was set to 1 mL/min.

**Statistical Analysis**

All statistical analyses were performed using SPSS 17.0 and GraphPad Prism 7.0. Logistic linear regression analysis was carried out to determine the covariates that influence RIF $C_{\text{max}}$. Pearson’s correlation was used to determine the linear dependency of $C_{\text{max}}$ on individual covariates. Chi-square analysis was used to determine the effect of categorical dichotomous variables. Based on the data skewness, parametric tests were used for group comparison. $P < 0.05$ was considered statistically significant throughout the study (95% CI).
RESULTS

Patient Demographics

All patients enrolled into the study were in the intensive phase of ATT and received either 450 mg or 650 mg of RIF thrice weekly in combination with isoniazid, ethambutol, and pyrazinamide. However, the data of five patients were not used for further statistical and pharmacokinetic analyses as the serum $C_{\text{max}}$ of RIF were below the limit of quantification.

The mean (SD) age of patients in the study was 44.78 years (10.45 years). The mean (SD) age of males was significantly higher than that of females. Age wise and gender wise distribution of studied patients are shown in Tables 1 and 2.

The mean (SD) body weight of the patients was 45.7 kg (7.8). The mean (SD) body weight of females was comparatively higher than that of males with values of 46.82 kg (8.5) and 45.34 kg (7.4), respectively ($P = 0.5181$). Similarly, PTB patients with diabetes mellitus displayed higher body weights than PTB patients who were non-diabetic with mean (SD) values of 50.87 kg (7.4) and 42.36 (5.9), respectively ($P = 0.024$). Other comorbidities observed are chronic bronchitis (15.4%), emphysema (9.2%), liver cirrhosis (8.3%), bronchiectasis (5.8%), hypertension (4.1%), oral candidiasis (1.4%), and alcoholic liver disease (0.8%). 19 (31.7%) patients had hypoalbuminemia, whereas 41 (68.3%) did not. Hepatic function was normal in 31 (51.7%) patients while 29 (48.3%) patients displayed decreased hepatic function. 34 (56.6%) patients were smokers, whereas 26 (43.3%) were non-smokers. 25 (41.7%) patients were alcoholic while 35 (58.3%) patients were non-alcoholic. 35 (58.3%) patients were relapse cases while 25 (41.6%) newly diagnosed with PTB.

Determination of Factors Influencing RIF $C_{\text{max}}$ - Multivariate Analyses

$C_{\text{max}} < 8$ mcg/mL was considered as decreased or subtherapeutic, whereas a $C_{\text{max}} > 8$ mcg/mL and within 24 mcg/mL was considered as within the therapeutic range. [16] The mean (SD) RIF $C_{\text{max}}$ observed in the studied population was 7.655 mcg/mL (3.44) with a median RIF $C_{\text{max}}$ of 8.318 mcg/mL. To determine the factors that alter RIF $C_{\text{max}}$ and ascertain their effect on $C_{\text{max}}$, logistic multivariate regression analysis was performed. 27 independent variables expected to alter RIF concentrations were regressed against the dependent variable, $C_{\text{max}}$. Results of the regression analysis are shown in Table 3. Variables of model 3 with the best regression coefficient of 0.833 have a significant influence on RIF $C_{\text{max}}$.

Effect of Body Weight on RIF $C_{\text{max}}$

Body weight was found to be the predominant factor that affects RIF $C_{\text{max}}$. Body weight inversely correlated with RIF $C_{\text{max}}$ in both PTB with DM ($r^2 = -0.686$, $P = 0.001$) and PTB without DM group ($r^2 = -0.558$, $P = 0.001$). Pearson correlation plot of body weight versus $C_{\text{max}}$ in both groups is shown in Figures 1 and 2.

Effect of Antacid Coadministration on RIF $C_{\text{max}}$

A similar influence on RIF $C_{\text{max}}$ was shown in the patients being administered with antacids concomitantly. The incidence of low serum peak concentrations of RIF was more in patients receiving

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**Table 1: Age wise distribution**

<table>
<thead>
<tr>
<th>Age range</th>
<th>Number of patients $n=60$ (%)</th>
<th>Mean±SD</th>
<th>Age quartile</th>
</tr>
</thead>
<tbody>
<tr>
<td>18–28</td>
<td>7 (11.7)</td>
<td>26.42±1.49</td>
<td>23–28</td>
</tr>
<tr>
<td>29–38</td>
<td>10 (16.7)</td>
<td>32.71±2.11</td>
<td>30–38</td>
</tr>
<tr>
<td>39–48</td>
<td>21 (35)</td>
<td>41.57±1.59</td>
<td>39–48</td>
</tr>
<tr>
<td>49–58</td>
<td>18 (30)</td>
<td>51±1.19</td>
<td>49–58</td>
</tr>
<tr>
<td>59–68</td>
<td>4 (6.6)</td>
<td>62.25±1.78</td>
<td>60–65</td>
</tr>
</tbody>
</table>

**Table 2: Gender wise distribution**

<table>
<thead>
<tr>
<th>Gender</th>
<th>Number of patients (%)</th>
<th>Age range</th>
<th>Mean±SD</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>43 (71.7)</td>
<td>23–65</td>
<td>43.17±11.07</td>
<td>0.4626</td>
</tr>
<tr>
<td>Female</td>
<td>17 (28.3)</td>
<td>27–55</td>
<td>45.41±8.48</td>
<td></td>
</tr>
</tbody>
</table>

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Figure 1: Correlation of body weight and $C_{\text{max}}$ in non-diabetic pulmonary tuberculosis patients
antacids when compared to those not on antacids ($P = 0.0009$, odds ratio $= 0.4167$, and relative risk $= 8.0$). $55\%$ of the patients coadministered with antacids displayed a mean (SD) RIF $C_{max}$ of 5.879 mcg/mL (0.5), whereas the mean (SD) RIF $C_{max}$ in patients who did not receive antacids was significantly high with a value of 9.785 mcg/mL (0.6) ($P < 0.0001$).

**Effect of Diabetes Mellitus on RIF $C_{max}$**

Diabetes mellitus was found to be a significant factor influencing RIF $C_{max}$. Incidence of decreased $C_{max}$ was found to be high in PTB patients with diabetes than patients without diabetes, suggesting that exposure to RIF is decreased in PTB patients with comorbid diabetes mellitus ($P < 0.0001$, odds ratio $= 13.81$, and relative risk $= 5.1$). Incidence of decreased $C_{max}$ between diabetic and non-diabetic patients is as shown in Figures 3 and 4. The mean (SD) RIF $C_{max}$ in PTB with and without diabetes mellitus was 4.71 mcg/mL (0.4) and 9.47 mcg/mL (0.4), respectively ($P < 0.0001$).

Univariate analyses were performed to determine the relationship between random blood glucose and RIF $C_{max}$. A near inverse correlation was obtained in diabetic patients ($r^2 = −0.734$, $P < 0.0001$); however, such a correlation was not observed in non-diabetic PTB patients, suggesting that the drug absorption is disfavored only under hyperglycemic conditions and linear relationship does not exist between blood glucose and RIF $C_{max}$ ($r^2 = −0.03$, $P = 0.412$). The RBS versus $C_{max}$ correlation plot of diabetic and non-diabetic PTB patients is shown in Figures 5 and 6, respectively.

**Effect of Hepatic Function on RIF $C_{max}$**

Irrespective of the nature of liver disease, patients with impaired liver function displayed higher serum $C_{max}$ of RIF than those with normal liver function ($P = 0.0003$). Aspartate transaminase (AST) was used as a measure of hepatic function, and a value $>40$ U/L was considered as impaired liver function. Patients with normal liver function displayed a mean (SD) $C_{max}$ of 6.244 (0.5) mcg/mL, whereas the mean (SD) $C_{max}$ of patients with impaired liver function was 9.478 (0.63) mcg/mL. Serum AST levels positively correlated with RIF $C_{max}$ in ($r^2 = 0.49$, $P < 0.0001$), suggesting that impaired hepatic function decreases the rate of biotransformation of RIF and thereby decreases the hepatic clearance. However, no statistically significant difference in incidence of decreased $C_{max}$ was found between alcoholic and non-alcoholic patients ($P = 0.2864$).

**Effect of hypoalbuminemia on RIF $C_{max}$**

Serum albumin levels were found to be inversely associated with RIF $C_{max}$ ($r^2 = −0.454$, $P < 0.0001$). Inverse association observed between serum albumin and $C_{max}$ could be attributed to the fact of that all patients were sampled at the $t_{max}$ range ($2^{nd}$ h postdose), where the rate of absorption is comparatively higher than the rate of elimination. Hence, higher unbound drug concentrations are observed in patients with hypoalbuminemia than patients with normal serum albumin levels with mean (SD) $C_{max}$ values of 10.33 mcg/mL (0.9) and 6.65 mcg/mL (0.4), respectively ($P = 0.0002$).

**Effect of PTB History on RIF $C_{max}$**

Statistically significant difference in incidence of decreased $C_{max}$ was not found between newly diagnosed

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### Table 3: Built multiple linear regression models

<table>
<thead>
<tr>
<th>Model</th>
<th>R</th>
<th>R²</th>
<th>Variables selected*</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>0.745</td>
<td>0.554</td>
<td>Body weight</td>
</tr>
<tr>
<td>2</td>
<td>0.811</td>
<td>0.658</td>
<td>Body weight, antacids</td>
</tr>
<tr>
<td>3</td>
<td>0.833</td>
<td>0.694</td>
<td>Body weight (−0.508), antacids (−0.261), comorbid DM (−0.250)</td>
</tr>
</tbody>
</table>

*Standardized coefficients of model 3 variables are given in brackets.
Effect of Smoking on RIF $C_{\text{max}}$
Statistically significant difference in incidence of decreased $C_{\text{max}}$ was not observed between smokers and non-smokers ($P = 0.1060$). 55% of the patients had a history of smoking with a mean (SD) RIF $C_{\text{max}}$ of 6.48 mcg/mL (0.6), whereas the mean (SD) $C_{\text{max}}$ of non-smokers was 8.86 mcg/mL (0.6) ($P < 0.0001$).

Effect of Gender on RIF $C_{\text{max}}$
Statistically significant difference in incidence of decreased $C_{\text{max}}$ was not observed between genders ($P = 0.5371$). The mean (SD) RIF $C_{\text{max}}$ in males and females was 7.512 mcg/mL (0.5) and 8.074 mcg/mL (0.8), respectively ($P = 0.6067$).

DISCUSSION
The pharmacokinetic profile of RIF is altered at various stages by diverse factors resulting in high interindividual variability. The mean (SD) RIF $C_{\text{max}}$ observed was 7.655 (3.45) mcg/mL which is less than the therapeutic range, suggesting a potential risk of treatment failure and emergence of drug-resistant strains in the studied population. However, the mean RIF $C_{\text{max}}$ in females was within the therapeutic range (8.074 mcg/mL), whereas it was subtherapeutic in males (7.512 mcg/mL) indicating males to be the high-risk groups. In spite of the various factors known to cause lower serum concentration of RIF, subtherapeutic concentration of RIF observed in males could be attributed to the high incidence of smoking. Polycyclic aromatic hydrocarbons in tobacco smoke are known to induce CYP450 enzymes including extrahepatic enzymes such as CYP1A1 and may thus increase the rate of biotransformation of CYP450 substrates.\[17\]

Variability in Absorption
Variability in absorption due to diabetes mellitus and antacids administration could be attributed to the high pH dependency of the drug.\[18\] Several clinical studies suggest that hyperglycemic state decreases the release of gastric acid from parietal cells and thereby increases gastric pH. Being highly pH dependent for solubility and well absorbed from an acidic pH, shift in the gastric pH due to hyperglycemia delays RIF absorption and thereby tends to prolong the $t_{\text{max}}$ and decreases the $C_{\text{max}}$.\[19\] Similarly, administration of antacids increase the gastric pH and may thereby disfavor the absorption of RIF. In addition, gastrointestinal ailments such as gastroparesis which are common in chronic diabetics may either delay or impair absorption of RIF leading to decreased systemic availability.\[20\]

Variability in Distribution
Body weight is known to be associated with variability in distribution by affecting the volume of distribution and relapse PTB patients ($P = 0.3772$). 26.7% of the studied population had prior history of PTB with a mean (SD) RIF $C_{\text{max}}$ of 7.18 mcg/mL (3.77). Whereas, the mean (SD) $C_{\text{max}}$ of RIF in newly diagnosed PTB patients was 7.48 mcg/mL (3.28) ($P = 0.6692$).
(\(V_p\)). Body weight was found to be inversely correlated with RIF \(C_{\text{max}}\) in both diabetic and non-diabetic PTB patients. Inverse correlation between body weight and RIF AUC\(_{0-24h}\) has also previously been reported emphasizing the WHO recommendation that RIF should carefully be dosed on the basis of body weight.\(^{[21]}\) RIF is 89% protein bound with a short half-life (\(1/2\)) of 3.35 ± 0.66 h.\(^{[20]}\) RIF attains peak serum \(C_{\text{max}}\) between 2 and 4 h after oral administration. In patients with hypoalbuminemia, unbound drug concentration is increased during the distribution phase after oral administration of RIF.\(^{[22]}\) However, such an effect is often transient since the intrinsic capacity of the liver to metabolize RIF is intermediate and an increase in free drug concentration increases the rate of hepatic elimination of RIF.\(^{[23]}\) Hence, higher unbound drug concentrations are observed in patients with hypoalbuminemia than patients with normal serum albumin levels with mean (SD) \(C_{\text{max}}\) values of 10.33 mcg/mL (0.9) and 6.65 mcg/mL (0.4), respectively (\(P = 0.0002\)).

**Variability in Metabolism**

Hepatic function has a direct effect on the rate of biotransformation of RIF. Irrespective of the nature of liver disease, patients with impaired liver function displayed higher serum \(C_{\text{max}}\) of RIF than patients with normal liver function. Decrease in hepatic clearance in impaired liver function contributes to higher \(C_{\text{max}}\). Decrease in hepatic clearance could possibly be attributed to the fact that CYP450 levels and enzyme activity are altered and reduced in patients with liver impairment.\(^{[24,25]}\) Further, it has been reported that in the cirrhotic patients, the mean \(t_{1/2}\) of RIF is significantly longer than that in healthy controls.\(^{[26]}\) In addition, RIF is a potent activator of PXR which regulates the transcription of multiple drug-metabolizing enzymes and drug transporters.\(^{[11]}\) Besides, increasing the rate of biotransformation of CYP450 substrates through enzyme induction, RIF induces its own metabolism on successive administrations. Therefore, the steady-state concentration (\(C_{ss}\)) of RIF falls critically beyond the initial \(C_{ss}\) on prolonged therapy. In addition, in patients who received 450 mg and 600 mg of RIF, the AUC\(_{0-24h}\) has been reported to decrease by 41% and 42%, respectively, after 28 days.\(^{[27]}\)

**CONCLUSION**

The pharmacokinetic profile of rifampicin is altered by various clinical, environmental, and genetic factors which may contribute decreased systemic availability of the drug. Decreased exposure of the mycobacterium bacilli to rifampicin due to subtherapeutic concentrations reached in serum may either lead to emergence of drug-resistant strains or prolong the length of treatment or treatment failure. Hence, identification of factors that tend to decrease systemic rifampicin concentrations and dosing individualization based on such factors could potentially aid providing a therapeutically safer and effective treatment strategy.

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**REFERENCES**


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