Addis Ababa University

School of Graduate Studies

Department of Pharmacology and Clinical Pharmacy

Pharmacokinetics and Pharmacogenetics Studies of Rifampicin and Isoniazid in Ethiopian Tuberculosis Patients

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Department of Pharmacology and Clinical Pharmacy

This is to certify that the thesis prepared by Tesemma Sileshi titled "**Pharmacokinetics and Pharmacogenetics Studies of Rifampicin and Isoniazid in Ethiopian Tuberculosis Patients**" and submitted in partial fulfillment for the requirements of the Degree of Doctor of Philosophy in Pharmacology complies with the regulation of University and meets the accepted standard concerning originality and quality.

Signed by the examining committee

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Chairperson of the Department

This thesis is based on the following papers

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2. Sileshi, T., Mekonen, G., Makonnen, E., & Aklillu, E. (2022). Effect of Genetic Variations in Drug-Metabolizing Enzymes and Drug Transporters on the Pharmacokinetics of Rifamycins: A Systematic Review. Pharmacogenomics and personalized medicine, 15, 561–571. https://doi.org/10.2147/PGPM.S363058
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Abstract

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Introduction: Tuberculosis (TB) is an ancient disease of mankind and remains a major public health problem despite tremendous efforts made to combat it. Globally, 10.6 million people fell ill and 1.13 million died from TB in 2022. Short-course regimens of first-line anti-TB drugs can cure about 90% of cases. However, the success of treatment appears to be on a declining trend over time. Unfavorable TB treatment outcomes might result from altered plasma exposure to antitubercular drugs. Rifampicin and isoniazid display wide between-patient pharmacokinetics variability. Ethiopia is among the 20 high TB and TB-HIV burdens countries. Ethiopians display significant genetic variation from other black African populations. Despite these facts, data on the extent of exposure to rifampicin and isoniazid, as well as inter-patient variability in plasma concentration, remains scarce among Ethiopian TB patients.

Objective: The study aimed to determine the plasma levels of rifampicin and isoniazid and investigate the effect of genetic polymorphism and socio-demographic characteristics on the pharmacokinetics of rifampicin and isoniazid in Ethiopian tuberculosis patients.

Methods: The study was conducted at the primary healthcare centers in Addis Ababa, Ethiopia. A total of one hundred forty-six adult patients with newly diagnosed TB who had received 2 weeks of first-line anti-TB therapy were enrolled. Venous blood samples were drawn at three-time points from the majority of the patients ranging from 1 to 7 h post-drug intake. Genotyping of *NAT2, SLCO1B1 (c.388A>G, c.521T>C), ABCB1 (c.3435C>T, c.4036A>G), AADACc.841G>A,* and *CES-2 (c.269-965A>G)* was done using TaqMan drug metabolism assay. Rifampicin, isoniazid, and its metabolite concentration were determined using validated liquid chromatography-tandem mass spectrometry (LC-MS/MS). Population pharmacokinetic (POPPK) modeling of rifampicin was done using NONMEM.

Results: The overall median isoniazid maximum plasma concentration (C_{max}) was 4.73 µg/mL and the area under the curve ($AUC_{0\text{-}7h}$) was 11.21µg.h/mL. The majority of patients 94(64.4%) had isoniazid C_{max} within the recommended therapeutic range (3-6 µg/mL), while only 19 (13%) had an isoniazid C_{max} of below 3 µg/mL. The median rifampicin C_{max} was 6.79µg/mL. Only 42

(29%) patients achieved the therapeutic efficacy threshold (≥ 8µg/mL). The median rifampicin $AUC_{0\text{-}7h}$ was 17.055µg.h/mL. The frequency of slow, intermediate, and fast *NAT2* acetylators genotypes was 74.2%, 22.4%, and 3.3%, respectively. The overall concordance between *NAT2* genotype and phenotype was 85%. The minor allele frequency for *SLCO1B1*1B (c.388A>G), SLCO1B1*5 (c.521T>C), ABCB1 c.3435C>T, ABCB1 c.4036A>G, AADAC c.841G>A* and *CES-2 c.269-965A>G* were 2.2%, 20.2%, 24.4%, 14.6%, 86.1% and 30.6%, respectively. *NAT2* acetylator genotypes alone accounted for 26.1% and 40.6% of the variability in isoniazid C_{max} and $AUC_{0\text{-}7h}$, respectively. *ABCB1 c.4036A>G* genotypes independently accounted for 7.4%, and 6.1 % of the variability in rifampicin C_{max} and $AUC_{0\text{-}7h}$, respectively. A two-compartment model coupled with a transit absorption model adequately fitted the rifampicin data. Subjects with *ABCB1 c.4036A>G GG* genotype were estimated to have 41% lower intrinsic clearance of rifampicin compared to subjects with *ABCB1 c.4036A>G AA* or *AG* genotypes. Similarly, subjects with *ABCB13435C TT* genotype were estimated to have a 100% higher absorption rate constant than those with *ABCB1 3435C>T CC* or *CT* genotypes.

Conclusion: There is high inter-patient variability in isoniazid and rifampicin exposure in Ethiopian TB patients. The majority of the patients attained therapeutic plasma concentration of isoniazid but not that of rifampicin. *NAT2* acetylation genotypes, dose, and sex are strong predictors of isoniazid exposure. Rifampicin exposure varied with sex, dose, *ABCB1 c.4036A>G*, and *ADAC c.841G>A* genotypes. The clinical significance of higher isoniazid and lower rifampicin exposure in Ethiopian TB patients needs further investigation.

Keywords: Rifampicin, isoniazid, pharmacokinetics, pharmacogenetics, Ethiopia, Tuberculosis

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Special thanks are extended to families, particularly my beloved mother and father, who unfortunately left us prematurely. Gratitude is also expressed to my wife and children for their unwavering support throughout this journey.

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List of Acronyms and Abbreviations

AADAC	Arylacetamide deacetylase
ABCB1	Adenosine triphosphate (ATP)-binding cassette, subfamily B member 1
AcHz	Acetyl hydrazine
AcINH	Acetyl isoniazid
AIC	Akaike information criterion
AUC	Area under the curve
BMRC	British Medical Research Council
CES	Carboxylesterases
CL	Clearance
CLi	Intrinsic clearance
C_{max}	Maximum concentration
CV	Coefficient of variation
DOTs	Directed observation therapy
DTV	Percent decrease in Vc after day 4
F	Bioavailability
FDC	Fixed-dose combination
GOF	Goodness of fit
HPLC	High-performance liquid chromatography
IIV	Inter-individual variability
INA	Isonicotinic acid
IND	Percent increase intrinsic clearance after day 4

IRB	Institutional Review Board
KA	Absorption rate constant
MDR-TB	Multidrug-resistant TB
MIC	Minimum inhibitory concentration
MTT	Mean transit time
NN	Number of transit compartments
OFV	Objective functional value
PAS	Para-Aminosalicylic acid
PcVPC	Visual predictive check
PK	Pharmacokinetic
POPPK	Population pharmacokinetic
Q	Inter-compartment clearance
RSE	Relative standard errors
SLCO	Solute carrier organic anion transporter
Vc	Central volume
Vp	Peripheral volume
WHO	World Health Organization
η	ETA

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Chapter 1: Introduction

1.1. Background

Tuberculosis (TB) is an ancient disease of mankind caused by *Mycobacterium tuberculosis* (*M. tuberculosis)*. It is a leading cause of death from infectious diseases. Globally 10.6 million people fell ill and 1.13 million died from TB in 2022 [1]. *M. tuberculosis*-infected about one-third of the global population. The majority of the infected individuals remain asymptomatic, while only 5–10% of the infected ones develop active TB [2]. TB infected 126 per 100,000 people in Ethiopia in 2022. Ethiopia is listed among the top twenty countries with the highest TB and TB-HIV burden [1].

TB is an airborne disease that primarily affects the pulmonary system. Patients with active pulmonary TB are the main source of transmission of *M. tuberculosis* [3]. TB infects the lungs in a majority of cases. It also affects other areas of the body. Extrapulmonary TB accounts for 15% of all TB infections. Lymphatic, central nervous, gastrointestinal, renal, and musculoskeletal systems are the most frequent manifestations of extrapulmonary TB [4].

The history of TB treatment dates back to 1940 when Albert Schatz discovered streptomycin. Unfortunately, resistance to streptomycin monotherapy emerged a few years after the introduction. Subsequently, a series of drugs including para-aminosalicylic acid, isoniazid, pyrazinamide, cycloserine, ethionamide, ethambutol, and rifampicin were rapidly developed [5, 6]. Most TB patients undergo treatment with a standard combination of isoniazid, rifampicin, pyrazinamide, and ethambutol for the initial two months, followed by isoniazid and rifampicin for an additional four months. These short courses of anti-TB drugs can cure more than 90% of cases. However, the cure rate with the first-line drug drops as low as 65% in certain regions [7]. The escalating drug resistance in TB poses a major threat to the control of TB worldwide.

The first treatment failure and drug resistance was observed in 1948 when mycobacterial isolates cultured from the relapsed patients showed resistance to streptomycin [8]. This pivotal observation prompted a shift towards combination therapy for TB treatment. The first combination was comprised of streptomycin and Para-aminosalicylic acid [9]. This dual therapy

followed with triple therapy after the discovery of isoniazid which comprises isoniazid, streptomycin, and Para-aminosalicylic acid.

In 1965, a significant milestone was reached with the introduction of rifampicin in TB treatment. This led to the formulation of a combination of therapy involving isoniazid, rifampin, ethambutol, and pyrazinamide and a continuation phase of isoniazid and rifampin [10]. Additionally, to boost adherence, directed observation therapy (DOTs) was implemented.

Despite the numerous achievements in combination therapy, the emergence of drug resistance has continued to grow and threatens the advancements in TB control and treatment worldwide [11]. Globally, For example, it was estimated that 3.6% of new and 18 % of previously treated TB cases had multidrug-resistant TB (MDR-TB) in 2021[1]. Similarly, in Ethiopia, a meta-analysis showed the prevalence of MDR-TB among newly diagnosed and previously treated TB patients to be 2% and 15% respectively [12].

Treatment failure has been linked to various factors, including medical risk factors like HIV infection, diabetes mellitus (DM), low body weight, cavitation on chest x-ray, high bacterial burden, drug resistance, positive culture after two months of treatment, and sociodemographic factors such as drug abuse, alcoholism, smoking, and poor treatment adherence as reported in several studies [13, 14]. Furthermore, findings from studies involving mice models [15], hollow fiber system (HFS) models [16], and population pharmacokinetics studies revealed a strong correlation between plasma concentration of first-line drugs and treatment outcome [17, 18].

1.2. Literature Review

1.2.1. An Overview of Tuberculosis

Tuberculosis is a global public health problem that remains the main cause of death from infectious diseases. In 2022 alone about 10·6 million people became ill with TB and 1.13 million death was reported. The TB incidence rate varies in different areas of the world (**Figure 1**). Ethiopia is the seventh top high-TB burden country globally and has an incident rate of 126 per 100,000 people in 2022 [1].

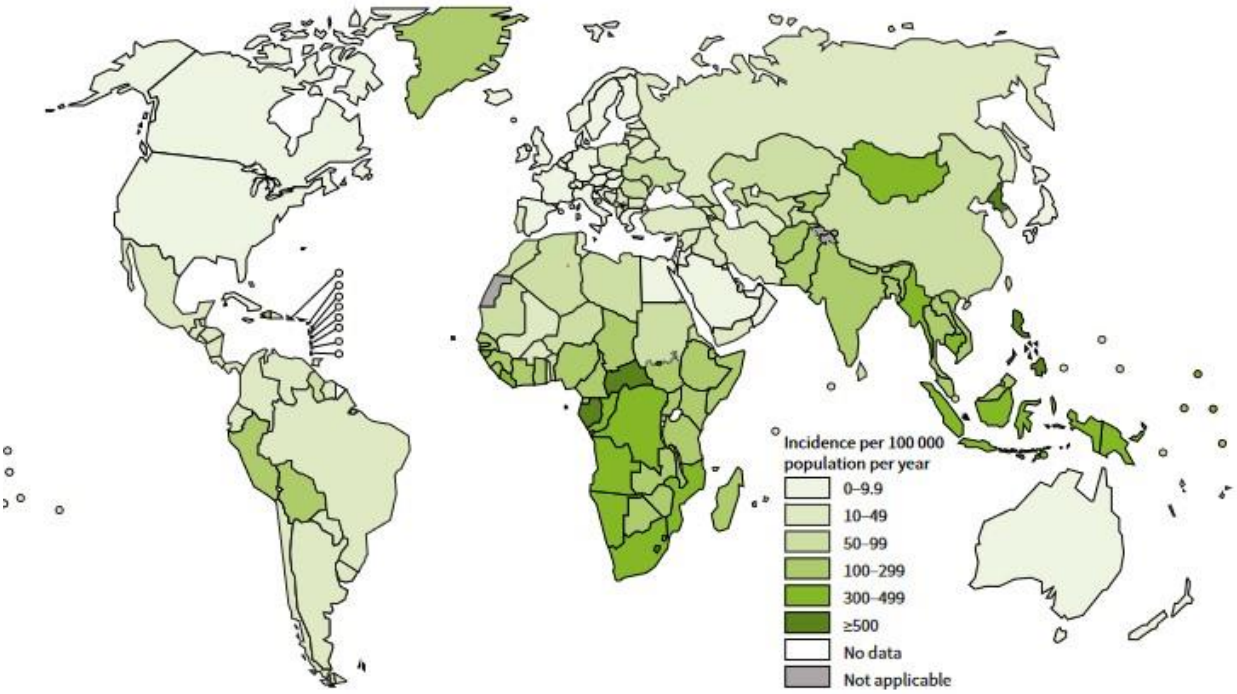

Figure: 1: Estimated global TB incidence rates in 2022. (Source WHO 2023 global tuberculosis report)

Tuberculosis is a preventable, and curable disease with initiatives in place to combat its prevalence. These include; the provision of TB treatment under directly observed therapy (DOTs), DOTS-Plus for MDR-TB programs for patients with MDR-TB, improving TB diagnosis, and ensuring an uninterrupted drug supply. A significant stride was taken when all WHO member states universally adopted the WHO END TB strategy during the World Health

Assembly in May 2014. This strategy aims to reduce TB incidence and mortality in 2035 by 90%, and 95%, respectively compared to the 2015 cases [19].

Despite the encouraging advancements in the reduction of TB incidence, morbidity, and mortality rates throughout the past two decades, the TB incidence rate rose by 3.6% between 2020 and 2021. The overall reduction from 2015 to 2021 was 10%, marking only halfway progress towards the initial milestone set by end TB strategy [1].

1.2.2. Treatment of tuberculosis

Robert Koch's discovery of *M. tuberculosis* as a causative agent of TB in 1882 marked a crucial turning point in the understanding of the disease. Despite this discovery, an immediate curative treatment option did not materialize soon. However, several sanatoriums were established in Europe and America to address the escalating TB epidemic.

The breakthrough in the effective treatment of TB was heralded by the discovery of streptomycin in 1944. Initial experiments demonstrated significant improvements in guinea pigs infected with virulent *M. tuberculosis* following streptomycin administration. However, the clinical use of streptomycin alone resulted in drug resistance which restricted its therapeutic application [8]. In response to these challenges, a combination of streptomycin and para-aminosalicylic acid (PAS) was introduced and found to greatly reduce the occurrence of drug resistance [20]. While this combination exhibited moderate effectiveness, it was not without significant adverse effects.

These early observations opened the modern era of antituberculous drug discovery. Consequently, isoniazid, pyrazinamide, ethionamide, ethambutol, and rifampicin were discovered [5, 21]. These drugs represented a crucial arsenal in the fight against TB, offering improved efficacy with reduced adverse effects compared to the earlier drugs.

Based on the clinical observation from the streptomycin and PAS combination, subsequent regimens evolved through additions and modifications as new drugs were discovered. The first such modifications were British Medical Research Council (BMRC) studies that investigated the combination of isoniazid with streptomycin or PAS. However, drug resistance remained a challenge. This led to the trial of triple therapy including streptomycin, isoniazid, and PAS. This combination was considered the first curative regimen and had relapse rates as low as 4% in cohorts treated for 1 to 2 years [22].

The remarkable ability of pyrazinamide and rifampicin to kill bacilli that persisted in mice organs led to further BMRC clinical trials. These trials demonstrated that the addition of pyrazinamide or rifampicin could radically reduce the relapse rate. Noteworthy clinical studies, incorporating pyrazinamide and rifampicin were conducted in East Africa, Hong Kong, and Singapore. These studies laid the foundation for the modern short-course treatment of TB [11, 23].

The modern short-course treatment of drug-susceptible TB utilizes a combination of rifampicin, isoniazid, pyrazinamide, and ethambutol. The standard treatment lasts for six months. The initial 2-month intensive phase involves isoniazid, rifampin, pyrazinamide, and ethambutol, followed by a 4-month continuation phase with isoniazid and rifampin. Isoniazid and rifampin are key components of TB chemotherapy. Isoniazid has early bactericidal activity while rifampicin has both early bactericidal and sterilizing effects [24].

Despite the high cure rates of the current four-drug combination therapy, several challenges persist. Among the challenges, the extended duration of therapy is one, which leads to poor adherence and increased toxicity. Poor adherence, on the other hand, increases the incidence of drug resistance and the prevalence of multidrug resistance (MDR). As a result, efforts are underway to decrease the duration of treatment so that improved adherence, low cost, and decreased adverse effects can be achieved. The use of high-dose rifampicin is one of the efforts made. High doses of rifampicin have been associated with an increased rate of sputum culture conversion, eradicating persistent bacteria and reducing relapse [25, 26].

1.2.3. Pharmacology of Rifampicin

Rifamycins, a class of antibiotics, were first isolated from a gram-positive- bacterium, Amycolatopsis rifamycinica in 1957. Among these antibiotics, rifampicin, a semi-synthetic derivative was discovered in 1963 and has since been used in clinical settings for treating various ailments. It has a complex chemical structure (**Figure 2**) [27]. It has bactericidal action and a potent sterilizing effect against *M. tuberculosis* in both cellular and extracellular environments [28]. Rifampicin mechanism of action involves inhibiting bacterial DNA-dependent RNA polymerase, thereby blocking the synthesis of mRNA and inhibiting protein synthesis [29].

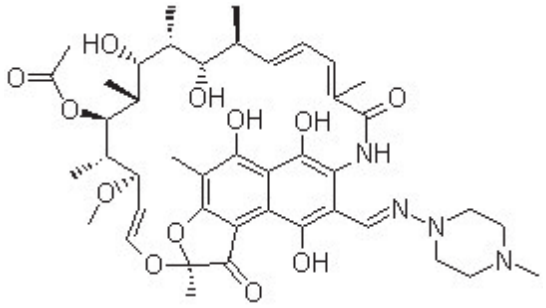

Figure 2: Rifampicin Chemical Structure

The antimycobacterial activity of rifampicin is a dose-dependent phenomenon [30]. The peak plasma concentrations (C_{max}) of rifampicin typically occur within 1 to 3 hours after oral administration of 10mg/kg. Rifampicin is nearly completely absorbed after oral administration when taken on an empty stomach, with a target C_{max} of 8-24μg/mL [31]. The bacterial killing activity of rifampicin correlates with AUC/MIC and C_{max}/MIC ratio [32].

The absorption, distribution, and excretion of various drugs are mediated by plasma membrane-bound drug transporters namely; the solute carrier (SLC) transporters and the adenosine triphosphate (ATP)-binding cassette (ABC) transporters [33]. The absorption, distribution, and excretion of rifampicin are also mediated by these drug transporters. Genetic polymorphism in *SLCO1B1* has been implicated in the inter-individual variation in drug response. For example, the missense mutation of rs4149056 (*c.521T>C*) is associated with an increased risk for statin-induced muscle toxicity [34]. Similarly, a few studies reported an association between rifampicin exposure and *SLCO1B1* genetic polymorphism. Patients who are carriers of homozygous wild type (*AA*) and heterozygous (*AG*) of rs2306283 (*388A>G*) were reported to have lower rifampicin exposure [35, 36]. A wild type (*CC*) of *SLCO1B1* rs11045819 has higher rifampicin exposure compared to the variant types [37]. On the other hand, patients who are homozygous mutant and heterozygous for rs4149032 have lower rifampicin bioavailability and C_{max} [38, 39].

Rifampicin is both a substrate and inducer of ABC drug transporter. The decline in rifampicin exposure throughout treatment can be partly explained by the induction of the *ABCB1* gene by rifampicin. P-glycoprotein (P-gp) which is a product of the *ABCB1* gene mediates the efflux of rifampicin. Limited studies have explored the effect of *ABCB1* genotypes on rifampicin exposure. Huerta-García *et al* found that the rs1045642 genotype is associated with variability in

the rifampicin pharmacokinetics whereas a *TT* genotype predicts about 34.8% of the variability in rifampicin C_{max} and 48.5% of the variability in $AUC_{0–24}$ h. However, Medellin-Garibay *et al* [40] did not observe the result of Huerta-García *et al*.

The metabolism pathway of rifampicin remains incompletely characterized but is thought to involve deacetylation by microsomal hepatic carboxylesterases (CES), and serine esterase aryl acetamide deacetylase (AADAC) leading to the formation of 25-deacetylrifampicin [41, 42]. The metabolite retains 25–50 % of the activity of rifampicin against clinical isolates. AADAC is primarily expressed in the liver. So far three *AADAC* variants, *AADAC*1*, *AADAC*2*, and *AADAC*3* have been reported. AADAC*2 and AADAC*3 have reduced enzymatic activity [42]. In the human liver, two CES isoforms (CES1 and CES2) are involved in the metabolism of endogenous and exogenous esters, such as fatty acid esters, environmental toxins, drug-containing esters, thioesters, or amide moieties [43, 44].

Limited studies have investigated the impact of genetic polymorphism of *AADAC* and *CES* on rifampicin metabolism. Song *et al* reported an association of *CES-2 c.269-965A>G* (rs4783745) and *c.1612 + 136G>A* with an increased exposure to rifampicin [45]. Patients carrying *CES-2* (rs8192925) *G* versus *A* allele have shown a 17.2% increase in rifapentine AUC0-24. Decreased exposure to rifapentine was observed for the *GG* variant of *AADAC* rs1803155 [46].

Literature is available on the population pharmacokinetics of rifampicin. Muda *et al* recently summarized the results of these studies in a systematic review [47]. The majority of studies described rifampicin population pharmacokinetics using one compartmental model while there are also studies reporting two compartmental models to best describe the rifampicin pharmacokinetic data. First-order [48, 49], lag time [50, 51], and transit compartment [52, 53] absorption models were reported to describe the absorption phases of rifampicin. The absorption rate constant varies from study to study and a lower absorption rate constant of 0.248/h and a higher absorption rate constant of 2.15/h were reported by Nishimura *et al*, and Seng *et al* respectively [50, 52]. About 80% of rifampicin is transported in blood bound to plasma proteins, mainly albumin.

The volume of distribution of rifampicin ranges from 30.9 to 87.2 L. The clearance of rifampicin ranges from 8.17 to 22.8 L/h and doubles after three weeks of treatment with rifampicin [54].

Increasing the dose of rifampicin results in a more-than-proportional increase in exposure to the dose, indicating saturable clearance at a high dose [55]. Rifampicin is eliminated extensively by hepatic metabolism. The drug and its metabolites are largely excreted in bile and the fraction is also eliminated via urine.

1.2.4. Pharmacology of Isoniazid

Isoniazid was first synthesized in 1912 by Hans Meyer and Josef Mally. The anti-tubercular effect of isoniazid was recognized after 40 years in 1951 by the independent work of three laboratories; Hoffmann-La Roche and E. R. Squibb and Sons in the U.S. and Bayer in Germany [56]. Isoniazid has a simple molecular structure (**Figure 3**), a low-molecular-weight and water-soluble compound.

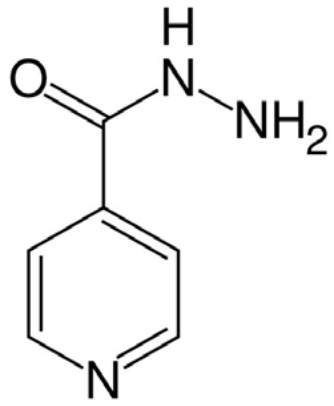

Figure 3: Chemical structure of isoniazid

Isoniazid is highly bactericidal against replicating *M. tuberculosis* in the early phases of TB treatment. Isoniazid is a prodrug activated by the bacterial catalase-peroxidase enzyme KatG. Once activated, isoniazid inhibits enoyl-acyl carrier protein reductase (InhA), which is essential for mycolic acid synthesis, a component of the Mycobacterium cell wall [57]. *Mycobacterium* is also capable of inhibiting isoniazid by an acetylation reaction catalyzed by its arylamine N-acetyltransferase (NAT) [58, 59]. The role of the mycobacterial NAT is not well characterized. However, the mutation in the *katG* gene is the major cause of isoniazid resistance, followed by *inhA, ahpC, kasA, ndh, iniABC,* and *fadeE* [57].

Isoniazid is a simple water-soluble molecule and is readily absorbed from the gastrointestinal tract after oral administration. The C_{max} is achieved after 1-3 hours post-administration. Various

factors affect the plasma concentration of isoniazid. *NAT2* genotype, age, comorbidities, sex, and co-administered food were among the factors reported in previous studies [60, 61]. Isoniazid is eliminated after being metabolized to inactive metabolites. The metabolism of isoniazid involves hepatic acetylation by NAT2 to acetylisoniazid (AcINH) and hydrolysis to produce isonicotinic acid (INA) and hydrazine through amidase (**Figure 4**). NAT2 also catalyzes the acetylation of acetyl hydrazine (AcHz), which is a metabolite of AcINH, to non-toxic diacetylhydrazine. AcINH can be further metabolized to produce acetyl-hydrazine, which has been proposed as the cause of isoniazid hepatotoxicity via a CYP2E1-mediated bioactivation pathway [62, 63]. Genetic polymorphisms in *NAT2* and *CYP2E1* have been associated with altered isoniazid-induced hepatotoxicity [64].

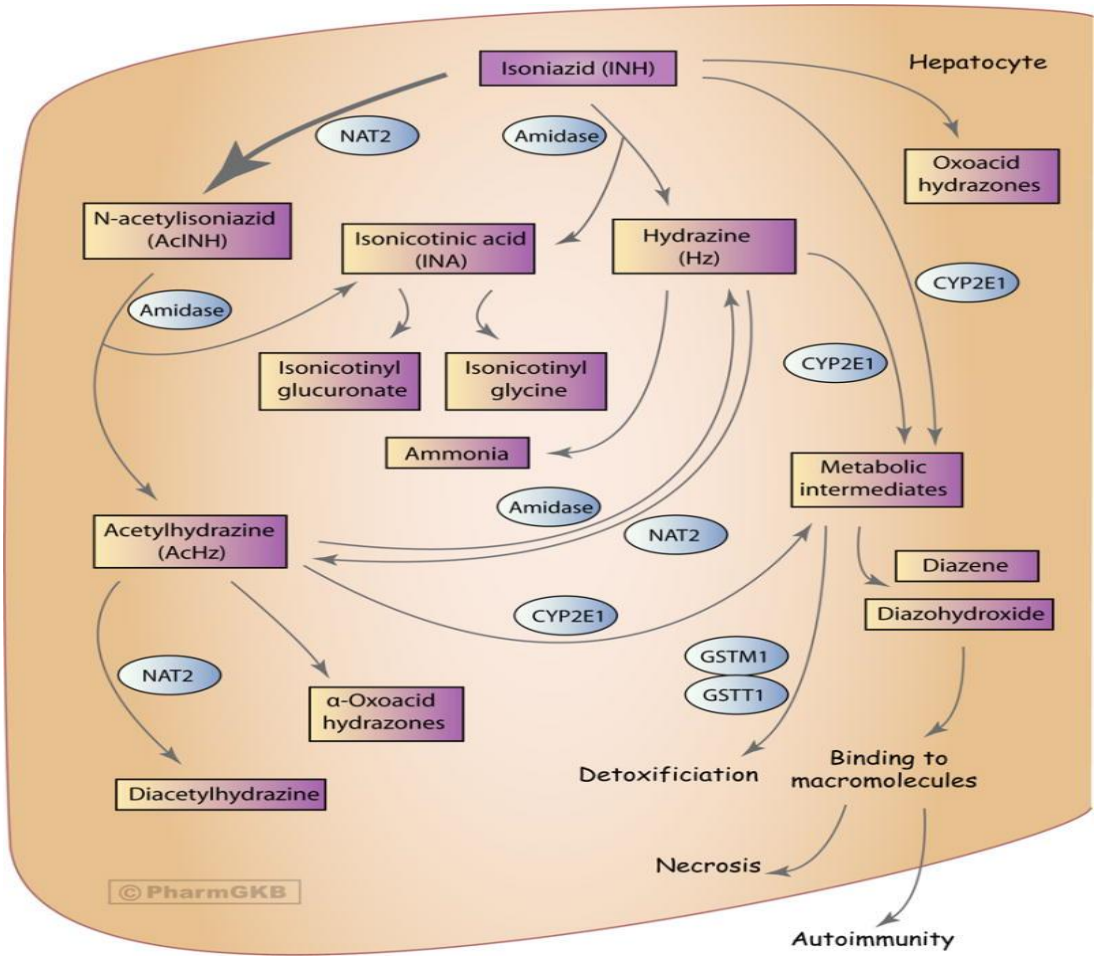

Figure 4: Metabolic pathway of isoniazid.

The figure is taken from PharmGKB and available at http://www.pharmgkb.org/pathway/PA166151813.

1.2.5. The Role of Pharmacokinetics in Tuberculosis Treatment

Pharmacokinetics involves the study of the time course of a drug concentration in different body compartments, encompassing four essential processes: absorption, distribution, metabolism, and excretion of the drug. In TB treatment, poor compliance with treatment has been reported as the major cause of treatment failure. Consequently, the DOTs strategy was adopted to improve adherence. However, recently low serum concentrations of anti-TB drugs have been associated with unfavorable treatment outcomes [16, 65].

The target concentration of first-line anti-TB drugs with favorable treatment outcomes is presented in **Table 1**. Data from clinical studies and hollow fiber system (HFS) models emphasize total exposure to TB drugs (the area under the plasma concentration AUC0-24) and the peak plasma concentration (Cmax) as key indicator parameters relevant for anti-TB drugs.

Determination of AUC for the 24 h after dosing ($AUC_{0–24}$) requires intensive blood. However, recent studies showed blood sampling at 1, 3, and 8hrs has also shown better AUC for the 24 hours after dosing [51]. Novel approaches to measuring drug exposure such as the use of dried blood spot methods to store and transport samples [66], and urine colorimetry to detect low rifampicin exposure have been reported [67]. To measure serum or plasma anti-TB drug concentrations, chromatography-based methods such as HPLC (high-performance liquid chromatography)-ultraviolet detection, HPLC-fluorescence detection, and HPLC–tandem mass spectrometry (MS/MS) have been introduced. LC-MS/MS is particularly useful as a simple and rapid analytical method for the simultaneous, quantitative determination of 20 anti-TB drugs including both first-line and second-line anti-TB drugs [68]. These approaches make the pharmacokinetic analysis simple and cost-effective.

Table 1: Some pharmacokinetic properties of first-line anti-TB drugs

Drug name	Dose	Serum Cmax (µg/ml)	Tmax (hr)	Serum T½ (hr)
Rifampicin	600mg	8-24	2	2-3
Isoniazid	300mg	3-6	0.75–2	1.5 fast 4 slow
Pyrazinamide	25-35mg/kg	20-60	1-2	9
Ethambutol	25mg/kg	2-6	2-3	Biphasic: 2–4, then 12–14

Several pharmacokinetic studies have associated plasma anti-TB drug concentration and drug efficacy. Increased exposure to rifampicin decreased time to culture conversion [69-71], decreased exposure associated with treatment failure [72], and delayed culture conversion. Similarly, observations were reported for isoniazid [16, 70], and pyrazinamide [16]. However, some studies did not report an association between pharmacokinetic variability and treatment response [73]. Furthermore, there is no rapid and accurate endpoint marker showing pharmacokinetic parameters and treatment outcome. Many studies used sputum culture conversion as a marker for treatment response. However, a molecular bacterial load assay has recently been developed, which reports the *M. tuberculosis* bacterial load by quantization of 16S ribosomal RNA within 72 h of sample processing [74]. A detail of the role of the impact of rifampicin and isoniazid pharmacokinetics on the treatment of TB is summarized in ***Paper I*** from literature.

1.2.6. Pharmacogenetics in Tuberculosis Treatment

Pharmacogenomics studies how the genetic makeup of an individual affects their drug response. Human genomic information can be used for forecasting disease risk, predicting drug toxicity and response, and helping optimize treatment (**Figure 5**). For infectious diseases, microorganisms' genetics also influence treatment outcomes. *Mycobacterial* genetics test is widely used in detecting drug-resistant strains [75]. With the advance in genotyping technology and reduced cost, pharmacogenomics has evolved as a precision medicine in clinical practice

[76]. Pharmacogenomics is most practiced in cancer therapeutics [77]. However, information is scarce regarding pharmacogenomics' contribution to TB treatment.

Among the studied genetic polymorphisms, *NAT2, CYP2E1*, and glutathione S-transferase (GST1) have been stated to increase isoniazid-induced hepatotoxicity [78]. American Food and Drug Administration indicated *NAT2* genotype as a biomarker for isoniazid in the table of pharmacogenomic biomarkers in drug labels [79]. There is no pharmacogenomics information for rifampicin, pyrazinamide, and ethambutol on the Pharm GKB website.

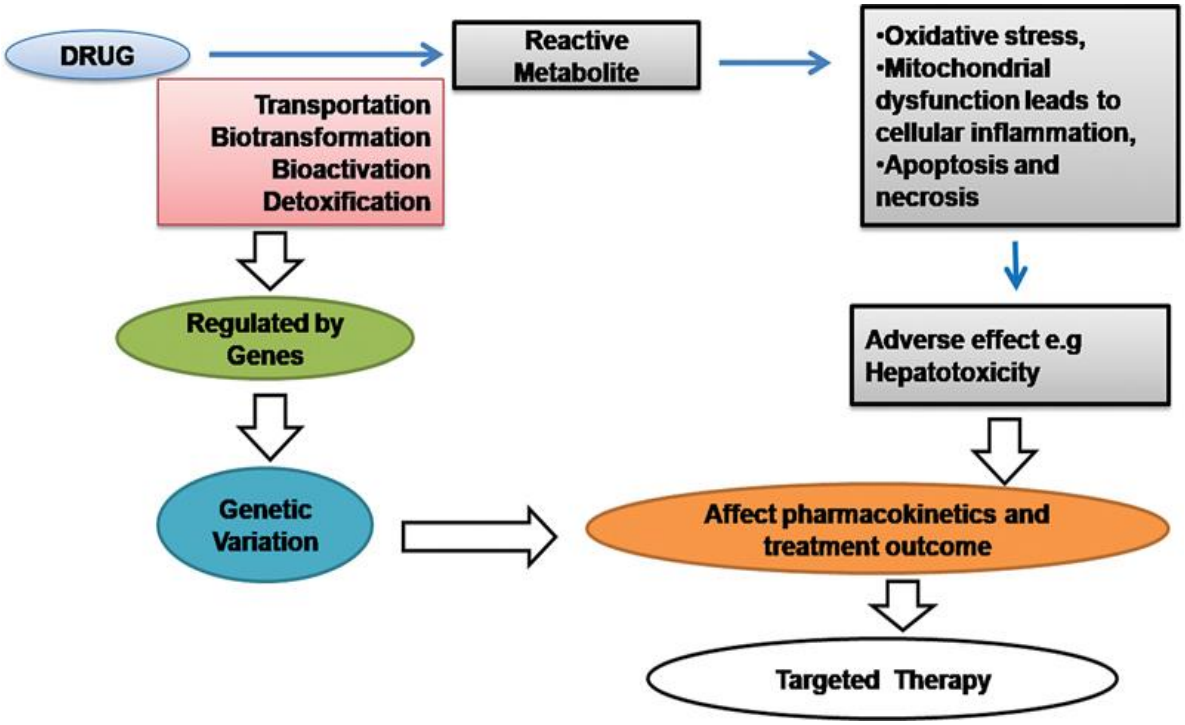

Figure 5: Schematic of genotype-based individualized therapy

Adopted from Genetic Variants and Drug Efficacy in Tuberculosis: A Step toward Personalized Therapy Global Medical Genetics Vol. 9 No. 2/2022 [80].

There are two *NAT* genes in humans namely, *NAT1* and *NAT2*. Both genes are located on chromosome 8 but vary in their substrate specificity and their distribution in various tissues [81]. NAT1 and NAT2 enzymes catalyze both N-acetylation and O-acetylation of aromatic and heterocyclic amine carcinogens. As a result, the polymorphisms in these enzymes have been associated with an increased risk of certain cancers [82-84].

The human *NAT2* gene encodes a 290-amino-acid protein. A total of 45 nucleotide variations have been reported till now, of which more than 27 are single nucleotide polymorphisms (SNPs). Some of these polymorphisms encode NAT2 proteins displaying a reduced enzyme activity [85]. Genetic variations in *NAT2* have been shown to correlate with metabolic activities, giving rise to three different phenotypes; slow, intermediate, and fast acetylators. Among the described SNPs *191G>A* (*2*14), 341T>C(2*5), 590G>A (2*6),* and *857G>A(2*7)* have an amino acid exchange that leads to a reduction in the enzyme activity display a high genotype and phenotype concurdance. Homozygotes for the *NAT2*4* wild-type allele are fast acetylators. SNPs at *190C>T (2*19), 364G>A (2*12D), 411A>T (2*5I), 434A>C (2*17),* and *499G>A (2*10)* have also enzyme activity altering capacity, however, the effect on NAT2 acetylation status varies among the population. The panel of four SNP is recognized as a reliable estimate to infer fast, intermediate, and slow acetylator phenotypes [86]. *NAT2* polymorphism has been implicated in variations of therapeutic response and adverse effects of isoniazid in different acetylators groups [85, 87].

NAT2 gene polymorphism also shows significant between population and ethnic groups variations in its expression and enzyme activity. A recent systematic review showed that East Asians and Native Americans have the highest frequencies of the fast phenotype, followed by South Europeans. Central Asia, the Middle East, and West European populations were the major carriers of the slow acetylator status [88]. Black Africans have higher variations in *NAT2* genotype frequencies and slow acetylator phenotypes than non-Africans. Ethiopians have also a high frequency of slow acetylator genotypes and phenotypes [89].

There is a mounting interest in the understanding of rifampicin pharmacogenetics. However, so far at least a single pharmacogenetic marker has not been identified. Hepatocellular uptake of rifampicin is mediated by organic anion transporting polypeptide 1B1 (OATP1B1), which is a product of solute carrier (SLC) organic anion transporter family member 1B1 (*SLCO1B1*) gene [90, 91]. The *SLCO1B1* gene is located on chromosome 12. Many variants of the *SLCO1B1* gene have been characterized. The genetic variations are associated with impaired transporter function of OATP1B1. As a result, altered effects of drugs were observed in patients carriers of various variants. The missense variants, *SLCO1B1 c.629G>T* cause a loss of function of OATP1B1 transport [92, 93]. This might alter the pharmacokinetics of the substrate drug, for instance,

statins, and increase the risk of drug toxicity [93, 94]. Rifampicin pharmacokinetics change was observed in *SLCO1B1 c.388A>G,* where patients who were homozygous wild type (AA) and heterozygous (AG) were reported to have lower rifampicin exposure. *SLCO1B1* rs4149032 polymorphism was found to be associated with lower rifampicin exposures. Homozygous wild types have higher rifampicin exposure compared to homozygous mutant and heterozygous for rs4149032 [39, 48]

Rifampicin is a substrate for P-gp. P-gp mediates drug efflux and is encoded by *ABCB1 genes*. The *ABCB1* gene is present on chromosome number 7. The efflux effect of P-gp dramatically affects the plasma concentration of therapeutics drugs and other xenobiotics and has been associated with drug resistance [95, 96]. The genetic polymorphism of *ABCB1* also changes P-gp expression to increase or decrease, which may affect the pharmacokinetics of drugs [97, 98]. There are also reports showing the association of *ABCB1* gene polymorphism with rifampicin pharmacokinetics. Patients with *CC* or *CT* genotypes of rs1045642 showed lower exposure compared to those with a *TT* genotype to rifampicin. Rifampicin induces the *ABCB1* gene and increases the expression and activity of P-gp [99]. The decrease in plasma concentration of rifampicin after two weeks of treatment may be linked to this.

Rifampicin is metabolized by esterase enzymes. Two esterases namely; hepatic carboxylesterases (CES), and serine esterase arylacetamide deacetylase (AADAC) metabolize rifampicin [41]. These enzymes convert rifampicin to 25-desacetylrifampicin, which also retains partial antimycobacterial activity. Two isoenzymes of CES, CES1, and CES2, are expressed in humans, and the gene encoding lies on chromosome 16. The metabolism of several drugs is affected by the genetic polymorphisms of the *CES1* and *CES2* genes. Dabigatran oseltamivir and clopidogrel metabolism have been reported to be modified by variations in the *CES1* gene. Similarly, *CES2* gene polymorphisms have been found to affect aspirin and irinotecan[100]. In the cases of rifampicin only Song *et al* reported increased plasma rifampicin concentrations with the *CES2 c.-22263A>G (g.738A>G)* variants [45].

AADAC enzyme is expressed in the liver and the gene is located on chromosome 3. AADAC catalyzes the hydrolysis of several clinically relevant drugs [101]. There are three AADAC variants; *AADAC*1* (wild-type), *AADAC*2*, and *AADAC*3*. The latter two are characterized by reduced enzymatic activity [41, 42]. No significant effect of *AADAC* polymorphism was

observed on rifampicin metabolism [102]. However, Francis *et al* and Weiner *et al* reported that *AADAC* rs1803155 genotypes have a significant effect on rifapentine metabolism. The wild-type (*GG*) carriers have shown decreased rifapentine exposure whereas, the variant of rs1803155 (AA) has decreased clearance of rifapentine [46, 103].

In addition to human genetics, *M. tuberculosis* genetics has evolved as a key tool to detect drug-resistant strains of *M. tuberculosis* and optimize TB treatment. Drug resistance in *M. tuberculosis* is linked to drug targets or pro-drug activators. Mutations in the so-called "hot-spot region" of 81-bp spanning codons 507–533 of the rpoB gene are responsible for about 96% of *M. tuberculosis* isolates resistant to rifampicin [104, 105]. Mutations in rpoB alter the beta subunit of RNA polymerase thereby reducing affinity for rifampicin.

Isoniazid is a prodrug and requires activation by an endogenous mycobacterial enzyme, the catalase-peroxidase KatG. The KatG gene encodes the KatG enzyme. Mutation in the katG gene at codon 315 (S315T) is found to occur in 40–90% of isoniazid-resistant isolates of *M. tuberculosis* [106]. In addition, mutations in several other loci, such as the alkylhydroperoxidase (AhpC) and the enoyl reductase (InhA), may contribute to isoniazid resistance. AhpC is a member of a large family of peroxidases, responsible for the antioxidant defense mechanism in *M. tuberculosis*. These antioxidant defense systems in *M. tuberculosis* have led to increased drug resistance and virulence [107, 108]. The enzyme InhA is encoded by the inhA gene and is involved in mycolic acid biosynthesis. Mutations in the InhA regulatory region account for 30%–35% of isoniazid-resistant isolates of *M. tuberculosis* [109].

1.3. Statement of the Problem

Short-course regimens of first-line TB drugs can cure about 90% of cases. However, the success of treatment appears to be on a declining trend over time. Various factors have been associated with treatment failure, including HIV co-infection, diabetes mellitus, and lower plasma concentrations of drugs. Notably, low serum concentrations of isoniazid or rifampicin have been shown to compromise the ability to kill *M. tuberculosis* isolate [17]. The pharmacokinetic threshold of TB drugs in drug-susceptible patients below which treatment failure may occur has been described [7].

Rifampicin exhibits concentration-dependent activity against *M. tuberculosis*. A study conducted in Korea showed that reduced rifampicin plasma level is associated with relapse. Similarly, a study done in Canada demonstrated that low plasma levels of rifampicin increased the risk of drug resistance and treatment failure [110, 111]. Peak levels of rifampicin are attained within 1-3 hours with a C_{max} ranging between 4 – 32µg/mL [112]. Various factors, including food intake, co-morbid conditions, genetic factors, and drug-drug interactions can influence rifampicin plasma concentration.

Rifampicin is metabolized by arylacetamide deacetylase (AADAC) to 25-deacetylrifampicin [41]. Shimizu *et al* reported that the *AADAC*3* allele has low hydrolase activity for rifampicin [42]. However, the impact of *AADAC* polymorphism on the pharmacokinetics of rifampicin and its implications for TB treatment outcomes remain inadequately elucidated.

Rifampicin is a substrate for OATP1B1, an influx transporter. Several studies have linked the expression levels of OATP1B1 with the pharmacokinetics of rifampicin. For example, investigations in South Africa and Uganda indicated that *SLCO1B1* (rs4149032 and rs11045819) are associated with reduced rifampicin exposure [37, 38]. In contrast, a study conducted in Malawi did not replicate this finding [102].

Isoniazid plays a key role in the early bactericidal activity of first-line drugs. It exhibits concentration-dependent activity against *M. tuberculosis*. Numerous studies have linked lower plasma concentrations of isoniazid to unfavorable treatment outcomes [113, 114]. Pharmacokinetic studies suggest a target of 3 -6 µg/mL for the peak concentration (Cmax) for favorable treatment outcomes.

Isoniazid undergoes metabolism by arylamine N-acetyltransferase- 2 (NAT-2) enzymes forming acetyl-isoniazid. NAT-2 enzyme exhibits genetic polymorphism, with three distinct genotypes - fast, intermediate, and slow acetylators, which are identified [115]. Fast acetylators tend to display low plasma concentrations of isoniazid.

Patients with diabetes and HIV infection face a higher risk of suboptimal plasma drug concentration, leading to poor treatment outcomes [116]. Impaired drug absorption and drug interaction with antiretroviral drugs were reported as causes of pharmacokinetics variation in this segment of the population.

Ethiopia is among the 20 high TB and TB-HIV burdens countries. Ethiopians display significant genetic variation from other black African populations. There are a few studies that assessed the pharmacokinetics and pharmacogenetics of first-line TB drugs in the Ethiopian population. Ibrahim *et al* investigated the pharmacokinetics of isoniazid in pediatric patients [115]. The effect of rifampicin on the pharmacokinetics of efavirenz has been studied in Ethiopian TB-HIV co-infected patients [117]. Petros *et al* explored genetic biomarkers associated with anti-tuberculosis and antiretroviral drug-induced liver toxicity in Ethiopian patients [118]. Despite these valuable data, studies assessing the pharmacokinetics and pharmacogenetics of rifampicin and isoniazid are lacking.

1.4. Significance of the Study

Ethiopia is characterized by a high prevalence of TB, and TB/HIV co-infection ranking among the top 20 countries globally burdened by TB and TB/HIV [119]. The alarming increase in MDR-TB further compounds the challenge that TB poses to public health. Isoniazid and rifampicin constitute key components of standard six-month treatment for drug-sensitive *M. tuberculosis*. The effectiveness of these drugs depends on the concentration reaching the bloodstream and the target organ where the bacteria reside.

As reported in existing literature, the suboptimal plasma concentration of ant-TB drugs is among the contributory factors for treatment failure [7]. In Ethiopia, there is an increased risk of treatment failure particularly prevalent among patients treated previously for tuberculosis [12]. Moreover, patients with HIV and diabetes face an increased risk of experiencing lower plasma drug levels [116, 120].

Ethiopians display a high degree of genetic heterogeneity relative to non-Africans [121]. These genetic variations can significantly influence drug response in several ways. Firstly, these variations may alter metabolizing enzyme expression. Secondly, drug transporter protein expression and activity may be changed. However, the Ethiopian population is among under-studied segments of the global population, with only a limited number of studies exploring genetic polymorphism with drug exposure or response. Therefore, investigations into genetic polymorphism and drug exposure are of paramount for public health importance.

Recognizing these gaps, the current study aims to describe the plasma exposure of key anti-TB drugs, specifically rifampicin and isoniazid. The study compares the plasma exposure of these drugs with currently acceptable ranges for favorable treatment outcomes. The study also explores the effect of genetic polymorphism in *NAT2, SLCO1B1 (c.388A>G, c.521T>C), ABCB1 (c.3435C>T, c.4036A>G), AADACc.841G>A, and CES-2 (c.269-965A>G)* on the plasma exposure of these drugs. The results of the study may contribute to the optimization of TB treatment in Ethiopian patients. Additionally, it identifies individuals at risk for treatment failure based on socio-demographic characteristics and genetic makeup. It also provides insight into whether therapeutic drug monitoring is necessary, considering observed plasma exposure differences among patients

1.5. Research Questions

Plasma concentrations of rifampicin and isoniazid below the target could lead to sub-optimal treatment outcomes. Furthermore, rifampicin and isoniazid show high inter-individual variability in pharmacokinetics. However, the extent of plasma exposure, the variation, and the causes for variation were not investigated in Ethiopian patients. This study aimed to answer the following questions.

1. Is the plasma concentration of rifampicin within the recommended therapeutic range in Ethiopian TB patients?
2. Is the plasma concentration of isoniazid within the recommended therapeutic range in Ethiopian TB patients?
3. How might socio-demographic characteristics and *NAT2* genetic polymorphism modify plasma levels of isoniazid in Ethiopian TB patients?
4. How might socio-demographic characteristics and *SLCO1B1, ABCB1, AADAC,* and *CES-2* genetic polymorphism modify rifampicin plasma levels in Ethiopian TB patients?
5. What are the population pharmacokinetics characteristics of rifampicin in Ethiopian TB patients?

Chapter 2: Objectives of the study

2.1. General objective

The study aimed to determine the plasma levels of rifampicin and isoniazid and investigate the effect of genetic polymorphism and socio-demographic characteristics on the pharmacokinetics of rifampicin and isoniazid in Ethiopian tuberculosis patients.

2.2. Specific objectives

- To determine plasma levels of isoniazid in Ethiopian tuberculosis patients undergoing treatment with first-line drugs (***Paper I and III***)
- To determine plasma levels of rifampicin in Ethiopian tuberculosis patients undergoing treatment with first-line drugs (***Paper I and IV***)
- To evaluate the effect of socio-demographic characteristics and *NAT2* genetic polymorphism on isoniazid plasma levels (***Paper III***)
- To evaluate the effect of socio-demographic characteristics and *SLCO1B1*, *ABCB1*, *AADAC*, and *CES-2* genetic polymorphism on rifampicin plasma levels (***Paper II, IV, and V***)
- To characterize population pharmacokinetics of rifampicin in Ethiopian tuberculosis patients (***Paper V***)

Chapter 3: Materials and Methods

3.1. Study Setting

The study was conducted at the primary healthcare centers in Addis Ababa, Ethiopia **(*Paper III-V*)**. Addis Ababa is the capital city of Ethiopia and its population is estimated to be 3,604,000 (1,703,000 male and 1,900,000 female) in the year 2019 as reported by the Ethiopian Statistical Service (available at http://www.statsethiopia.gov.et/population-projection/). Currently, 13 hospitals are run by the government and more than 40 private hospitals are available in the city. Additionally, 98 government-operated health centers are providing primary healthcare services across various sub-cities of Addis Ababa.

According to the current TB treatment approach in Ethiopia, the delivery of anti-TB drugs takes place at the primary healthcare level. The primary healthcare centers included in the study are Beletshachew, Teklehymanote, Kazanchis, Areda, Adis Raiy, and Woreda 02 Health Centres.

3.2. Study design and period

A prospective observational study was conducted in Addis Ababa, Ethiopia ***(Paper III-V).*** The study was conducted from October 2019 to November 2021.

3.3. Treatment

Newly diagnosed patients with drug-susceptible *Mycobacterium* TB were enrolled in the TB clinics at the aforementioned primary health care centers. Treatment was given according to Ethiopian treatment guidelines for drug-sensitive TB. Participants were administered a fixed-dose combination (FDC) compromising rifampicin, isoniazid, pyrazinamide, and ethambutol daily ***(Paper III-V)***. Patients with a body weight greater than 55kg received four FDC tablets daily. Those with a body weight ranging between 40 and 55 kg received three FDC tablets daily, while those below 40 kg received two FDC tablets daily. Each FDC tablet contained 150, 75, 400, and 275 mg of rifampicin, isoniazid, pyrazinamide, and ethambutol respectively. Treatment was provided under directly observed therapy at the health center mentioned.

3.4. Eligibility Criteria

3.4.1. Inclusion criteria

The study included treatment naïve TB patients who have either pulmonary TB or extrapulmonary TB. Both male and female participants aged 18 to 65 years were recruited to the

study. For the analysis of the influence of genetic makeup on the pharmacokinetics of rifampicin and isoniazid, patients diagnosed only with TB were included. Furthermore, the participants who were able to understand the given information during consenting and were willing to sign the informed consent form were included.

3.4.2. Exclusion Criteria

Pregnant women, individuals who were critically ill, patients who had hemoglobin levels below 7g/dl, and those with a history of liver disease, recent vomiting, and diarrhea were excluded from the study. For the analysis of the influence of genetic makeup on the pharmacokinetics of rifampicin and isoniazid, patients diagnosed with either diabetes or HIV were excluded.

3.5. Blood sample collection

Blood samples were obtained at three different time points, although for a few patients, blood samples were drawn at two time points. Patients collected the medications in the morning at the TB clinic and ingested the drug on an empty stomach under direct observation. Blood was collected in an EDTA tube. For pharmacokinetics analysis plasma was separated immediately and stored at -80°C at the Department of Pharmacology and Clinical Pharmacy at Addis Ababa University. The samples were later transported to Karolinska Institutet in Stockholm, Sweden for analysis on dry ice. Similarly, whole blood for pharmacogenetics analysis was stored and transported under the same conditions ***(Paper III-V)***.

3.6. DNA extraction and SNP Genotyping

Genomic DNA was extracted from whole blood samples using the QIAmp DNA Blood Midi Kit (QIAGEN GmbH, Hilden, Germany) following the manufacturer's protocol. The protocol is used for genomic DNA extraction from 0.5-2ml of whole blood. DNA was quantified spectrophotometrically using a NanoDrop spectrophotometer and stored at -20˚C until performing the genotyping assay **(*Paper III-V*)**.

Genotyping was performed using TaqMan^{R} drug metabolism assay reagents for allelic discrimination (Applied Biosystems Genotyping Assays) with the following ID numbers for each SNPs: C___8911003_1 for *AADAC2* (c.841G>A, rs1803155), C__31760486_10 for *CES2* (c.269-965A>G, rs4783745), C___7586657_20 for *ABCB1* (3435C>T, rs1045642), C__11711730_20 for *ABCB1* (c.4036A>G, rs3842), C___1901697_20 for *SLCO1B1*

(c.388A>G, rs2306283), C__30633906_10 for *SLCO1B1* (c.521T>C, rs4149056), C___1204093_20 for *NAT2*5* (c.341T>C, rs1801280), C___1204091_10 for *NAT2*6* (c.590G>A, rs1799930), C____572770_20 for *NAT2*7* (c.857G>A, rs1799931), C____572770_20 for *NAT2*14* (191G>A, rs1801279). The final volume for each reaction was 10 µL, consisting of 9 µL TaqMan® fast advanced master mix (Applied Biosystems, Waltham, MA, United States), DNA/RNA free water, TaqMan 40X for *SLCO1B1*, *ABCB1*, and all *NAT2*, and TaqMan 20X for *AADAC* and *CES2* drug metabolism genotyping assays mix (Applied Biosystems) and 1 µL genomic DNA. Genotyping was performed by real-time Q-PCR (Applied Biosystems) equipped with 7500 software V2.3 (Life Technologies Corporation) for allelic discrimination. The PCR conditions consisted of an initial step at 60°C for 30 s, holding the stage at 95°C for 10 min and the PCR stage for 40 cycles, step 1 at 95°C for 15 and step 2 at 60°C for 1 minute, and after reading stage with 60°C for 30 s.

3.7. Drug concentration determination

Rifampicin, isoniazid, and actylisoniazid concentrations were simultaneously determined using a liquid chromatography-tandem mass spectrometry (LC-MS/MS) system ***(Paper III-V)***. The system comprised an Acquity Ultra Performance LC-system coupled to a Xevo TQ-S Micro (Waters, Milford, MA, USA). Quantifications of the drugs were done at the routine therapeutic drug-monitoring laboratory, Department of Clinical Pharmacology, Karolinska University Hospital; Stockholm, Sweden as previously described [122]. The chromatographic column utilized was the YMC-ultraHT hydrosphere C18, 2µm (particle size), 100 (length) × 2mm (internal diameter), reversed-phase column (Waters). The mobile phase gradient consisted of 0.1% formic acid in Milli-Q pure water; 100% methanol, methanol: Milli-Q pure water containing 0.1% formic acid (10:90:0.1); methanol: Milli-Q pure water: isopropanol: containing 0.1% formic acid (70:20:10:0.1); Methanol: Milli-Q pure water (10:90).

Plasma sample preparation involved protein precipitation with acetonitrile containing deuterated rifampicin, isoniazid, and acetylisoniazid as an internal standard. The process consisted of diluting 100 µL plasma samples with a 300 µL solution containing the internal standards dissolved in acetonitrile. After shaking for 30 seconds using Hamilton and 5 minutes of centrifugation, 150 µL supernatant was transferred to another plate using Hamilton. The supernatant dried for 30 minutes at 35ºC and the dried sample was re-suspended with 15 µL

methanol and 275µL 0.1% formic acid for injection. The chromatography run time was approximately 3 min for each sample. The quantification range was 0.1 – 40 µg/mL, 0.05 – 20 µg/mL, 0.05-10 µg/mL for rifampicin isoniazid and acetylisoniazid respectively. The method was validated according to European Medicines Agency (EMA) guidelines [123].

3.8. Statistical Analysis

The C_{max} was determined based on the available plasma concentration; with the highest observed concentration identified as C_{max}. The AUC_{0-7h} was calculated using the trapezoidal rule and Graphpad prism was used for AUC_{0-7h} calculations. The normality of data was assessed using the Shapiro-Wilks test. Non-normally distributed data were expressed as median (interquartile range, IQR), and normally distributed data were presented as mean (standard deviations SD). To evaluate the difference between observed and expected Hardy-Weinberg equilibrium, and genotype-phenotype concordance the Chi-square test was applied. Kruskal-Wallis tests were performed to see the variations of C_{max} and AUC_{0-7h} between genotypes. Additionally, Univariate and multivariate linear regression analyses were performed to identify predictors of C_{mx} and AUC_{0-7h}. Variables with p-value <0.2 from the univariate analysis were included in the multivariate regression analysis. Data were analyzed using SPSS version 25, and a p-value of less than 0.05 was considered statistically significant ***(Papers III and IV)***.

3.9. Population Pharmacokinetic Analysis

Population pharmacokinetic (POPPK) modeling was done using NONMEM (Version 7.5.0, Icon Development Solutions, http://www.iconplc.com, Ellicott City, MD, USA) with PsN (version 5.2.6; https://uupharmacometrics.github.io/PsN/) and Pirana (version 2.9.9; https://www.certara.com/software/pirana-modeling-workbench/) as modeling and simulation workbench. Data preparation and NONMEM results post-processing and visualization were conducted using the R statistical software (version 4.2.0; www.r-project.org) ***(Paper V).***

The POPPK model was developed by adapting a reference model from literature; following the recommended best practices for using the PRIOR subroutine in NONMEM [124]. The maximum likelihood estimation method (FOCE-I in NONMEM) was used for parameter estimation. Structural model parameters for individuals (P_i) were derived from the equation

$$P\_i=P\_TV\times e^{\eta}, \text{ where } P\_TV$$

Represents the typical population parameter value and η (ETA) is randomly sampled from a normal distribution with a mean of 0, and variance of ω^2, i.e., η_i=N(0,ω^2).

Proportional (prop), additive (add), or combined (add+prop) residual error models were tested to account for within-subject variability, experimental errors, and model misspecification. Models were evaluated using log-likelihood ratio test, goodness of fit (GOF) plots, parameter uncertainty, and visual predictive checks. The difference in OFV > 3.84 units (equivalent to p-value ≤ = 0.05 for χ2 distribution) between nested models was considered to be a statistically significant improvement in model fit. Uncertainties of model parameters were measured by relative standard errors (RSE) or bootstrap confidence intervals for the final model.

Inter-individual variability (IIV) of model parameters, as measured by a coefficient of variation (CV %), were calculated from ω^2 using the formula: CV%=100×√ (e^ (ω^2)-1). Potential covariates were identified by visual inspection of covariates versus η plots. However, only biologically plausible covariates were added to the model by stepwise forward inclusion and backward elimination. First, all plausible parameter-covariate relations were separately included in the model. Then, all relations resulting in a > 3.84 decrease in OFV (i.e., p-value ≤ 0.05) were jointly included in the model. At this step, some covariate coefficients shrunk toward zero. In the next iterations of the model, covariates with the greatest decrease in coefficient values or with relative standard error (RSE) > 50% were eliminated in a step-wise manner. The tested covariates are presented in the table of subject characteristics.

The final model was qualified by goodness of fit (GOF) plots, visual predictive check (PcVPC), and non-parametric confidence intervals (CI) obtained by fitting the final model to bootstraped samples of the current dataset (Bootstrap confidence intervals).

3.10. Ethical considerations

Ethical approval was obtained from the Institutional Review Board of the College of Health Science, Addis Ababa University (Ref number 080/17/IM), and the national research ethics review committee (Ref. number MoSHE/RD/401/10975/20). The study was conducted following the ethical principle of the Helsinki Declaration. All participants received a detailed explanation of the study protocol and provided written informed consent ***(Paper III-V)***.

Chapter 4: Results

4.1. Overview

From the desk review, isoniazid and rifampicin show high inter-individual variability in pharmacokinetics. This pharmacokinetics variation of first-line antitubercular drugs can influence treatment outcomes ***(Paper I).*** Genetic variation is among the factors contributing to inter-individual variation in pharmacokinetics. ***Paper II*** summarized the current literature available on the role of pharmacogenetics in the inter-individual variability of rifampicin and other rifamycins.

Overall, a total of 146 newly diagnosed TB patients were enrolled. Rifampicin pharmacokinetics data is available for 145 patients. The majority of patients 94 (64.4%) had isoniazid C_{max} within the recommended therapeutic range (3-6 µg/mL) (***Paper III),*** but only 42 (29%) patients achieved the therapeutic range of > 8µg/mL for rifampicin, (***Paper IV)***. The majority (74.2%) of the study participants were slow acetylators. *NAT2* genotype significantly influences the pharmacokinetics of isoniazid and the metabolic ratio. The overall concordance between *NAT2* genotype and phenotype was 85% ***(Paper III).***

Considerable variability between patients in rifampicin pharmacokinetics parameters was observed (***Paper IV and V***). Variability in the pharmacokinetics of rifampicin might be in part explained by *ABCB1 c.4036A>G, ABCB13435C>T,* and *AADAC2 c.841G>A* genotypes *(**Paper II, IV, and V***). For instance, *ABCB1 c.4036A>G* genotypes independently accounted for 7.4%, and 6.1 % of the variability in rifampicin C_{max} and $AUC_{0\text{-}7h}$, respectively ***(Paper IV***) and 41% variability in clearance ***(Paper V)***. Similarly, the *ABCB1 3435C>T TT* genotype was estimated to have a 100% higher absorption rate constant than those with *CC* or *CT* genotypes. A two-compartment model coupled with a transit absorption model adequately fitted the rifampicin data ***(Paper V)***.

In addition to the genotypes described above, the plasma exposure of both isoniazid and rifampicin significantly increases as the dose increases ***(Paper III and IV)***. Sex is also another socio-demographic characteristic associated with the variability in plasma exposure to isoniazid and rifampicin. Generally, females had better exposure compared to males. However, variables

such as age, smoking, khat use, alcohol consumption, diabetes mellitus, and HIV did not influence plasma exposure to rifampicin and isoniazid ***(Paper III, IV, and V).***

4.2. Study Participants Characteristics

A total of 146 newly diagnosed TB patients, with a mean age of 31.25 years and a mean body weight of 55.82kg were enrolled in the study. The majority of the patients had no co-morbid conditions; however, 11 patients (7.5%) and 10 (6.7%) patients had diabetes and HIV comorbidity respectively. Of the total 146 patients, 102 patients (69.86%) had pulmonary TB, and 44 (30.14%) had extrapulmonary TB. The male participants account for a higher proportion (55.5%) compared to females (44.5%). Cigarette smoking, khat use, and alcohol use were reported by 14.38%, 19.17%, and 19.17% respectively as shown in **Table 2**.

Overall, 96.57% (N=141) of participants completed treatment; two (1.37%) were lost to follow-up, two (1.37%) were transferred to another health facility and one patient died before the completion of treatment. Treatment failure was noted for one patient.

According to the Ethiopian treatment guidelines, the doses of rifampicin and isoniazid recommended are 10mg/kg and 5mg/kg with maximum daily doses of 600mg and 300mg respectively. The mean dose of rifampicin received was 9.4 mg/kg (SD, 0.96) while the mean dose of isoniazid received was 4.7 mg/kg (SD, 0.48).

Table 2 Socio-demographic and clinical characteristics of Ethiopian tuberculosis patients (N=146)

Variables		Pulmonary TB	Extrapulmonary TB	Total
Sex	Male	62	19	81(55.5)
	Female	40	25	65(44.5)
Marital status	Single	64	14	78 (53.42)
	Divorced	5	1	6 (4.1)
	Married	30	27	57 (39)
	Widowed	3	2	5 (3.42)
Treatment outcome	Completed	33	44	77 (52.7)
	Cured	63	0	63 (43.1)
	Died	0	1	1 (0.7)
	Failed	1	0	1 (0.7)
	Not assessed	2	0	2 (1.4)
	Referred	2	0	2 (.4)
Smoking	Yes	19	2	21 (14.38)
	No	83	42	125 (85.6)
Khat Chewer	Yes	25	3	28 (19.17)
	No	77	41	118 (80.8)
Alcohol	Yes	24	4	28 (19.18)
	No	78	40	118 (80.8)
Age (years), (mean, range)		30.88 (18-65)	32.1 (19-60)	31.25 (18-65)
Body weight in Kg, (mean, range)		54.05 (34-82)	59.95 (35-81.5)	55.82 (34-82)
TB-HIV (n, %)		9	1	10 (6.7)
TB-DM (n, %)		9	2	11(7.5)
Dose of rifampicin received (mg/kg, SD)		9.46 (0.97)	9.28 (0.95)	9.4 (0.965)
The dose of isoniazid received mg/kg mean (SD)		4.73(0.487)	4.64 (0.47)	4.7 (0.48)

4.3. Genotypes

Genotyping of *NAT2, SLCO1B1 (c.388A>G, c.521T>C), ABCB1 (c.3435C>T, c.4036A>G), AADACc.841G>A, and CES-2 (c.269-965A>G)* was performed to investigate the pharmacogenetic effects on pharmacokinetics of isoniazid and rifampicin. For all genotypes, no significant variation between observed and expected genotype frequencies according to Hardy-Weinberg equilibrium was observed.

Genotyping for the four most common functional variant alleles of *NAT2 rs1801280 (c.341T>C), rs1799930 (c.590G>A), rs1799931 (c.857G>A),* and *rs1801279 (c.191G>A)* was done for 120 TB patients. The frequency distribution of *NAT2*4, *5, *6, *7,* and **14* alleles were 14.6%, 47.1%, 31.3%, 5.4%, and 1.7%, respectively. There were twelve *NAT2* genotype groups observed among the study participants. The most frequent genotype was *NAT2 *5/*5* followed by *NAT2 *5/*6,* and *NAT2 *6/*6*. The frequency of the homozygous wild type (*NAT2 *4/*4* genotype) was rare.

A panel of four SNP can estimate acetylator genotype groups of about 98% [86]. Among 120 patients enrolled in the study, we identified 4, 27, and 89 individuals with fast, intermediate, and slow acetylator genotypes, respectively. The overall frequencies of genotype-predicted slow, intermediate, and fast acetylators were 74.2%, 22.4%, and 3.3% respectively. The genotype distribution and inferred phenotypes of the *NAT2* are presented in ***Paper III*** **(Table 2)**.

Genotyping for drug transporters, including *SLCO1B1c.388A>G, SLCO1B1 c.521T>C, ABCB1 c.3435C>T, ABCB1 c.4036A>G,* and enzyme *AADAC c.841G>A,* and *CES-2 c.269-965A>G* was done to assess their impact on rifampicin pharmacokinetics. The observed genotype and allele frequency distributions among the 119 study participants are presented in ***Paper IV*** **(Table 2).**

The variant allele *SLCO1B1c.388A>G* occurred at a higher frequency (62.2%), while the defective variant allele *SLCO1B1c.521T>C (*5)* occurred at a lower frequency (20.2%). The minor allele variant *ABCB1 c.3435T* and *ABCB1 c.4036G* were 24, 4%, and 14.6% respectively. Conversely, the variant *AADAC c.841A* allele occurred at a much higher frequency (86.1%), whereas the *CES-2 c.269-965G* allele occurred at 30.6%.

4.4. Pharmacokinetics of Isoniazid

Plasma samples were obtained from 146 patients for the analysis of isoniazid pharmacokinetics. Blood was collected at varying intervals ranging from 1 hour to 7 hours post-drug intake on an empty stomach during the intensive phases of therapy. In total, 428 samples were collected. Plasma sampling took place three times for the majority of patients 137 (93.8%), two times for 8 (5.48%) patients, and one time for 1 (0.68%) patient.

4.4.1. Peak plasma concentration of Isoniazid (C_{max})

The C_{max} of isoniazid was determined by selecting the highest measured isoniazid plasma concentrations. In general, the median C_{max} of isoniazid is 4.73µg/mL. Literature shows that the C_{max} of isoniazid is usually achieved within 1-2 hours post-dosing. Similarly, in the majority of subjects who participated in the study, the C_{max} was achieved between 1 and 2 hours post-dosing. **Figure 6** shows the time at which C_{max} was observed and the regression line in the figure shows that the highest C_{max} is achieved when the plasma is sampled earlier and gradually decreases as the time of sampling increases.

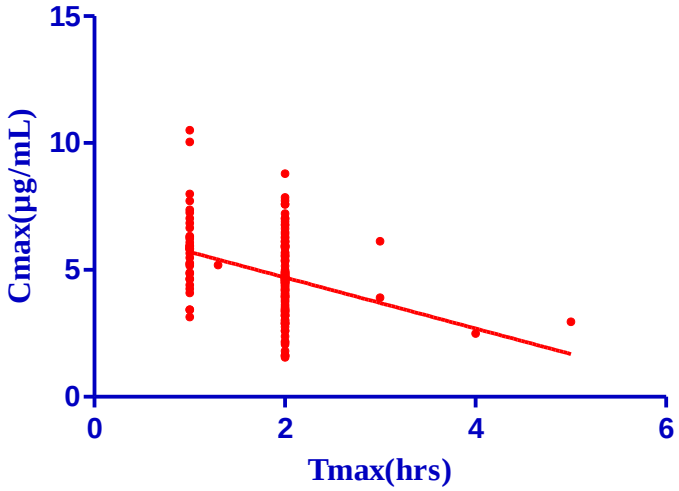

Figure 6: C_{max} of isoniazid compared to the time at which C_{max} achieved (N=146)

The median C_{max} of isoniazid is higher in females (5.48 µg/mL) compared to males (4.54 µg/mL) ($p<0.001$). Higher median C_{max} was also observed in patients with extrapulmonary TB (5.55 µg/mL) compared to patients with pulmonary TB (4.66 µg/mL). **Figure 7** depicts box-and-

whisker plots showing the distribution of isoniazid C_{max} in males and females. The line in the middle of each box displays the median, and the lower and upper end of the box shows the 25th and the 75th quartiles respectively. The length of the box represents the IQR.

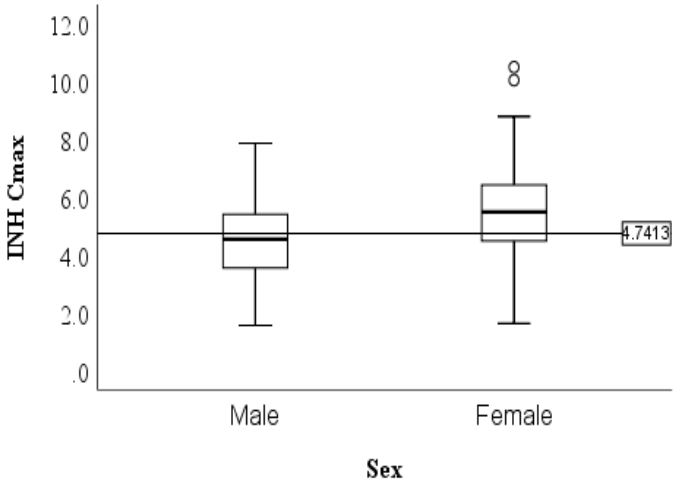

Figure 7: Box-and-Whisker plots showing isoniazid (INH) C_{max} in males and females.

A range of 3 to 6 µg/mL C_{max} of isoniazid is considered optimal for a favorable treatment outcome. The majority of patients 94(64.4%) had isoniazid C_{max} within the specified range (3 to 6 µg/mL), 19 (13%) had an isoniazid C_{max} of below 3 µg/mL (low C_{max}), and 33 (22.6%) had a C_{max} of above 6µg/mL (high).

4.4.2. Effect of *NAT 2* genotypes on isoniazid C_{max}, acetyl-isoniazid, and metabolic ratio

There was a significant difference in median values of C_{max} of isoniazid and acetyl isoniazid among the three *NAT-2* genotype groups. Specifically, a significant difference in the C_{max} value of isoniazid was observed between fast and slow acetylators (p=0.04) and intermediate and slow acetylators (p<0.001). However, no significant difference in the C_{max} of isoniazid was observed between fast acetylators and intermediate acetylators (p=0.81). **Figure 2** and **Figure 3** in ***Paper III*** show the comparison of the median C_{max} of isoniazid and the metabolic ratio of acetyl isoniazid/isoniazid among fast, intermediate, and slow acetylators genotypes respectively.

The C_{max} concentration of acetyl-isoniazid exhibits significant variation among the three *NAT2* genotypes. A substantial difference in acetyl-isoniazid concentration was observed between slow and intermediate (p<0.001) and slow and fast (p=0.001) acetylators. The variation in the

AcINH/INH metabolic ratio among the three genotype groups is highly significant ($p<0.001$), indicating distinct metabolic patterns for the genotype group.

At the time of $C_{max,}$ ten slow acetylators and two intermediate acetylators had undetectable acetyl isoniazid concentrations. The pattern of $C_{max,}$ $AUC_{0-7,}$ and AcINH/INH of the three metabolizer groups is shown in ***Paper III*** **(Table 3).** A pronounced difference in isoniazid $AUC_{0–7h}$ was observed among acetylator genotype groups. The median isoniazid AUC_{0-7h} for slow, intermediate, and fast acetylators was 13.09 µg.h/mL, 6.09µg.h/mL, and 3.73µg.h/mL, respectively. The difference in $AUC_{0–7h}$ between the slow genotype group and the other two groups is statistically significant ($p<0.001$).

4.4.3. NAT2 genotype - phenotype concordance

The concordance between phenotype and genotype was evaluated through conventional phenotype classification. The overall concordance between *NAT2* genotype and phenotype was 85%. In ***Paper III (*****Table 5*****),*** the alignment between genotype-inferred acetylator status and measured NAT2 acetylator phenotypes is presented. The *NAT2* genotype accurately predicted the acetylator phenotype in 92 patients. Nearly all slow acetylator genotypes (98.3%) were precisely predicted, whereas 13.88% of fast acetylators genotypes were misclassified as slow acetylator phenotypes. Only one *NAT2*5/*5* slow acetylator genotype was predicted as a fast acetylator phenotypically. Heterogeneity in acetylation status was evident for *NAT2*4/*5* and **4/*6* genotypes, with more than half of *NAT2*4/*5* (62.5%) and *NAT2*4/*6* (57%) genotypes carriers manifesting a slow acetylator phenotype.

4.4.4. Predictors of isoniazid C_{max} and AUC_{0-7}

We conducted univariate and stepwise multivariate linear regression analysis using log-transformed C_{max} and AUC_{0-7hr} as a dependent variable to identify predictors of isoniazid C_{max} and AUC_{0-7hr}. The variables considered in the analysis were age, cigarette smoking, khat chewing, alcohol use, gender, drug dose, the three *NAT2* acetylators genetic variants, and comorbid condition to identify a predictor of isoniazid C_{max} (**Table 3**).

In the univariate analysis, both sex ($p=0.002$) and *NAT2* acetylator genotypes ($p<0.001$) emerged as significant predictors of isoniazid C_{max}. The results of stepwise regression analysis revealed

that *NAT2* acetylator genotypes alone accounted for 26.1% of the variability in isoniazid C_{max}. When considering sex and *NAT2* acetylator genotypes together, the explained variability increased to 32.7%. Furthermore, 36.2% variability in isoniazid C_{max} was observed when drug dose (mg) was added to the sex and *NAT2* acetylator genotypes. These findings suggest that, among the examined variables, *NAT2* acetylator genotype, and sex and drug dose play significant roles in predicting isoniazid $C_{max.}$

Similarly, sex ($p=0.011$), and *NAT2* acetylators genotypes ($p<0.001$) showed significant association with variations in isoniazid $AUC_{0\text{-}7h}$. In the multivariate stepwise analysis, sex, *NAT2* acetylator genotypes and drug dose (mg) collectively account for 46.8% variation in $AUC_{0\text{-}7h}$ of isoniazid. *NAT2* acetylator genotypes alone were responsible for 40.6% of isoniazid $AUC_{0\text{-}7h}$ variation and this variation increased to 43.1% with the addition of sex.

Table 3: Univariate and multivariate analysis showing factors associated with $logC_{max}$ and $logAUC_{0-7}$ of isoniazid (n=146) (predictors in the bracket are a reference variable)

PK parameter	Predictor	Univariate analysis		Multivariate analysis	
		Adjusted Beta Coefficients	*P*	Adjusted Beta Coefficients	*P*
C_{max}	Sex (female)	-0.25	0.002	-0.255	<0.001
	Age	0.00	0.99	-	-
	Smoking (smoker)	0.022	0.8	-	-
	Khat (chewer)	0.024	0.77	-	-
	Alcohol (user)	0.007	0.93	-	-
	Dose (mg)	0.123	0.14	0.165	0.013
	Acetylator genotypes (fast)	0.517	< 0.001	0.487	<0.001
	Co-morbidity (TB only)	-0.056	0.484	-	-
AUC_{0-7h}	Sex (female)	-0.162	0.05	-0.141	0.045
	Age	-0.089	0.286	-	-
	Smoking (yes)	-0.074	0.376	-	-
	Khat (yes)	-0.096	0.246	-	-
	Alcohol (yes)	-0.066	0.434	-	-
	Dose (mg)	0.117	0.159	0.169	0.015
	Acetylator genotypes (fast)	0.86	0.00	0.667	< 0.001
	Co-morbidity (TB only)	-0.014	0.86	-	-

4.5. Pharmacokinetics of Rifampicin

There was considerable between-patient variability in rifampicin C_{max}, ranging from1.90 to18.57 µg/mL. The median and interquartile ranges (IQR) of rifampicin C_{max} were 6.79µg/mL (5.59µg/mL to 8.44µg/mL). Only 29% (42 patients) of participants achieved the therapeutic efficacy threshold (≥ 8µg/mL) [30]. C_{max} below 4µg/mL associated with increased risk for treatment failure was observed in 7 (4.8%) patients. Similar between-patient variability in rifampicin AUC_{0-7} was observed. The AUC_{0-7} ranges from 3.61 to 47.05µg.h/mL. The median and interquartile ranges (IQR) of rifampicin AUC_{0-7} were 17.055µg.h/mL (13.9 to 22.23µg.h/mL).

4.5.1. Effect of Genotype on Rifampicin C_{max} and AUC_{0-7}

Table 3 in ***Paper IV*** presents a comparison of the median (IQR) and geometric mean (± standard error) of rifampicin C_{max} and AUC_{0-7h} between the different genotypes. While the genotypes of *SLCO1B1*1B* and *SLCO1B1*5* did not significantly contribute to variation in rifampicin C_{max} and AUC_{0-7h}, patients homozygous for *SLCO1B*5/*5 (C/C)* exhibited a C_{max} below the target concentration. Variability in rifampicin C_{max} and AUC_{0-7h} was shown to be significantly correlated with the genotype *ABCB1 c.4036A>G*. Compared to heterozygous (*A/G*) and wild type (*A/A*) genotypes, patients homozygous for the *G* variant allele had considerably greater C_{max} and AUC_{0-7h} as illustrated in **Figure 1A** in ***Paper IV***. There was no significant difference in C_{max} and AUC_{0-7} among the various *ABCB1 c.3435C>T* genotype groups.

In contrast, a significant correlation was found between *AADAC c.841G>A*, and rifampicin C_{max} with a similar trend observed for $AUC_{0-7h.}$ Notably, the values were lower among the individuals with the wild type (*G/G*) genotype compared to heterozygous (*A/G*) or homozygous variant allele (*A/A*) as depicted in **Figure 1B** in ***Paper IV***. On the other hand, no significant association of *CES 2 c.269-965A>G* genotype with rifampicin C_{max} and AUC_{0-7h} was identified.

4.5.2. Predictors of rifampicin C_{max} and AUC_{0-7}

Univariate and multivariate linear regression analyses using log-transformed C_{max} were performed to explore the predictor of rifampicin C_{max} including age, sex, drug dose, alcohol use, khat use, cigarette smoking, genotype, and co-morbidity. In the univariate linear regression analysis, sex (*p=0.005*), drug dose (p=0.024), *ABCB1 c.4036A>G* (p=0.003), and *AADAC c.841G>A* (*p=0.016*) genotypes were significant predictors of rifampicin C_{max} (**Table 4**). All the

variables that were significant predictors of rifampicin C_{max} in univariate analysis remained significant predictors in the multivariate analysis. The stepwise regression analysis demonstrated that *ABCB1 c.4036A>G* genotypes independently accounted for 7.4% of the variability in rifampicin C_{max}. Combining *AADAC c.841G>A* and *ABCB1 c.4036A>G* genotypes increased the explained variability to 12.2%. Additionally, 13.7% variability in rifampicin C_{max} was observed when a drug dose (mg) was added to the two genotypes. The overall, variability in rifampicin C_{max} explained by the two genotypes, drug dose, and sex was 17.9%.

Table 4**:** Univariate and Multivariate linear regression analysis of factors associated with rifampicin C_{max} in Ethiopian adult tuberculosis patients (n=145) (predictors in the bracket are a reference variable)

Variables	**C max**			
	Univariate		Multivariate	
	Adjusted Beta Coefficients	*p*	Adjusted Beta Coefficients	*p*
Age	0.017	0.837		
Sex (female)	-0.233	0.005	-0.223	0.01
Drug dose (mg)	0.188	0.024	0.2399	0.07
Alcohol use (yes)	0.037	0.657		
Khat chewing (yes)	0.038	0.65		
Smoking (yes)	0.007	0.93		
Co-morbidity (TB only)	-0.031	0.71		
SLCO1B1c.388A>G	0.013	0.886		
SLCO1B1c.521T>C	0.035	0.702		
ABCB1 c.3435C>T	0.089	0.335		
ABCB1 c.4036A>G	0.272	0.003	0.234	0.006
AADAC c.841G>A	-0.22	0.016	-0.224	0.009
CES-2 c.269-965A>G	0.001	0.99		

Univariate and multivariate linear regression analyses were also done on a log-transformed $AUC_{0\text{-}7hr}$ to explore the predictor of rifampicin $AUC_{0\text{-}7hr}$ including age, sex, drug dose, alcohol use, khat use, cigarette smoking, genotype, and co-morbidity (**Table** 5). In the univariate linear regression analysis, sex (p=0.023), drug dose (p=0.036), and *ABCB1 c.4036A>G* (p=0.021), genotypes were significant predictors of rifampicin $AUC_{0\text{-}7hr.}$ In the multivariate analysis, sex

(p=0.043), drug dose (p=0.018), and *ABCB1 c.4036A>G* (p=0.05), and *AADAC c.841G>A* (p=0.036) genotypes were significant predictors of rifampicin $AUC_{0\text{-}7hr}$. Overall, *ABCB1 c.4036A>G* genotypes explained 6.1 % of the variability in rifampicin $AUC_{0\text{-}7hr.}$ With the sequential addition of sex, drug dose, and *AADAC c.841G>A* to the model, the variability in rifampicin $AUC_{0\text{-}7hr}$ increased to 10.1%, 15.8%, and 19.3% respectively. These findings underscore the significant roles of *AADAC c.841G>A* and *ABCB1 c.4036A>G* genotype, along with sex and drug dose in predicting rifampicin C_{max} and $AUC_{0\text{-}7hr}$ among the variables examined.

Table 5: Univariate and Multivariate linear regression analysis of factors associated with rifampicin $AUC_{0\text{-}7hr}$ in Ethiopian adult tuberculosis patients (n=145) (predictors in the bracket are a reference variable)

Variables	**$AUC_{0\text{-}7hr}$**			
	Univariate		Multivariate	
	Adjusted Beta Coefficients	*p*	Adjusted Beta Coefficients	*p*
Age	0.001	0.47		
Sex (female)	-0.066	0.023	-0.062	0.043
Drug dose (mg)	0.001	0.036	0.001	0.018
Alcohol use (no)	0.011	0.76		
Khat chewing (no)	-0.002	0.967		
Smoking (no)	-0.012	0.786		
Co-morbidity (TB only)	0.006	0.81		
SLCO1B1c.388A>G	0.007	0.76		
SLCO1B1c.521T>C	0.006	0.82		
ABCB1 c.3435C>T	0.006	0.81		
ABCB1 c.4036A>G	0.071	0.021	0.007	0.05
AADAC c.841G>A	-0.06	0.055	-0.063	0.036
CES-2 c.269-965A>G	0.008	0.765		

4.6. Population Pharmacokinetics of rifampicin

The dataset for POPPK modeling consisted of 145 subjects with a total of 427 pharmacokinetic samples collected between days 10 to 54 and between 1 to 7 hours postdose. The number of subjects who underwent pharmacokinetic sample collection at 10 to 14-, 15 to 21-, 22 to 28-, and

greater than 28 days following the initiation of tuberculosis treatment were 27, 70, 25, and 23, respectively. Most subjects had the samples taken at 1, 2, 4, and 6 hours after dose. We evaluated 3 POPPK models of rifampicin from literature two of which reported 1-compartment disposition kinetics for rifampicin. The adequacy of the models to describe the observed pharmacokinetic in the current dataset was assessed using GOF plots, OFV, and AIC (Akaike information criterion) obtained with MAXEVAL = 0 in NONMEM, and NPDE (normalized prediction distribution error) statistics. We selected Svensson's 2019 model as the reference model for our modeling [125]. In addition to its relatively lower OFV/AIC compared to other models, Svensson's model had better GOF and good precision of parameter estimates. The comparison of the three reference models is presented in ***Paper V*** (**Figure 1** and **Table 2)**.

Svensson's 2019 population pharmacokinetic model described 2-compartment disposition kinetics coupled with transit absorption kinetics. Clearance was modeled using a well-stirred model with saturable intrinsic clearance described by Michaelis Menten kinetics. The volume of distribution appeared to decrease after day 4 of treatment. The parameters of the model were oral bioavailability (F), saturable intrinsic clearance (CLi), Michaelis–Menten constant (Km), percent increase in CLi after day 4 (IND), central volume (Vc), percent decrease in Vc after day 4 (DTV), inter-compartment clearance (Q), peripheral volume (Vp), absorption rate constant (Ka), mean transit time (MTT), number of transit compartments (NN). Allometric scaling was applied to clearance (CL, Q) and volume (Vc, Vp) parameters with fixed allometric exponents (e.g.,$(CL)_i = (CL)_{pop} \times \left(\frac{WT}{70}\right)^{0.75}$, and $(Vc)_i = (Vc)_{pop} \times \left(\frac{WT}{70}\right)$).

Refitting the model with pharmacokinetic data from adult Ethiopian subjects (after supplying priors and weights of the priors) led to a decrease in OFV ($\Delta OFV = -113$). The weights of the priors were calculated from the RSE reported in the reference model. The resulting model with priors converged successfully but the NONMEM covariance step was aborted because the covariance matrix was non-positive semi-definite indicating over-parameterization of the model. To remove over-parameterization, 5 parameters including Q, Vp, IND, DTV, and F were fixed to prior values while the OMEGA degree of freedom was recalculated based on half of RSE from the reference model. As the new model converged and the NONMEM covariance step was successful, it was used as a base model for further development.

The next step involved identifying parameters that could be stably estimated without supplying priors. This was achieved by comparing RSE from the base model with those reported in the reference model, followed by sensitivity analysis. Comparisons of RSE indicated a RSE ratio of ≤ 0.5 for CLi, V, Ka, and NN, therefore we conducted a sensitivity analysis for these parameters.

The sensitivity analysis showed that despite a 50% change in the prior values, the estimates of CLi, V, and Ka changed by ≤ 10% indicating that these parameters were less sensitive to their priors. Therefore, priors for these parameters were removed in the subsequent covariate model development. Although the resulting model converged, the covariance step failed indicating that the model was still over parameterized. Therefore IIV of Q and IOV of MTT were fixed to prior values. The resulting model was used for covariate model development. Except for η of CL and F1, η for other parameters indicated high shrinkage and correlation. Although covariates versus η plots indicated parameter-covariate relations, only biologically plausible relations were tested in the model as described in the methods section. Relations resulting in a significant decrease in OFV when added individually to the model were: Sex on CL ($\Delta OFV = -11$), HIV on CL ($\Delta OFV = -4$), *ABCB1 rs3842* on CL ($\Delta OFV = -10$), *AADACc.841G>A* on F ($\Delta OFV = -6$), and *ABCB1 CT3434* on KA ($\Delta OFV = -8$). When all these covariates were jointly added, the model did not converge due to rounding errors and OFV decreased by 20 units. Stepwise backward elimination removed sex on CL and *AADACc.841G>A* on F without a significant increase in OFV.

The final POPPK parameter estimates and bootstrap confidence intervals are presented in ***Paper V*** (**Table 3)**. The RSE is consistent with the narrow bootstrap confidence intervals and indicates that the parameters were estimated with good precision. Genetic polymorphisms in *ABCB1*, namely *ABCB1 (c.4036A>G)* and *3435C>T* were identified as covariates on CL and KA, respectively. According to the final model, subjects with *ABCB1 (c.4036A>G) GG* genotype are estimated to have 41% lower CLi compared to subjects with *ABCB1 (c.4036A>G) AA* or *AG* genotypes. Similarly, subjects with an *ABCB1 (c.3435C>T) TT* genotype were estimated to have 100% higher KA than those with *ABCB1 (c.3435C>T) CC* or *CT* genotypes. Based on the VPC (**Figure 2** in ***Paper V)***, the developed model provides an adequate description of the observed data from the present study.

Chapter 5: Discussion

Since the pharmacokinetics of rifampicin and isoniazid may modify the drug effect and TB treatment outcomes [18], several studies described their pharmacokinetics in different populations ***(Paper I).*** However, data from sub-Saharan Africa, in particular, Ethiopia are scarce. On the other hand, sub-Saharan African countries are highly burdened by TB and TB-HIV co-infections. Furthermore, the African population has high levels of genetic and phenotypic diversity [126]. In this study, we investigated the plasma exposure of isoniazid and rifampicin, the effect of the *NAT2* genotype on isoniazid, and its metabolite (acetyl isoniazid) exposure, the effect of pharmacogenetic variations in drug transporter proteins (*SLCO1B1* and *ABCB1*) and metabolizing enzymes relevant for rifampicin disposition (*AADAC2* and *CES-2*) on rifampicin exposure, and developed population pharmacokinetics in a cohort of newly diagnosed Ethiopian tuberculosis patients.

The main findings of our study include: 1. High variability of isoniazid and rifampicin exposure where a majority (87%) of patients had isoniazid peak plasma concentration within or above the range of therapeutic efficacy (≥3µg/mL), and 71% of patients had a rifampicin peak plasma concentrations below the recommended target concentration (≥8µg/mL) ***(Paper III and IV);*** 2. High prevalence of slow acetylators genotypes and a significant association of *NAT2* acetylator genotype with variability in isoniazid exposure ***(Paper III)***; 3. High concordance between (85%) *NAT2* genotype inferred and phenotypically measured acetylation status of isoniazid ***(Paper III)***; 4. Between patient variability in rifampicin, exposure may be explained by dose, *ABCB1c.4036A>G* and *AADACc.841G>A* genotypes and to some extent sex ***(Paper IV)***; 5. Population pharmacokinetics of rifampicin is best described by two compartments and genetic polymorphisms in *ABCB1*, *rs3842A>G* and *3435C>T* were identified as covariates on clearance and absorption rate constant, respectively ***(Paper V)***.

Previous studies in mice [15], hollow fiber system models, and recent clinical pharmacokinetic studies [127, 128] reported the role of rifampicin and isoniazid exposure in predicting TB treatment outcomes. A target of 3-6 µg/mL following a 300mg daily dose of isoniazid and 8-24µg/L following a 600mg daily dose of rifampicin C_{max} is considered optimal for a favorable treatment outcome [72, 129]. Interestingly, we found a high proportion of participants with isoniazid concentrations within the recommended threshold (64.4%) and above the threshold for

22.6% of participants. However, an alarmingly high proportion of participants (71%) had a rifampicin C_{max} below the recommended threshold ***(Paper IV).***

These results are consistent with several other studies that reported low C_{max} of rifampicin in TB patients [36, 130, 131] and the recent report also showed low exposure to rifampicin even at high doses [132]. Similarly, previous studies showed low isoniazid plasma exposure [15, 130, 133]. However, we observed high exposure (C_{max} and AUC_{0-7h}) to isoniazid in Ethiopian tuberculosis patients ***(Paper III)***. Ibrahim *et al* also reported a similar pattern of isoniazid plasma concentration in Ethiopian pediatric TB patients [115]. The large proportion of slow acetylators genotypes in our study participants may be responsible for this increased exposure to isoniazid. Previous reports show that slow acetylators achieve the highest plasma concentration of isoniazid [134] and are known to be at risk of isoniazid-induced toxicity, whereas fast acetylators may experience treatment failure [135]. However, the clinical benefit and risk of increased isoniazid plasma exposure in this population may need further investigation.

Rifampicin plasma levels below the threshold are associated with unfavorable outcomes, and risk for development of drug resistance [136, 137]. Indeed, a recent meta-analysis showed a high prevalence of rifampicin resistance in Ethiopia[138]. A higher dose of rifampicin or therapeutic drug monitoring in selected patients could be beneficial as suggested previously [139]. On the other hand, reports of high-dose rifampicin clinical trials are emerging. High-dose rifampicin increased exposure to rifampicin to shortened treatment duration and increased efficacy [140-142]. Therefore, the investigation of a higher dose of rifampicin may be relevant for the Ethiopian population.

Several factors which are associated with the variability in exposure to isoniazid have been reported. Factors such as sex [133], HIV co-infection [143], diabetes [144], and *NAT2* genetic polymorphism are widely implicated in the variability of isoniazid plasma concentrations [145, 146]. Plasma isoniazid C_{max} and AUC_{0-7} increased as the isoniazid dose increased. Sex, *NAT2* acetylator genotype status, and isoniazid dose were predictors of isoniazid C_{max} and AUC_{0-7}. Females, being slow acetylator, and patients receiving higher doses of isoniazid had higher C_{max} and AUC_{0-7} compared to males, and other genotype groups ***(Paper III).***

NAT2 gene shows significant between population variations in its expression and enzyme activity. As a result, the frequency distribution of the different acetylator genotypes varies between populations. For example, Asians have the highest frequencies of the fast acetylator phenotype, followed by Native Americans and South Europeans. Central Asia, the Middle East, and West European populations were the major carriers of the slow acetylator status [88]. Africans show high variations in *NAT2* genotype frequencies. The fast acetylators are predominant in West Africa [135].

In the present study, the overall frequencies of *NAT2* acetylators genotypes are 74.2%, 22.4%, and 3.3% for slow, intermediate, and fast acetylators respectively ***(Paper III)***. Prior research conducted in the Ethiopian population similarly observed a predominance of slow acetylator genotypes and phenotypes [89, 117]. These findings align with our study's result, suggesting the high frequency of slow acetylators *NAT2* genotype in the Ethiopian population.

Various levels of NAT2 genotype-phenotype concordance were reported in different populations [147, 148]. The overall NAT 2 genotype-phenotype concordance in the present study is 85% ***(Paper III)***. In contrast, Aklillu *et.al* [89] reported somewhat a lower (75%) but significantly higher NAT2 genotype-phenotype concordance in healthy Ethiopians using caffeine as a probe drug for NAT2 enzyme activity. Notably, our findings in Ethiopian tuberculosis patients differed from those in a recent study among Zulu-speaking South Africans, where a lower percentage (55%) of genotype-phenotype concordance was reported [149]

Overall, the current study revealed higher plasma exposure, and slow acetylation status which could be predicted using both phenotypically and genotypically in Ethiopian TB patients ***(Paper III)***. Previous studies reported an association between slow acetylation status and anti-TB drug-induced hepatotoxicity [150]. Similarly, high rates of anti-TB and antiretroviral treatment-induced liver toxicity in Ethiopian TB-HIV coinfected patients, particularly in slow acetylators is reported previously[151]. Furthermore, studies have indicated that among the three NAT2 acetylator phenotypes, fast acetylators achieve the lowest, while slow acetylators achieve the highest plasma level of isoniazid [134]. Interestingly, TB patients who have *NAT2* slow acetylators have been reported to have a comparatively higher early bactericidal activity of isoniazid than fast acetylators [152]. *NAT2* genotype-guided isoniazid administration reduced

toxicity and improved treatment outcomes in Japanese trials [153], suggesting the need for further investigation of the clinical relevance of this higher plasma exposure in this population.

Significant between-patient variability in rifampicin plasma concentration was observed, ranging from 1.90 to 18.57µg/mL ***(Paper IV)***. Similar high variability in rifampicin plasma concentrations was reported in previous studies [154, 155]. The observed variability in the plasma concentration of rifampicin is associated with several factors. Population variations in the plasma concentration of rifampicin have been linked to sex [155-157], age [154, 158], human immunodeficiency virus (HIV) co-infection, and diabetes [47]. Furthermore, concomitant food [159] and drug administration [160] have been recognized as contributing factors for rifampicin pharmacokinetics variation. Food impairs the absorption of rifampicin and reduces absolute bioavailability and maximum concentration [60].

Genetic polymorphisms in genes encoding for drug-metabolizing enzymes and transporters may modify pharmacokinetics, which can also be associated with significant differences in drug efficacy, and safety [161]. Similarly, rifampicin hepatocellular uptake is mediated by OATP1B1, which is a product *SLCO1B1* gene [90, 91] and the efflux is mediated by drug efflux i.e. P-gp which is encoded by *ABCB1* genes [91].

Several studies evaluated the effect of *SLCO1B1* and *ABCB1* gene polymorphism on rifampicin exposure. However, there are inconsistent results on whether genetic polymorphism in these genes can be responsible for the variation in exposure to rifampicin. Thus, we evaluated the effect of pharmacogenetic variations in drug transporter proteins (SLCO1B1 and ABCB1) on rifampicin exposure. In the present work, we did not find a significant impact of *SLCO1B1 c.388A>G* and *SLCO1B1 c.521T>C* genotypes on rifampicin C_{max} and $AUC_{0\text{-}7h}$ (*Paper IV*). However, South African and Ugandan studies reported an association of the *SLCO1B1* genotype with variability in rifampicin pharmacokinetics [37]. Chigutsa *et al* reported lower rifampicin bioavailability for patients heterozygous and homozygous for *SLCO1B1* rs4149032 polymorphism and similar results were seen for C_{max} in the Gengiah *et al* study. In Weiner *et al* studies a 36% lower $AUC_{0–24}$ of rifampicin was observed among participants with *SLCO1B1* genotype *c.463CA* than among participants with *SLCO1B1* genotype c.*463CC*. In addition, Kim *et al* also reported lower oral clearance and higher rifampicin exposure to rifampicin in patients'

carriers of homozygous wild-type *SLCO1B1* rs4149032 [48]. However, the findings reported in these studies were not replicated in other studies [53, 102] and the present study.

While rifampicin is an inducer of the *ABCB1* gene and substrate for the gene product, in contrast, few studies investigated the influence of *ABCB1* gene polymorphism on rifampicin exposure. The effect of *ABCB1(c.3435C>T)* and *ABCB1(c.4036A>G)* genotypes on rifampicin pharmacokinetics was investigated in this work. Interestingly, subjects with a *3435C TT* genotype were estimated to have a 100% higher absorption rate constant than those with *ABCB1 3435C>T CC* or *CT* genotypes. However, we did not find significant variation in rifampicin C_{max} and $AUC_{0\text{-}7h}$ for *ABCB1 c.3435C>T* ***(Paper IV)***. Notably, *ABCB1 3435C>T* and rifampicin exposure association were reported by Huerta-García *et al* in which patients with *CC* or *CT* genotypes of *ABCB1 (c.3435C>T)* had lower C_{max} and AUC_{24} compared to those with a *TT* genotype. The *TT* homozygous genotype carrier showed lower P-gp expression in the small intestine and consequently had a lower efflux capacity [162].

On another hand, for the *ABCB1c.4036A>G* genotype, we observed a significant variability of rifampicin C_{max} and $AUC_{0\text{-}7h}$. Rifampicin $AUC_{0\text{-}7h}$ was significantly higher in homozygous variant genotype (*GG*) carriers compared to the homozygous wild type. Although the frequency of the homozygous variant genotype (*GG*) occurs at a low frequency in our study, the trends in Figure 9 show that the *G* allele carriers have better exposure to rifampicin ***(Paper IV)***. Furthermore, from the population pharmacokinetic study, subjects with *ABCB1c.4036A>G GG* genotype are estimated to have 41% lower clearance compared to subjects with *ABCB1c.4036A>G AA* or *AG* genotypes ***(Paper V)***. A lower frequency of *ABCB1c.4036A>G GG* genotype was also reported previously [163].

The metabolism of rifampicin involves an esterase enzyme. Two esterases namely; hepatic carboxylesterases (CES), and serine esterase arylacetamide deacetylase (AADAC) metabolize rifampicin [41, 42]. Few studies have assessed the influence of *CES* genetic polymorphism on rifampicin exposure ***(Paper II)***. Several drug metabolisms are modified due to genetic polymorphisms in the *CES1* and *CES2* genes. For example, polymorphisms of the *CES1* gene have been reported to affect the metabolism of dabigatran, methylphenidate, oseltamivir, imidapril, and clopidogrel, whereas variants of the *CES2* gene have been found to affect aspirin and irinotecan [164]. In the case of rifampicin, few studies reported an association between *CES*

genetic polymorphism and rifampicin pharmacokinetics. Increased plasma rifampicin concentrations with the *CES2 c.-22263A>G (g.738A>G)* variants were reported by Song *et al* [45]. Patients who carry the *CES2* (rs8192925) *G* versus *A* allele had a 17.2% increase in rifapentine $AUC_{0\text{-}24.}$ In contrast, we did not find a significant association between the *CES2 c.269-965A>G* genotypes and rifampicin C_{max} or $AUC_{0\text{-}7}$ ***(Paper IV)***. Studies from Ghanaian children [36] and Malawian TB patients [102] did not also show a significant effect of *CES2* polymorphism on rifampicin exposure.

Studies reporting an association of *AADAC* polymorphism with pharmacokinetics variation are available for rifapentine than rifampicin. Francis *et al* reported that patients with the *AA* genotype of *AADAC* rs1803155 had a 10.4% lower rifapentine clearance in South African patients [103]. Similarly, the Weiner *et al* study found that the *G* variant allele has lower rifapentine AUC, particularly in black patients than the *A* allele [46]. However, a study from Malawi did not report what was observed for rifapentine in patients receiving rifampicin [102]. In our study, *AADAC c.841G>A* significantly influenced variability in rifampicin C_{max}. C_{max} is significantly higher among those with homozygous *AA* than heterozygous *GA* genotypes or *GG* genotypes ***(Paper IV).***

The frequency distribution of *AADAC*2 (c.841G>A)* exhibits considerable variability across races and populations. Notably, reported allele frequencies of *AADAC*2* among European American, African American, Japanese, and Korean populations was around 60% [42], contrasting sharply with the 99.9% prevalence in Peruvian tuberculosis patients [165] where wild type variant almost missing. Our study on Ethiopian tuberculosis patients reveals *AADAC*2* allele frequencies of 86% and the wild-type *G* variants were less prevalent with only three individuals exhibiting homozygosity for the *GG* genotype ***(Paper IV)***. High interindividual variability in both *AADAC* mRNA levels and hydrolysis activities was observed in human lungs [166]. These findings suggest the important role of *AADAC* pharmacogenetics in tuberculosis drug therapy and the need for further investigation in populations where the *AADAC c.841G* variant occurs at higher frequencies.

Population pharmacokinetics has several advantages over classical pharmacokinetics. Population pharmacokinetics can handle spare pharmacokinetic data, easily incorporate covariates, and identify and quantify sources of variability in drug concentration in the patient population [167].

We developed a population pharmacokinetic model based on clinical observations for standard doses of rifampicin in Ethiopian TB patients. A two-compartment model coupled with a transit absorption model adequately fitted the rifampicin data. In contrast, several previous models described the population pharmacokinetics of rifampicin using a one-compartmental model [53, 70, 157]. However, the transit absorption model was described by the majority of previous model reports which is similar to our model [53, 168, 169].

Due to the sparseness of our pharmacokinetic data, the prior from the previous model [125] was used to stabilize the estimation of the model's parameter estimates. The model predicted absorption rate constant is 1.863/h with a 74% inter-individual variability ***(Paper V)***. Several previous studies reported a range of 0.236 to 2.15/h absorption rate constant for rifampicin [47]. The absorption rate constant is higher than reported by Gao *et al, l* [170], *and* Zvada *et al* [169], but lower compared to the Ugandan study.

Intrinsic clearance was saturable and well described by Michaelis Menten kinetics with a KM of 15.05mg/L ***(Paper V)***. Thus, numerous population pharmacokinetics models showed changes in clearance over time. The autoinduction of rifampicin has been well-recognized from the early days of its introduction into clinical use. Autoinduction accelerates clearance and reduces rifampicin concentration after multiple doses of therapy. A 48% induction was observed in our model. Previous reports vary on when the induction starts and ends. Smythe *et al* described the need for 8 days and 40 days for the first half-life of induction and to full induction respectively [171]. However, Chirehwa *et al* indicated that 90% of the induction could be reached after 2 weeks of oral therapy, consistent with a half-life of 4.5 days [55]. The results of our model incline towards Chirehwa *et al* because our study subjects received rifampicin daily in contrast to Smythe *et al* where the rifampicin dosing was intermittent.

The simulated central volume of distribution was 5.823L and the peripheral volume of distribution was 26.2L which is higher compared to a report by Mukonzo *et al* [122]. The bioavailability of rifampicin was 77.6% nearly similar to previous reports. The mean transit time in our study is 0.67h ***(Paper V)*** which is lower than one observed in healthy Asians, and South African children [169] but nearly similar to high dose rifampicin study [70].

Rifampicin is characterized by high pharmacokinetic inter-individual variability. Different levels of inter-individual variability in pharmacokinetic parameters were reported. For example, a recent systematic review revealed a 5.5–64.5% median of inter-individual variability for clearance [47]. We investigated the effects of polymorphisms of drug-metabolizing enzymes, drug transporters, sex, rifampicin dose, and demographic variables on the population pharmacokinetics of rifampicin.

Many drugs have been reported to show faster clearance and larger volumes of distribution in males compared to females because males have greater body weight, body mass, body water, plasma volume, organ blood flow, and larger intravascular volume [172-174]. The influence of sex on rifampicin clearance and volume of distribution has been reported previously [157, 175]. However, the covariates modeling in our present work did not show any influence of sex on the clearance and volume of distribution of rifampicin. Furthermore, none of the pharmacokinetics parameters evaluated in the study varied based on sex.

Several studies evaluated the effect of *SLCO1B1* and *ABCB1* gene polymorphism on rifampicin pharmacokinetics. There are conflicting results from different previous studies on the effect of *SLCO1B1* gene polymorphism on rifampicin pharmacokinetics. Uganda and South Africa studies reported a significant effect of *SLCO1B1* rs4149032 and rs1104581983 genetic polymorphism on rifampicin pharmacokinetics [37, 38], whereas studies conducted in South Indian[176] and other African populations [102] did not report significant effects of *SLCO1B1* rs11045819, and rs4149032 polymorphism on the pharmacokinetic parameters of rifampicin. Likewise, the two *SLCO1B1* i.e *SLCO1B1*1B(c.388A>G)* and *SLCO1B1*5 (c.521T>C)* which were investigated in this study did not affect any pharmacokinetic parameters of rifampicin in Ethiopian TB patients ***(Paper IV and V)***.

P-gp contributes to marked variability in the oral absorption of rifampicin [177]. Few studies evaluated the effect of *ABCB1*, polymorphism on rifampicin pharmacokinetic parameters. Huerta-García *et al* demonstrated that the *ABCB1* rs1045642 *TT* genotype is a predictor that explains 34.8% of the variability in rifampicin C_{max} and 48.5% of the variability in $AUC_{0–24}$ in the Mexican population. However, Medellin-Garibay *et al* [40] and Chigutsa *et al* [38] studies did not report *ABCB1* gene polymorphism on rifampicin pharmacokinetic parameters. In contrast to Medellin-Garibay *et al* [40] and Chigutsa *et al* [38], in the present study, subjects with

ABCB1 c.4036A>G GG genotype are estimated to have 41% lower intrinsic clearance compared to subjects with *ABCB1 c.4036A>G AA* or *AG* genotypes. Similarly, subjects with *ABCB1* 3435C>T *TT* genotypes were estimated to have a 100% higher absorption rate constant than those with *ABCB1 3435C>T CC* or *CT* genotypes ***(Paper V).*** Patients who are carriers of *ABCB1 c.4036A>G GG* genotype showed higher C_{max} of rifampicin and previous study in the Ethiopian and Tanzanian populations revealed higher efavirenz plasma concentration for the *GG* genotypes group [178] suggesting a significant role of this genotype in predicting drug pharmacokinetics in this population.

Diabetes [179] and HIV are well-recognized risk factors for developing TB infection [180] and studies linking rifampicin exposure and HIV and diabetes are also available. However, in the population pharmacokinetic study of Ethiopian TB patients, we did not observe the effect of HIV and diabetes on rifampicin pharmacokinetic parameters. In contrast to our findings, previous reports have indicated low rifampicin plasma concentration in diabetic patients [181] and HIV patients[182]. For instance, in Korean TB patients, diabetes affected the absorption rate constant and the volume of distribution of rifampin[183].

Chapter 6: Limitations of the Study

This study has several merits. Firstly, it leverages a substantial sample size for plasma exposure study. The timing of plasma sampling was meticulously chosen, aligning with the literature to capture C_{max} effectively, and blood collection was done post-attainment of steady state.

However, the study has also certain limitations:

1. Blood sampling took place from 1 to 7 hours, with only three samples collected. While this approach facilitates the capturing of C_{max}, it may not fully describe the AUC. Intensive blood sampling is more informative, but it poses a high cost and may not be practical for all sick patients. Thus, several studies recommended a limited sampling strategy to capture the $AUC_{24h.}$ Among the recommended limited sampling strategies, 1, 3, 8 hours [51]; and 2, 4, 8 hours [184] are well recognized for rifampicin. Our plasma sampling times are closely aligned with these previously established points.
2. C_{max}/MIC and AUC/MIC best describe the pharmacokinetic-pharmacodynamic relationship of anti-TB drugs. Our study lacks MIC and the pharmacokinetic results we obtained may not predict treatment outcome. Nevertheless, C_{max} and AUC when compared to the established range are useful signatures to predict the exposure-response relationship of first-line anti-TB drugs.

Chapter 7: Conclusion and Recommendation

7.1. Conclusion

There is high inter-patient variability in isoniazid and rifampicin exposure in study participant Ethiopian TB patients. The majority of the patients attained therapeutic plasma concentration of isoniazid for a favorable treatment outcome. In contrast, the exposure to rifampicin is very low in a large proportion of patients who participated in the study. We observed a high prevalence of the slow *NAT2* acetylator genotype and high concordance between genotype-inferred acetylator status and measured NAT2 acetylator phenotypes.

NAT2 acetylation status and the female sex are strong predictors of isoniazid exposure. Rifampicin exposure varied with sex, dose, *ABCB1 c.4036A>G*, and *AADAC c.841G>A* genotypes. *AADAC c.841G>A GG* and *ABCB1 c.4036A>G AA* genotype groups and male patients had a higher risk of low rifampicin plasma exposure. On the other hand, slow acetylators and females are at a higher risk of concentration-dependent isoniazid toxicity. Therefore, the risk of dose-dependent toxicity in the cases of isoniazid and treatment failure from lower rifampicin exposure is apparent.

7.2. Recommendation

Pharmacokinetic studies are highly valuable in the optimization of drug therapy. Pharmacogenomics studies are also considered as a tool for individualized drug therapy. However, these studies are scarce in the Ethiopian population. Therefore, based on the results of our present study we recommend;

1. Intensive pharmacokinetic study to fully characterize the pharmacokinetic parameters of rifampicin and isoniazid.
2. High plasma concentration of isoniazid may be associated with isoniazid dose-dependent toxicity. Therefore, high-dose isoniazid in DR-TB therapy should be cautiously considered and pyridoxine supplementation is highly recommended to mitigate peripheral neuropathy.
3. The impact of low rifampicin exposure on treatment outcomes needs further investigation in Ethiopian TB patients. Our findings may have important clinical implications and warrant studies on whether high-dose rifampicin improves therapeutic efficacy.

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List of Appendices

Patients Information sheet

1. **Purpose of the study**

The purpose of this study is to investigate the pharmacokinetics of rifampicin and isoniazid, which will help understand the pharmacokinetics of rifampicin and isoniazid in Ethiopian tuberculosis patients.

2. **Procedures to be followed**

If you agree to participate in the study, the study nurse will give you a routine medical examination and ask you some questions according to standard clinic procedure. He/she will take about 5ml of blood at three-time points after ingestion of the drug on the date after two weeks of anti-TB drug initiation. We will use your sample for different tests. The blood sample is used to determine the pharmacokinetics of rifampicin and isoniazid. Some genetic makeup tests that are believed to affect pharmacokinetics will be also carried out. Treatment for tuberculosis is given as per the treatment guidelines of Ethiopia.

3. **Voluntary participation**

During the study, you can choose not to answer any particular question or provide blood and sputum specimens. A decision not to participate will not affect the care you will receive at the hospital or health centers in any way. If you do agree to become a study participant, you can withdraw from the study at any time without any consequence in your treatment.

4. **Discomfort and risks**

You may feel a slight discomfort while repeated blood sample is taken and you may have some bruising at the place where the sample is taken. You may also be required to stay or need to come at the time of blood collection at the study site on the day of blood sample collection which could affect your daily activity.

Benefits: Your participation does not have monetary benefits. You will, however, receive a standard of care while participating in the trial. Your participation in this study will help generate information on how patients will benefit while using the drugs for treatment.

5. **Confidentiality statement**

The records concerning your participation are to be used only for this research project. Your name will not be used on any study forms or labels on laboratory specimens or in any report resulting from this study. At the beginning of the study, we will give you a study identification number and this number will be used on the forms and the laboratory specimens. Any information obtained in connection with this study will be kept strictly confidential. Only members of the study team will have access to information linking your name with your study number.

6. **Questions on the study**

You may contact any of the study staff if you have questions about the research, Tesemma Sileshi's Telephone number is 0911550975

Informed consent forms

I have been informed about the purpose of this study and understood the information given. I also know whom to contact if I need more information. I understand that confidentiality will be protected. I understand that I am free to withdraw from the study at any time without affecting the care I normally receive at the health institution. I agree to participate in this study.

Name of volunteer_____Signature _____ Date _____

Name of witness_____ Signature _____Date_____

Investigator's statement

I, the undersigned, have explained to the volunteer in a language he/she understands, the procedures of this study, its aims, and the risks and benefits associated with his/her participation.

I have informed the volunteer that confidentiality will be preserved and that she/he is free to withdraw from the study at any time without affecting the care she/he will receive at the hospital.

Following my definitions and explanations the volunteer agrees to participate in this study.

Name of investigator _____Signature _____Date _____

Name of witness_____ Signature _____Date_____

ስለጥናቱ ማስተዋወቅያ

1. የጥናቱ ዐለማ

የዚህ ጥናት ዐለማየቲቢ መዲሃንት በሆኑት ሪፋምፕስንና ኣይሶኒያዛየድ በኢትዮጲያን የቲቢ ህሙማን ደም ዉስጥ ያለዉን መጠን ለመረዳት ነዉ።

2. ጥናቱ የሚከተለዉ ህደት

በጥናቱ ላይ ለመሳተፍ ፈቃደኛ ከሆኑ ፤ የጤና ባለሙያ ቀለል ያሉ ምርመራና አንዳነድ ጤያቄዎችን ልጠይቆዎት ይችላሉ። በተጨማር እስከ 5ml ደም መድሓንት ከጀማሩ ከሁለት ሳምንት በኋለ አንድ ቀን ላይ መድሓንት ከወሰዱ በኋለ በተለያየ ሰዓት ሶስት ጊዜ ይወሰዳል። የተወሰደዉ ደም የቲቢ መዲሃንት በሆኑት ሪፋምፕስንና ኣይሶኒያዛየድ በኢትዮጲያን የቲቢ ህሙማን ደም ዉስጥ ያለዉን መጠን ለማጥናት ይዉላል። በተጨማር በደም ዉስጥ ያለዉ የሪፋምፕስንና ኣይሶኒያዛየድ ና የርሶ ዘረመል በቲቢ ሕክምና ዉጤት ላይ ያለቸዉ ተጽኖ ለማጥናት የገለግላል። የቲቢ ሕክምና የኢትዮጵያን የህክሚና ስርዓትን በተከተለ ዘዴ ይሰጦታል።

3. በፈቃደኝነት ስለመሳተፍ

በጥናቱላይመሣተፍበርሶሙሉፍቃደኝነትላይየተመሰረተስሆን፤ በጥናቱ ባለመሳተፎ ወይንም ፍቃደኛ ባላመሆኖ በሚደረግሎት ህክምና ላይ ተጽኖ ኣያደርስም።ከጥናቱም ከፈለጉ በፈለጉት ጊዜ እራስዎን ማግለል ይችላሉ።

4. ሊደረስ የሚችል ጉዳት

በጥናቱ በመሳተፈዎ ሚክንያት የሚመጣበዎት ምንም ኣይነት ችግር ኣይኖርም። ነገር ግን ናሙናዉን ለመዉሰድ መርፌ ሲገባ ከሚፈጥረዉ የቅጽበት የህመም ስሜት በስተቀር የጎላችግር ኣያመጣም። በተጨማር የደምናሙና በምወሰድበት ቀን በጤና ተቋም መቆት ልያስገድድ ይችላል።

5. ጥቅምን በተመለከተ

በጥናቱ በመሳተፈዎ ሚክንያት የሚያገኙት የገንዘብ ጥቅም ኣይኖርም። ነገር ግን ጥራቱን የጠበቀ የህክማ አገልግሎት ያገኛሉ። በተጨማርም ከጥናቱ የሚገኛዉ ዉጤት ህሙማን ከምወስዱት መድሃንት ምንያህል እየተጠቀሙ እነደሆነ ለመረዳት የስችላል።

6. ምስጥር መጠበቅ

በጥናቱ ውስጥ ስምዎ በማንኛውም ሁኔታ ኣይገለጽም፤ ስለሆነም የሚሠጡት መረጃና የሚገኘዉ የላቦራቶሪ ምርመራ ዉጤት ሙሉ በ ሙሉ ሚስጢራዊነቱ የተጠበቀ ነዉ።

7. በጥናቱ ላይ ጥያቄ

በጥናቱ ወቅት ጥያቄ ካለዎት የጥናቱን አስተባባር የሆኑትን አቶ ተሰማ ስለሽን በአካል ወይም በስልክ ቁጥር 0911550975 በመደዋል መጠየቅ ይችላሉ።

የዉል ስምምነት

1. የተሳታፊ ስምምነት ማረጋገጫ

ከላይ የተጠቀሰውን መረጃ አንብቢያለሁ ወይም በቃል የተሰጠኝን ማብራሪያ ተረድቻለሁ፡፡ በዚህ መሰረት ከእኔ የሚጠበቅብኝን ድርሻ በሚገባ አውቄያለሁ፡፡ እናም በዚህ ጥናት ላይ በመሳተፌ ሊከሰቱ የሚችሉትን ሁኔታዎች ተገንዝቢያለሁ፡፡ ከዚህ ጥናት በማንኛውም ሠዓት ያለምንም ቅድመ ሁኔታ ና ምክንያት እራሴን ከተሳታፊነት የማግለል ሙሉ መብት እንዳለኝ ተረድቻለሁ፡፡ ይህን ውሳኔዬን ተከትሎ በእኔም ላይ በምፈልገው የጤናአገልግሎት ላይ ምንም አይነት አሉታዊ ተጽዕኖ እንደማይደርስብኝ ተረድቻለሁ፡፡

የተሳታፊስም_____ፊርማ_____ ቀን _____

የምስክርስም_____ ፊርማ _____ቀን _____

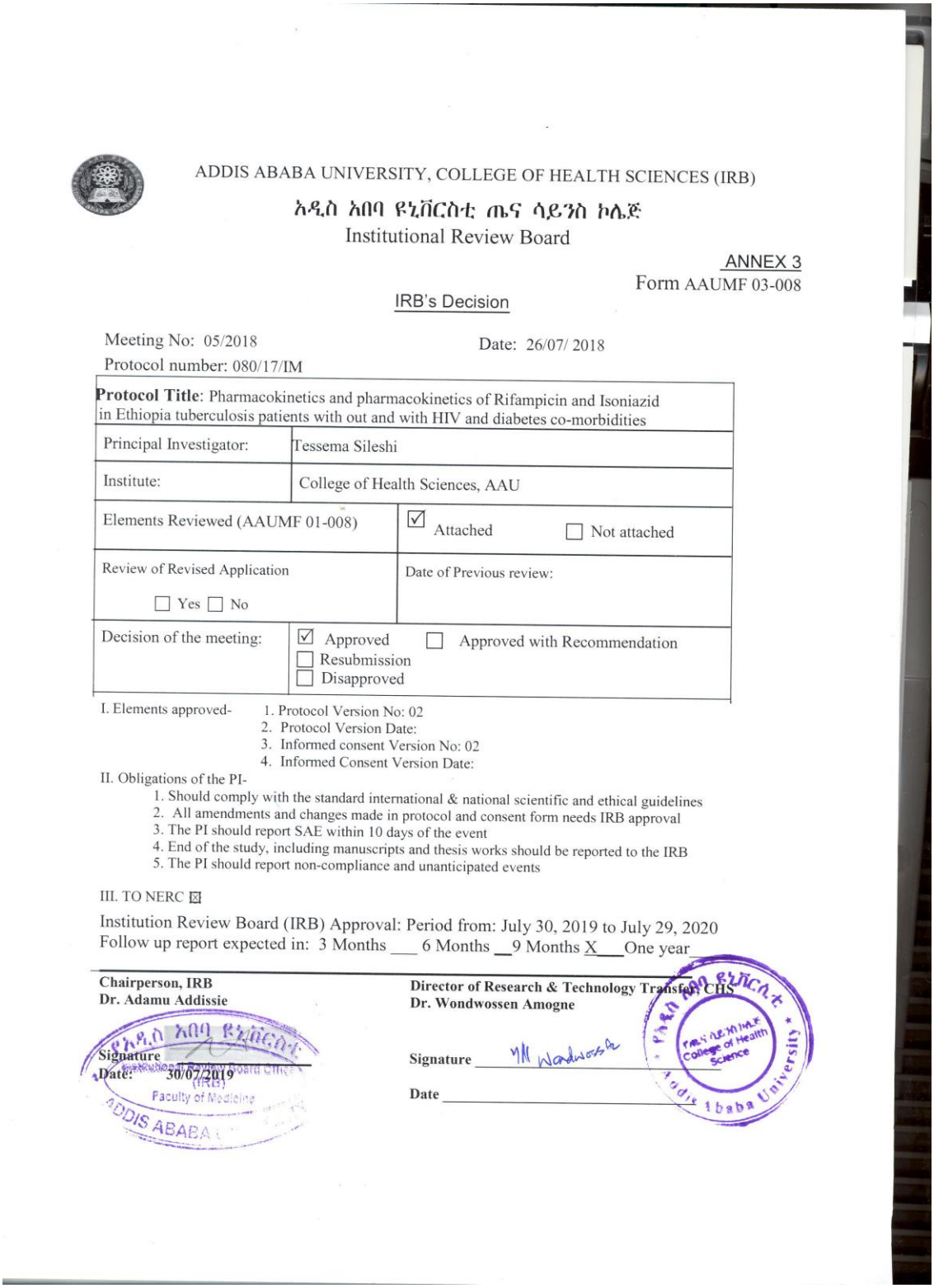
2. የአጥኝዉ ስምምነት ማረጋገጫ

ስሜ ከዚህ በታች የተጠቀሰዉ ለጥናቱ ተሳታፍ ስለጥናቱ ዐላማ፡ ጥቅምና ጉዳት ለተሳታፍዉ ግልጽ በሆናዉ ቋንቋ አስረድቻለሁ፡፡ ከተሳታፍዉ የሚገኘዉ መረጃና የላቦራቶሪ ምርመራ ዉጤት ለጥናቱ ዐላማ ብቻ እንደሚዉልና በምስጥር እንደሚጠበቅ ገልጫለሁ፡፡ አንዲሁም ለተሳታፍዉ በጥናቱ ምኞት ካልተሰማዉና ላለመሳተፍ ከፈለጉ በማንኛውም ጊዜ ማቋረጥ እንደሚችሉ አስረድቻለሁ፡፡ ይህንን ተረድተዉ ተሳታፍዉ በሙሉ ፍላጎቱ በጥናቱ ለመሳተፍ አረጋግጦልኛል፡፡

የአጥኝዉ ስም_____ ፊርማ _____ ቀን _____

የምስክር ስም_____ ፊርማ _____ ቀን _____

Ethical approval letters



ADDIS ABABA UNIVERSITY, COLLEGE OF HEALTH SCIENCES (IRB)

አዲስ አበባ ዩኒቨርስቲ ጤና ሳይንስ ኮሌጅ

Institutional Review Board

ANNEX 3

Form AAUMF 03-008

IRB's Decision

Meeting No: 05/2018 Date: 26/07/ 2018

Protocol number: 080/17/IM

Protocol Title: Pharmacokinetics and pharmacokinetics of Rifampicin and Isoniazid in Ethiopia tuberculosis patients with out and with HIV and diabetes co-morbidities	
Principal Investigator:	Tessema Sileshi
Institute:	College of Health Sciences, AAU
Elements Reviewed (AAUMF 01-008)	☑ Attached ☐ Not attached
Review of Revised Application ☐ Yes ☐ No	Date of Previous review:
Decision of the meeting:	☑ Approved ☐ Approved with Recommendation ☐ Resubmission ☐ Disapproved

I. Elements approved-
1. Protocol Version No: 02
2. Protocol Version Date:
3. Informed consent Version No: 02
4. Informed Consent Version Date:

II. Obligations of the PI-
1. Should comply with the standard international & national scientific and ethical guidelines
2. All amendments and changes made in protocol and consent form needs IRB approval
3. The PI should report SAE within 10 days of the event
4. End of the study, including manuscripts and thesis works should be reported to the IRB
5. The PI should report non-compliance and unanticipated events

III. TO NERC ☒

Institution Review Board (IRB) Approval: Period from: July 30, 2019 to July 29, 2020

Follow up report expected in: 3 Months ___ 6 Months __9 Months X___One year_

Chairperson, IRB
Dr. Adamu Addissie

Signature _____
Date: 30/07/2019

Faculty of Medicine
ADDIS ABABA

Director of Research & Technology Transfer, CHS
Dr. Wondwossen Amogne

Signature W/M Wondwossa

Date _____

Addis Ababa University College of Health Science

የሳይንስና ከፍተኛ ትምህርት ሚኒስቴር
Ministry of Science and Higher Education - Ethiopia

Ref.No. MoSHE//RD/141/1097/2020
Date: 09 MAR 2020

Addis Ababa University College of Health Science (AACHS)
Addis Ababa

Subject*: *Letter of Approval

The Ministry of Science and Higher Education (MoSHE) via its National Research Ethics Review Committee has reviewed "**Pharmacokinetics and Pharmacogenetics of Rifampicin and Isoniazid in Ethiopian Tuberculosis Patients without and with HIV and Diabetes Comorbidities**" project protocol in an expedited manner. We are writing to advise you that MoSHE has granted full approval to the above named project, for a period of **one year (March 08, 2020- March 07, 2021)**.

All your most recently submitted documents have been approved for use in this study. The study should comply with the international and national scientific and ethical standard guidelines. Any change to the approved protocol or consent material must be reviewed and approved through the amendment process prior to its implementation. In addition, any adverse or unanticipated events should be reported within 24-48 hours to MoSHE. Please ensure that you submit biannual progress report to MoSHE once in six months and annual renewal application 30 days prior to the expiry date.

We, therefore, request you as PI and your esteemed organization to ensure the commencement and conduct of the study accordingly and wish for the successful completion of the project.

Sincerely

Solomon Senor Belay (PhD)
Director General for Science and Research Affairs

Cc.

- Office of the State Minister (Sector for Science, Research and Community Service)
- Science and Research Affairs Directiorate General
- Research Ethics Directorate
  MoSHE
- Mr.Tesemma Sileshi (PI)
  AACHS

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Annex

Paper I: Sileshi, T., Tadesse, E., Makonnen, E., & Aklillu, E. (2021). The Impact of First-Line Anti-Tubercular Drugs' Pharmacokinetics on Treatment Outcome: A Systematic Review. Clinical pharmacology: advances and applications, 13, 1–12. https://doi.org/10.2147/CPAA.S289714

Paper II: Sileshi, T., Mekonen, G., Makonnen, E., & Aklillu, E. (2022). Effect of Genetic Variations in Drug-Metabolizing Enzymes and Drug Transporters on the Pharmacokinetics of Rifamycins: A Systematic Review. Pharmacogenomics and personalized medicine, 15, 561–571. https://doi.org/10.2147/PGPM.S363058

Paper III: Sileshi, T., Telele, N. F., Burkley, V., Makonnen, E., & Aklillu, E. (2023). Correlation of N-acetyltransferase 2 genotype and acetylation status with plasma isoniazid concentration and its metabolic ratio in Ethiopian tuberculosis patients. Scientific reports, 13(1), 11438. https://doi.org/10.1038/s41598-023-38716-3

Paper IV: Sileshi, T., Makonnen, E., Telele, N. F., Barclay, V., Zumla, A., & Aklillu, E. (2024). Variability in plasma rifampicin concentrations and role of SLCO1B1, ABCB1, AADAC2 and CES2 genotypes in Ethiopian patients with tuberculosis. Infectious Diseases, 56(4), 308–319. https://doi.org/10.1080/23744235.2024.2309348

Paper V: Tesemma Sileshi, Eliford Ngaimisi Kitabi, Nigus Fikrie Telele, Victoria Barclay, Alimuddin Zumla, Eyasu Makonnen, Eleni Aklillu (2024). Population Pharmacokinetics of Rifampicin in Ethiopian Adults undergoing treatment of Tuberculosis. To be submitted

Clinical Pharmacology: Advances and Applications

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ORIGINAL RESEARCH

The Impact of First-Line Anti-Tubercular Drugs' Pharmacokinetics on Treatment Outcome: A Systematic Review

This article was published in the following Dove Press journal:
Clinical Pharmacology: Advances and Applications

Tesemma Sileshi[1,2]
Esayas Tadesse[1]
Eyasu Makonnen[2]
Eleni Aklillu[3]

[1]Ambo University, Department of Pharmacy, Ambo, Ethiopia; [2]Addis Ababa University, College of Health Sciences, Addis Ababa, Ethiopia; [3]Karolinska Institutet, Department of Laboratory Medicine, Stockholm, Sweden

Background: Tuberculosis remains the major public health problem besides tremendous efforts to combat it. Most tuberculosis patients are treated with a standard dose of first-line anti-TB drugs. The cure rate, however, varies from patient to patient. Various factors have been related to anti-TB treatment failure. In recent years, studies associating lower plasma concentrations of anti-TB drugs with poor treatment outcomes are emerging although the results are inconclusive.

Objective: Investigate the impact of first-line anti-tubercular drugs pharmacokinetics on treatment outcome.

Methods: A systematic search of Pubmed, EMBASE, Web of Science, and the Cochrane Library for articles published in the English language between January 2010 to June 2020 was conducted to identify eligible studies describing associations of first-line anti-tubercular drug pharmacokinetics with treatment outcomes. The primary outcomes considered were pharmacokinetics parameter results and its association with treatment outcome.

Results: The search identified 1754 articles of which twelve articles; ten prospective observational studies and two controlled clinical trials fulfilled the eligibility criteria. The majority of the studies showed target concentrations for the first-line anti-tubercular drugs below the current standard range. Among the twelve studies, eleven studies assessed rifampicin pharmacokinetics of which eight reported association of drug concentration and treatment outcomes. Similarly, four out of eight and three out of seven reported drug concentration and treatment outcome association for isoniazid and pyrazinamide, respectively. Despite the low plasma concentration, a favorable treatment outcome was achieved for the bulk of the patients. Irrespective of the inconsistency, an increase in exposure to rifampicin improved the outcome, and lower rifampicin, isoniazid, and pyrazinamide concentration are associated with poor outcome. No data are available for ethambutol associating its pharmacokinetics with treatment outcomes.

Conclusion: The pharmacokinetics of first-line antitubercular drugs can influence treatment outcomes. Further controlled clinical studies are, however, required to establish these relationships.

Keywords: tuberculosis, pharmacokinetics, treatment outcomes, anti-TB drugs

Introduction

Tuberculosis (TB), an infectious disease caused by *Mycobacterium Tuberculosis*, stays the main health problem globally. TB is one of the top 10 causes of death and the leading cause of death from infectious diseases worldwide. According to the world health organization (WHO) 2019 tuberculosis report, TB caused an estimated

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1.2 million deaths among HIV-negative people and 251 000 deaths among HIV positive people in 2018.[1] The aspiring strategy of WHO to END TB aims to reduce TB incidence and mortality in 2035 by 90%, and 95%, respectively compared to the 2015 cases.[2] The first line anti-TB drugs which include rifampicin (RMP), isoniazid (INH), pyrazinamide (PZA), and ethambutol (EMB) have a cure rate of up to 95% in early clinical trials,[3] but the success rates drop as low as 65% in some areas.[4] Drug resistant TB poses a key threat to control TB globally with the first-line drugs. For example, the incidence of multi-drug resistance (MDR-TB) and extensive multi-drug resistance (XDR-TB) is increasing over time, for instance, 484,000 cases of MDR-TB cases were reported in 2018 which could decrease the success achieved.[2]

Many factors might be associated to treatment failure. Health factors such as HIV infection, diabetes mellitus, low body weight, cavitation on chest x-ray, high bacterial burden, drug resistance, positive culture after two months of treatment; and sociodemographic factors like drug abuse, alcoholism, smoking, and poor treatment adherence were reported in several studies.[5–10] Data from mice,[11] and hollow fiber system (HFS) models,[12] emphasized pharmacokinetic studies to predict tuberculosis treatment outcome. Furthermore, WHO recognized pharmacokinetics (PK) and pharmacodynamics (PD) studies to play a key role to establish the most appropriate dose of anti-TB medications.[13]

PK describes the time course of a drug concentration in different body compartments, such as blood, plasma, brain, lungs, and other tissue. It deals with what the body does to the drug; absorption, distribution, metabolism, and excretion.[14] Poor compliance to treatment has been considered as the major cause for treatment failure in tuberculosis treatment historically.[15] However, in recent years, several studies associated low serum concentrations of anti-TB drugs with poor treatment outcomes. The reference range for various first-line anti-TB drugs with therapeutic cut-offs is given in Table 1.[16] PK parameters especially the total exposure to anti-TB drugs (the area under the plasma concentration vs time curve (AUC0-24) and/or the peak plasma concentration (Cmax) appear to be relevant for anti-TB drugs.[17]

Systematic reviews and meta-analyses describing relations of PK and treatment outcome have been published previously.[18–22] However, the objectives and conclusions of these reviews or meta-analyses were not consistent (Table S1). For example, Pasipanodya et al reviewed the shreds of evidence on the association of isoniazid pharmacokinetic variability with either microbiological failure or acquired drug resistance and concluded pharmacokinetic variability of isoniazid was significantly associated with failure of therapy and acquired drug resistance in patients.[18] However, reviews focused on the association of drug concentration and treatment outcomes have contradicting conclusions. Perumal et al have shown that low PZA concentration probably increased the risk of poor outcomes; low RMP concentration might slightly increase the risk of poor outcomes; whereas low concentrations of INH and EMB had no clear effect on the treatment outcome.[22] On another hand, Sekaggya-Wiltshire et al and Wilby et al failed to reach on a conclusion that plasma concentration of first-line anti-tubercular drugs affects treatment outcome.[19,21]

Table 1 Basic Pharmacokinetics of First-Line Anti-Tubercular Drugs[16]

Drug Name	Dose	Serum Cmax (μg/mL)	Tmax (hr)	Serum T½ (hr)
Rifampicin	600mg	8–24	2	2–3
Isoniazid	300mg	3–6	0.75–2	1.5 fast 4 slow
Pyrazinamide	25–35mg/kg	20–60	1–2	9
Ethambutol	25mg/kg	2–6	2–3	Biphasic: 2–4, then 12–14

In addition to variation in the conclusion, the majority of the previously published reviews included all types of studies ranging from controlled clinical trials to case reports leading to a varying degree of evidence.[23] This systematic review was, therefore, designed to evaluate the recent evidence on the effects of pharmacokinetics in particular plasma concentration on tuberculosis treatment outcome by reviewing only prospective cohort studies and randomized clinical trials that have a better level of evidence.

Methods

Literature Search Strategy

We conducted the review according to the Preferred Reporting Items for Systematic Reviews (Table S2) and Meta-Analyses (PRISMA) statement. The protocol was registered at PROSPERO (Registration number CRD42019138544.[24]

We identified studies among tuberculosis patients in which all or any of the first-line anti-tubercular drug pharmacokinetic data or drug concentration and tuberculosis treatment outcomes were reported. A systematic search of Pubmed, EMBASE, and Web of Science for articles published in the English language over the last ten years (January 1, 2010- June 8, 2020) was done. We applied the search strategy including the next terms: "antitubercular agents" OR antituberculosis OR antimycobacterial, OR Isoniazid OR Pyrazinamide OR Rifampicin OR Rifampin OR Ethambutol AND "pharmacokinetics" or "concentration" AND "treatment outcomes" OR "sputum conversion" OR "culture conversion". We hand search reference lists from relevant studies, to identify further eligible articles not found by the systematic search. The search included only adult human studies.

Eligibility Criteria

The following inclusion criteria were used to select studies: patients (15 years and above) and treated with first-line anti-tubercular drugs (INH, RMP, PZA, and EMB) according to WHO treatment guidelines and at least one of whose PK data described. Only prospective cohort studies and controlled clinical trials were included. However, studies reporting pharmacokinetics effect on treatment outcome in children were excluded. Besides, population modeling, review article, retrospective studies, case-control, and case series studies were excluded. There is no limitation based on gender and other socio-demographic characteristics of study participants.

Data Quality Assessment

The included study quality was assessed using appropriate tools. Blended Cochrane's Risk of Bias assessment of Randomized Controlled Trials for controlled clinical trial studies with the Newcastle-Ottawa Quality Assessment Scale for cohort studies was used to assess the quality of the included studies. Quality assessment of the studies was done by one author (TS) using the prepared checklist (Table S3).

Data Extraction

A pre-designed data extraction form was used to review relevant studies (Table S4). Two authors (TS and ET) independently extracted data. Included articles were read and screened for eligibility criteria. From the studies included in the review, data were collected on study design, participant characteristics, pharmacokinetic parameters (Cmax, AUC or Cmax/MIC, AUC/MIC), treatment outcomes (cure, relapse, failed, culture conversion, smear conversion), the correlation between PK parameters, and treatment outcomes. The pharmacokinetics parameter results and its' association with treatment outcome were the primary outcomes considered strictly during data extraction for each study included. Disagreements between the two reviewers during data extraction were resolved through discussion. Similarly, if any ambiguity on the study methods or results was encountered the two reviewers discussed the issue together and resolved the ambiguity. No synthesis of data was done and contact of authors was not found to be relevant during the review for original data.

Results

Study Characteristics

As shown in Figure 1, a total of 1754 articles was identified. Of which, 47 duplicates and 1665 articles evaluated by their titles and abstracts were excluded. The remaining 42 studies were further evaluated through the full reading of their texts. 30 studies were excluded further because they are either review article, population pharmacokinetic modeling, retrospective studies, not having treatment outcome reported, or luck of pharmacokinetics data either AUC, Cmax, or both. The remaining 12 studies were selected for the final qualitative analysis. The geographic location of the included studies was; six from Africa,[25–30] one from Europe,[31] two from Latin America,[32,33] and the remaining three from Asia.[34–36] The majority of the studies were observational prospective in design. Only two of the studies included in this review were randomized clinical trials as shown in Table 2.[30,32] The median (mean) age of study participants ranges from 25 to 42. In all studies, some participants had comorbid conditions; either HIV or diabetes mellitus.[29,33–36] The PRISMA Flow diagram is shown in Figure 1.

Quality of Included Studies

All studies were assessed for the following parameters; 1. Representativeness of the selected cohorts (treatment of tuberculosis was with first-line anti-tubercular drugs) 2. The outcome of interest was not presented at the start of the study (no reported drug resistance at the beginning of treatment) 3. Compatibility of study participant 4. Assessment of outcome (treatment outcome listed above) 5. Adequacy of the duration of follow-up (at least for two

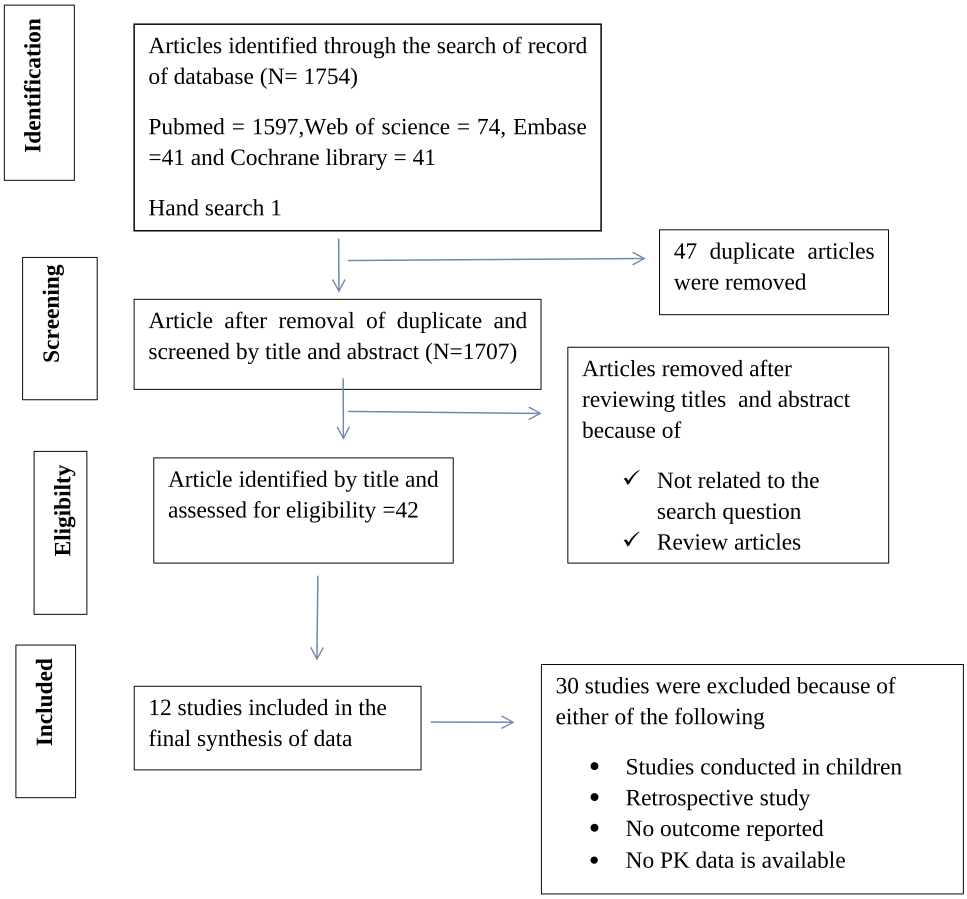

Figure 1 PRISMA flow diagram showing the literature search for studies which described drug pharmacokinetics of first line tuberculosis drugs and treatment outcomes.
Notes: PRISMA figure adapted from Liberati A, Altman D, Tetzlaff J, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. *Journal of Clinical Epidemiology.* 2009;62(10). Creative Commons.
Abbreviations: PK, pharmacokinetics; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses.

months) and for controlled randomized clinical trials, a randomization process was considered. Ten of the twelve studies were observational prospective studies. As shown in Table 2 all prospective observational studies have a low risk of bias. Similarly, in the remaining two randomized controlled clinical trials, although the proportion of patients with low PK and poor treatment outcomes was not indicated, they had a low risk of bias.

Pharmacokinetic Data and Treatment Outcome

Among the twelve studies evaluating PK parameters with treatment outcome, four assessed RMP only,[26,27,30,32] one INH only,[33] five three drugs (RMP, INH, and PZA)[25,29,34–36] and two all drugs (RMP, INH, PZA, EMB).[28,31] All studies reported clinical outcomes and their association with some kind of pharmacokinetics parameters. However, the proportion of patients with a low level of drug concentration was not reported in four studies.[27,30,32,36] Eight studies reported the proportion of study participants who had low plasma concentration. Strikingly, in one study, 100% of participants have low plasma Cmax measured at 2.5-hour post-dose.[26] In the majority of the studies that reported plasma levels of the drug, a large percentage of participants had a lower plasma concentration of RMP (up to 91%) and INH (up to 88%). However, the PZA level seems better in plasma availability (Table 3). Studies varied considerably at the time of blood sampling. This ranged from single-point sampling; at 2hours post-dose,[31,34,35] and 2.5 hours post-dose[26] to 9-time sampling to construct 24 hours AUC.[27] However, all studies used standard methods for quantification of plasma level of the drug. The pharmacokinetics characteristics and associated treatment outcomes of each drug are presented below.

Rifampicin

Among the twelve studies, eleven studies assessed RMP pharmacokinetics of which eight reported treatment

Table 2 Characteristics of Included Studies

Author	Country	Mean (Median) Age of Study Participant	Study Design	Sample Size	Population Characteristics	Risk of Bias
Aarnoutse, (2017)[30]	Tanzania	33.5	Randomized Controlled Trial	150	15 HIV positive	Low
Burhan (2013)[34]	Indonesia	35	Prospective cohort	181	44 diabetic 19 HIV positive	Low
Pasipanodya (2013)[25]	South Africa	36	Prospective cohort	142	15 HIV positive 98 have prior tuberculosis	Low
Prahl (2014)[31]	Denmark	42	Prospective cohort	32	2 HIV positive	Low
Requena-Méndez (2014)[33]	Peru	29	Prospective cohort	107	25 Diabetic 30 HIV positive	Low
Rockwood (2017)[29]	South Africa	33	Prospective cohort	100	65 HIV positive 4 diabetic	Low
Sekaggya-Wiltshire (2018)	Uganda	34	Prospective cohort	227	All HIV positive	Low
Svensson (2018)[27]	Tanzania and South Africa	34	Prospective cohort	97	2 patients HIV positive	Low
Vela´ squez (2018)[32]	Peru	25	Randomized Controlled Trial	180 randomized to three arms 10, 15, and 20 mg/kg	2 patients HIV positive	Low
Gengiah (2014)[26]	South Africa	33	Prospective cohort	57	All are HIV positive	Low
Ramachandran (2017)[35]	India	38	Prospective cohort	1912	19 HIV positive 53 diabetic	Low
Ramachandran (2020)[36]	India	39.5	Prospective cohort	404	27 HIV infected 113 Diabetics	Low

outcomes.[27,28,30–32,35,36] Ramachandran et al found that 91%, of the patients, had suboptimal concentrations of RMP (8 g/mL). This study evaluated factors influencing tuberculosis treatment outcomes in adult patients treated with thrice-weekly regimens. Lower RMP concentration was among the factors responsible for poor treatment outcome.[35] However, the study assessed multi-factors and the treatment was not according to current WHO recommendation. Similar authors recently have shown that low RMP concentrations were associated with poor outcomes.[36] Sekaggya-Wiltshire et al demonstrated that patients with both low RMP and INH Cmax have a moderately increased risk of unfavorable treatment outcomes, including death, treatment failure, loss to follow-up, and default.[28] This study had a large sample size, but all study participants were HIV positive and there was no evidence on the effect of HIV on treatment outcome. Pasipanodya et al, compared treatment outcome using culture conversion at two months and long term outcome at two years for RMP peak concentration above and below 6.6 mg/L. Among the patients who have a peak concentration below 6.6mg/L, 19% have culture-positive at two months while only 1% have a culture-positive for the patient group who have a peak concentration above

Table 3 Pharmacokinetic Characteristics and Treatment Outcome

Author	Study Drug	Pharmacokinetics Bio-Analytical Methods	PK Parameter Considered	PK Sampling Time	Duration for Which the Study Participant Followed	Outcome Measured	The Proportion of Patient with Low PK	The Proportion of Patients with Poor Outcome	Conclusion on the Predictive of PK on Treatment Outcome at the End of Treatment
Aarnoutse, (2017)[30]	RMP	ultraperformance liquid chromatographic	Cmax	1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, and 24 hours	12 weeks	Time to culture conversion on different days	NA	72.2% from 600mg 62.9 from 900mg 73.8% from 1200mg have culture conversion on 84 days	Higher exposure to RMP was observed as the dose increase., but did not result in an improved bacteriological response in patients with pulmonary TB
Burhan (2013)[34]	INH, RMP, PZA	HPLC	Cmax	2 hours	8 weeks	Culture conversion at 8 weeks	INH= 88% RMP= 49% PZA=2%	11/155 have a positive culture at week 8	No association was found between drug concentration and 8 weeks of culture conversion
Pasipanodya (2013)[25]	INH, RMP, PZA	HPLC with UV detection for RMP, INH, and PZA; mass spectrometry for EMB	24-hours AUC 1	0.5, 1, 1.5, 2, 2.5, 3, 4, 6, and 8 hours	For up to 2 years	Culture conversion at two months and long term outcome up to two years	PZA=69.7% RMP =70.7% INH =81%	11/142 did not convert culture at two months 25% of 142 patients had poor long-term outcomes	From CART analysis Low drug AUCs are predictive of clinical outcomes in tuberculosis patients PZA: AUC < 363 RMP: AUC <13 INH: AUC < 52
Prahl (2014)[31]	INH, RMP, EMB, PZA	HPLC with tandem mass spectrometry	2 hours of concentration	2hours	For up to one year after completion of treatment	Failure at six months or a relapse of TB within 1 year after the end of treatment	INH= 71%, RMP=57.6% EMB= 46% PZA=10%	5/28 failure during one year follow up	Lower INH and RMP are observed in treatment failure

Requena-Méndez (2014)[33]	INH	HPLC with a triple-quadrupole TSQ Quantum Access mass spectrometer	Cmax, and AUC(0–6h)	2 and 6 hours	6 months after completion of treatment	outcomes at the end of therapy and 6 months after the end of therapy	34% during the intensive phase and 33.3% during the continuation phase	4/41 (2 death, 1 relapse, and 1 prolonged treatment)	Unable to demonstrate a clear relationship between the Cmax of INH and treatment outcome
Rockwood (2017)[29]	INH, RMP, PZA	HPLC with Tandem mass spectrometry	Cmax AUC_{24} AUC0-24/ MIC, Cmax/ MIC	1, 2, 3, 4, 6, and 8 hours	2 months culture conversion	Culture conversion at two months	INH 43% Cmax <3 mg/L) and 6% <1.5 mg/L. RMP 80% Cmax <8 mg/L and 17% <4 mg/L. PZA 53% Cmax <35 mg/L and 1% <20 mg/L	13% overall treatment success without failure/ relapse was observed	None of these Cmax cutoff values for INH or RMP predicted 2-month culture conversion and/or failure/relapse but did predict failure/ relapse for PZA
Sekaggya-Wiltshire (2018)[28]	INH, RMP, PZA EMB	HPLC	Cmax and AUC	1, 2, and 4 hours	Up to 24 weeks after initiation of TB treatment	Cure, death, failure	INH =83.7% RMP = 77.5% PZA =2.64%a EMB=30.8%	Cure=158 Death = 11 Failure =8 Default = 2 Lost to follow up = 17	Patients with both low RMP and INH Cmax have moderately increased risk of unfavorable treatment outcomes, including death, failure, loss to follow-up, and default
Svensson, (2018)[27]	RMP	ultraperformance HPLC	AUC_{24}	0.5, 1, 2, 3, 4, 6, 8, 12, and 24 hours	Up to 26 weeks	time to stable sputum culture conversion (TSCC)	NA	NA	Increasing RMPampicin exposure to modestly shorter TSCC at week 8, TSCC increased from 39% to 55% with RMP AUC0–24h increasing from 20 to 175 mg/L·h

(*Continued*)

Table 3 (Continued).

Author	Study Drug	Pharmacokinetics Bio-Analytical Methods	PK Parameter Considered	PK Sampling Time	Duration for Which the Study Participant Followed	Outcome Measured	The Proportion of Patient with Low PK	The Proportion of Patients with Poor Outcome	Conclusion on the Predictive of PK on Treatment Outcome at the End of Treatment
Vela´ squez (2018)[32]	RMP	NA	AUC_{24}/MIC	NA	Up to 12 months	Change in elimination rate of M. tuberculosis log10 colony-forming units- and culture conversion at 8 week and unfavorable outcome at 12 months	NA	At 12 month cure 10mg/kg =44/60, 15 mg/kg= 46/60 And 20mg/kg =46/60	Increasing the dose enhanced rapid sputum sterilization
Gengiah (2014)[26]	RMP	Tandem HPLC mass spectrometry	Cmax	2.5 hours	Up to 6 months	Sputum at six months	All	8/55 sputum positive at two months 2/51 sputum positive at six months	No evidence, but in all patients Cmax is below the standard target
Ramachandran (2017)[35]	RMP INH, and PZA	HPLC	2 hours plasma concentration	2hours	Up to end of therapy	Outcome at the end of treatment	RMP= 91%, INH= 16%, PZA = 17%	264 (14%) had an unfavorable outcome	Low RMP concentration is among the factors associated with treatment outcome
Ramachandran (2020)[36]	RMP INH, and PZA	HPLC	2 hours of plasma concentration	2hours	Up to 2 years (24 months)	Outcome during the follow-up period	NA	77 (19%) patients have an unfavorable outcome	Low RMP and PZA concentrations were associated with poor outcomes

Abbreviations: NA, nonapplicable; RMP, rifampicin; INH, isoniazid; PZA, pyrazinamide; EMB, ethambutol; PK, pharmacokinetics; AUC, area under the curve; HPLC, high-performance liquid chromatographic.

6.6mg/L. A similar difference was also observed for a long outcome for patient group dichotomized to above and below 13 mg/L•h AUC, 12% and 33% poor outcome respectively.[25] Evidence on drug exposure and treatment outcome relationship was reported from two recent controlled clinical trials.[30,32] In both studies increasing the dose of RMP increased drug exposure, however, improved faster culture conversion in the high exposure group was observed only in one study.[32]

Svensson et al found an association between RMP concentrations and time to stable sputum culture conversion.[27] The proportion of patients with stable culture conversion on liquid medium at week 8 increased from 39% to 55%, with the RMP AUC increased from 20 to 175 mg/L·h. This study used only two dose levels, 10mg/kg and 35mg/kg, and the safety of 35mg/kg was not elucidated. Prahl et al found that treatment failure was observed at lower INH and RMP concentrations.[31] The remaining three studies have not shown any evidence on the association of RMP concentration and treatment outcome.[26,29,34]

Isoniazid

The impact of plasma concentration of INH on treatment outcome was reported in eight studies. Requena-Méndez et al investigated the effects of dose, comorbidities, and food on INH pharmacokinetics in Peruvian tuberculosis patients. Although 34% of patients during the intensive phase and 33.3% during the continuation phase had lower INH concentration, no association between plasma concentration and treatment outcome was observed at the end of therapy.[33] Burhan et al evaluated the association of 2hour plasma concentration of INH and treatment outcome, however, no association was found.[34] Similarly, Rockwood et al reported no association between two months of culture conversion and pharmacokinetics. On the other hand, in Prahl's study, patients with treatment failure had a significantly lower 2hour plasma concentration of INH than those who were cured.[31] Pasipanodya et al, compared treatment outcomes using culture conversion at two months for INH peak concentration above and below 8.8 mg/L. Among the patients who have a peak concentration below 8.8mg/L, 13% have culture-positive at two months while no patients have a culture-positive for the patient group who have a peak concentration above 8.8mg/L. For the long-term treatment outcome assessment, the patient group who have AUC of INH above and below 52mg/L•h have 20% and 70% poor treatment outcomes respectively.[25] Furthermore, Sekaggya-Wiltshire et al reported an association of low INH and RMP concentration with poor culture conversion.[28]

Pyrazinamide

The clinical impact of PZA drug concentrations was assessed in seven studies. However, only three studies reported the association between PZA plasma concentration and treatment outcome.[25,34,36] Pasipanodya et al reported an association of AUC less than 363 mg·h/L with poor long-term outcomes. Moreover, the highest predictor of 2-month sputum conversion among all clinical factors examined was PZA peak concentration. Burhan et al evaluated the relationship between the plasma concentration of INH, RMP, and PZA and treatment outcome. No association was found between RMP and INH plasma concentration and treatment outcome.[34] However, low pyrazinamide drug concentrations may be associated with a less favorable bacteriological response. Similarly, Rockwood et al found no association between Cmax of PZA and 2-month culture conversion culture but did predict failure or relapse.[29] Another recent study reported that a 1-μg/mL decrease in pyrazinamide concentrations was associated with recurrence.[36]

Discussion

The WHO End TB Strategy has set an ambitious target to reduce TB deaths by 90% and to cut new cases by 80% between 2015 and 2030.[2] To achieve these ambitious targets requires various interventions. First, achieving the WHO end TB targets will necessitate better, and early detection of TB which could halt TB transmission and hasten the decline in TB incidence and mortality,[37,38] Second, we need safer, shorter, and more efficacious treatment for all forms of TB. Third, a vaccine would be the ultimate solution if found to be highly effective, safe, able to prevent pre-exposure, infection as well as reactivation.[39,40] Since the existing anti-tubercular regimens made before the current advance in pharmacokinetic-pharmacodynamic (PK–PD), we are lacking evidence of exposure-response relationships even in today's tuberculosis pharmacotherapy.[41] Owing to this gap WHO developed a technical report on the PK and PD of drugs used for tuberculosis treatment.[13] This review examined the literature published over the last ten years reporting pharmacokinetics in particular plasma concentration of first-line anti-tubercular drug association with treatment outcome.

The pharmacokinetics properties of first-line anti-TB drugs at which treatment success achieved have been published previously.[16] The most common PK-PD measures used to describe anti-TB activities are the ratio of the Cmax relative to the MIC and the ratio of the area under the concentration-time curve at the end of the dosing interval relative to the MIC (AUC0 – 24/MIC).[4] However, in several PK-PD studies, AUC0 – 24 or/and Cmax of the first-line drug was used as a measure of exposure and response. Similarly, nine of the eleven studies reported in this review used either Cmax, AUC, or both. Only one study described AUC24/MIC[32] and one another both AUC24/MIC and Cmax/MIC.[29] Besides, there is a variation in methods of determination of Cmax and AUC. For instance, Prahl et al[31] measured the Cmax at 2hours post-dose, but Gengiah et al measured it at 2 1/2 hours post-dose.[22] For accurate prediction of AUC, although recent studies recommending a spare sample of plasma are emerging,[42] intensive blood sampling is essential. But estimation of AUC is also done using different approaches.

The prevalence of low concentration in all studies is high. Previous systematic review and meta-analysis have also shown a high prevalence of low plasma concentration.[20–22] However, they failed to find a strong association between low concentration and treatment outcomes. The type of studies included, the reliability of the current therapeutic range and variation on the report of treatment outcome were listed as factors responsible for lack of association. Owing to this the present review assessed only prospective and controlled clinical trials.

Only eight of the eleven studies have found the association between drug concentration and treatment outcome for RMP.[25,27,28,30,32,35,36] Aarnoutse et al observed faster culture conversion in the higher RMPexposure group;[30] Pasipanodya et al demonstrated AUCs of PZA, RMP and INH are predictive of clinical outcomes in tuberculosis patients;[25] Prahl et al observed lower INH and RMP concentration in treatment failure;[31] Sekaggya-Wiltshire et al reported patients with both low RMP and INH Cmax have delayed culture conversion;[28] Svensson et al[27] and Vela´squez et al[32] have shown increasing RMP exposure to modestly shorter time to stable sputum culture conversion, and Ramachandran reported low RMP concentration as a predictive of treatment outcome.[35] Similarly, a recent report of Ramachandran et al identified lower peak concentration or AUC as a predictor of culture conversion at two months and long-term treatment outcome respectively. From these findings, one could best describe that increase in exposure to RMP increases anti-mycobacterial effects. However, using the current reference range it is difficult to dichotomize tuberculosis patients into non-respondents and respondents to anti-mycobacterial drug therapy based on the concentration.

The prevalence of low drug concentration is high for INH and PZA. The previous meta-analysis has shown that pharmacokinetics variability of INH mediates acquired drug resistance.[43] Similarly, four studies reported the lower INH drug concentration associated with unfavorable treatment outcomes.[25,28,31,33] Of the seven studies that assessed PZA concentration and treatment outcome Burhan et al and Pasipanodya et al identified low PZA concentration as a primary cause of unfavorable outcome.[25,34] The results are consistent with the recent meta-analysis that needs attention in tuberculosis care.[44]

From this qualitative review, it is clear that increased exposure to drugs can improve the treatment outcome. However, further studies are required to validate this observation because of the following reasons. First, some patients are respondents to low and very low concentrations of the drug.[26] Second, in most studies, the participants have a comorbid condition that could affect the outcome. Thus, to establish exposure-response relationship studies controlling confounding factors are important. Third, data are emerging on the effect of the strain of Mycobacterium tuberculosis variation on the clinical outcome [46] and variation on the critical concentration inhibiting wild-type Mycobacterium tuberculosis [47]. Therefore, to integrate the concentration of drug measurement into tuberculosis care and treatment better characterization of Cmax, AUC, Cmax/MIC, and/or AUC/MIC are needed.

Conclusion

This systematic review attempts to link drug exposure and treatment outcome. Although a limited number of prospective observational studies and controlled clinical trials are available for the review, RMP, PZA, and INH concentration have shown a link with treatment outcomes. An increase in exposure to RMP improved the outcome. A lower concentration of NH and PZA is observed in unfavorable treatment outcomes. On the other hand, a better outcome was observed in patients who have low exposure to these drugs. Further studies addressing the validity of the current reference range, plausible pharmacokinetics parameter, bacterial, and host factors are,

however, are needed to predict drug concentration and treatment outcome association.

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Disclosure

The authors report no conflicts of interest for this work.

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REVIEW

Effect of Genetic Variations in Drug-Metabolizing Enzymes and Drug Transporters on the Pharmacokinetics of Rifamycins: A Systematic Review

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Background: Rifamycins are a novel class of antibiotics clinically approved for tuberculosis chemotherapy. They are characterized by high inter-individual variation in pharmacokinetics. This systematic review aims to present the contribution of genetic variations in drug-metabolizing enzymes and transporter proteins to the inter-individual variation of rifamycin pharmacokinetics.

Method: We followed Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement guidelines. The search for relevant studies was done through PubMed, Embase, Web of Science, and Scopus databases. Studies reporting single nucleotide polymorphism in drug transporters and metabolizing enzymes' influence on rifamycin pharmacokinetics were solely included. Two reviewers independently performed data extraction.

Results: The search identified 117 articles of which 15 fulfilled the eligibility criteria and were included in the final data synthesis. The single nucleotides polymorphism in the drug transporters *SLCO1B1* rs4149032, rs2306283, rs11045819, and *ABCB1* rs1045642 for rifampicin, drug metabolizing enzyme *AADAC* rs1803155 for rifapentine and *CES2 c.-22263A>G (g.738A>G)* for rifampicin partly contributes to the variability of pharmacokinetic parameters in tuberculosis patients.

Conclusion: The pharmacokinetics of rifamycins is influenced by genetic variation of drug-metabolizing enzymes and transporters. Controlled clinical studies are, however, required to establish these relationships.

Keywords: rifamycin, pharmacokinetics, pharmacogenetics, enzymes, transporters

Introduction

Tuberculosis (TB) is an infectious disease, which remains a major public health problem globally. In the year 2020, the estimated number of people who died from tuberculosis is 1.3 million among HIV-negative people and 214,000 among HIV-positive.[1] Current pharmacotherapy of tuberculosis involves a combination of at least four drugs. Rifamycins are key components of pharmacotherapy for both active and latent TB.

Rifamycins are a class of antibiotics isolated from Amycolatopsis in 1957. Four distinct semi-synthetic rifamycin analogs (rifampicin, rifabutin, rifapentine, and rifaximin) are approved for clinical use. Rifampicin, rifabutin, and rifapentine are used for the treatment of TB and chronic staphylococcal infections.[2] Rifapentine given once weekly for 12 weeks with isoniazid is effective and well tolerated in the treatment of latent TB.[3] Rifaximin is poorly absorbed from the gastrointestinal tract and is indicated for the treatment of traveler's diarrhea, functional bloating, irritable bowel syndrome, and small bowel bacterial overgrowth.[4]

Variable exposure to anti-TB drugs may be associated with unfavorable treatment outcomes.[5] Factors associated with drug exposure variability of anti-TB drugs, such as age, gender nutritional status, human immune-deficiency virus, diabetes, and genetic polymorphism, were described in various previous studies.[6–9] There has been a notable development in recent years on how genetic variations in drug-metabolizing enzymes and transporters contribute to variation in

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exposure and response to the drugs.[10,11] As the local and systemic concentrations of anti-TB drugs are affected by genetic variations in drug-metabolizing enzymes and transporters, pharmacokinetic and pharmacogenetic studies are increasingly performed to optimize TB treatments.[12,13]

Rifamycins are thought to be metabolized by microsomal hepatic carboxylesterases (CES), and serine esterase arylacetamide deacetylase (AADAC) to 25-deacetylrifamycins.[14,15] The uptake, distribution, and excretion of rifampicin are mediated by membrane drug transporters. There are two transporters superfamilies; the solute carrier (SLC) transporters and the adenosine triphosphate (ATP)-binding cassette (ABC) transporters.[16] SLC superfamily consists of more than 400 membrane-bound family proteins. Multiple studies revealed that the *SLCO1B1* sinusoidal influx transporter influences rifampicin influx,[17,18] and the *SLCO1B1 *15* haplotype is associated with rifampin-induced liver injury.[19] Most ABC transporters in eukaryotic cells mediate the efflux of the substrate from the cells. ABC transporters influence the hepatocellular concentration of rifampicin.[20–23] Rifamycins are substrates of P glycoprotein (P-gp), coded for by the polymorphic *ABCB1* gene.[24] Rifampicin also induces *ABCB1* gene expression.[25] Although *SLCO1B1* and *ABCB1* gene products have been reported to influence rifamycins pharmacokinetics, there is no candidate gene identified so far for therapeutic drug monitoring.

Recently, advances in technology and scientific discoveries in the medical arena have enabled the practitioner to individualize drug therapy. The keen interest to personalize TB treatment has been a point of discussion over the last decade.[26–29] The use of pharmacokinetics and pharmacogenetics of anti-tubercular drugs as tools for TB treatment optimization has been discussed previously.[13,18] However, there is a scarcity of comprehensive data on the pharmacogenetics of rifamycins. This systematic review was, therefore, designed to evaluate the influence of genetic polymorphism in rifamycins metabolizing enzymes and transporters on their pharmacokinetics.

Methods

This systematic review was carried out following Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statements (Table S1). The protocol has been registered at PROSPERO with registration number CRD42020206029.

Search Strategy

Relevant studies were identified through a search of PubMed, Web of Science, Embase, and Scopus databases. The following combination of words was used: pharmacokinetics OR concentration OR "drug concentration" AND rifamycins OR rifampin OR rifampicin OR rifabutin OR rifapentine OR rifaximin AND *SLCO1B1* OR *ABCB1* OR carboxylesterase OR *CES* OR Arylacetamide deacetylase OR *AADAC* AND "Genetic polymorphism" OR pharmacogenetics OR pharmacogenomics OR "single nucleotide polymorphisms" OR SNP. Further, a hand-search was done from reference lists of studies included to identify eligible studies. There was no limitation on the dates of publication or publication status. Publications available only in the English language were included. The search was refined to studies of human participants.

Eligibility Criteria

The following were the eligibility criteria for the inclusion of studies: 1. Human participant studies; 2. Studies that reported on pharmacokinetic parameters of rifamycins; 3. Studies in which study participants were genotyped for rifamycins metabolizing enzyme or transporters gene; and 4. Studies that reported on the pharmacokinetic parameters of rifamycins and the effect of genetic variation on pharmacokinetics.

Quality Assessment

Validated tools exist for genetic association studies methodological quality assessment. We used the quality of genetic association studies (Q-Genie)[30] tool to assess the quality of included studies. Using the checklist adopted (Table S2) from Q-Genie TS assessed the quality of selected studies.

Data Extraction

Two (TS and GM) independently extracted data from all included publications using a pre-prepared data extraction format which included items as follows: first author, publication year, study drug, sample size, type of pharmacokinetic parameters assessed, a country in which the study was conducted, participant characteristics, genetic polymorphism investigated, pharmacokinetic parameter results and its association with genetic polymorphism. The disparity between the two reviewers during data extraction was resolved through discussion.

No contact with the authors was done for missing data and the data presented in this review were extracted from the articles.

Results

Included and Excluded Study

A total of 115 articles related to genetic polymorphism of drug-metabolizing enzymes and drug transporters with the pharmacokinetics of rifamycins were retrieved from PubMed, Web of Science, Scopus, and Embase databases. Hand search identified two additional articles which were not obtained during the database search. As shown in the PRISMA flowchart (Figure 1) 51 duplicates were removed. The remaining 66 articles were screened by title and abstract for predefined criteria, and 47 were excluded. The reasons for exclusion of studies from titles and abstracts were (1) review articles (N=3); (2) studies focusing on drugs other than rifamycins (N=26); (3) studies that did not have information on the pharmacokinetics of rifamycins but only genetic information reported (N=8); and (4) studies in which only pharmacokinetics data were reported without genetic information (N=10). Furthermore, four articles were excluded after reading them fully. Of the four articles excluded; one article did not contain rifamycins data, one study was done on healthy participants and the other two articles did not contain pharmacokinetic parameters.

Characteristics of Included Studies

Of the 15 articles selected for qualitative data synthesis, most of the studies (N=14) focused on *SLCO1B1* gene polymorphism association with the pharmacokinetics of rifamycins (Table S3). Specifically, seven studies evaluated

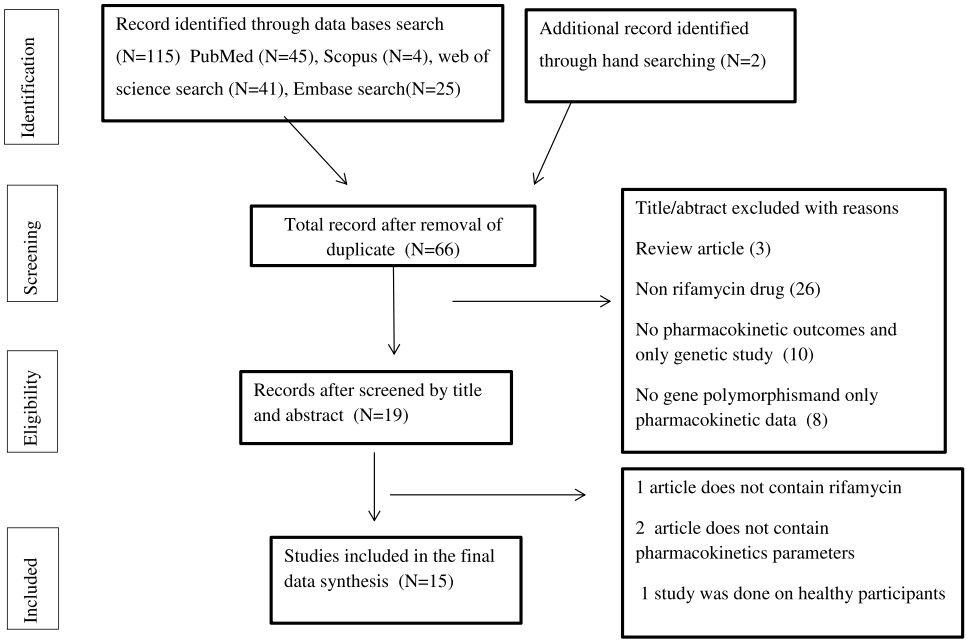

Figure 1 PRISMA flow diagram showing the literature search for studies that investigated the effect of genetic variations in drug metabolizing enzymes and drug transporters on the pharmacokinetics of rifamycins.

Notes: PRISMA figure adapted from Liberati A, Altman D, Tetzlaff J, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration. *Journal of clinical epidemiology.* 2009;62(10). Creative Commons.

the association of *SLCO1B1* gene polymorphism and pharmacokinetics,[31–37] three studies *SLCO1B1* and *ABCB1* gene polymorphism with pharmacokinetics,[38–40] one study *SLCO1B1* and AADAC gene polymorphism with pharmacokinetics,[41] one study *SLCO1B1*, and CES gene polymorphism with pharmacokinetics,[42] and two studies *SLCO1B1, AADAC*, and *CES* gene polymorphism with pharmacokinetics.[43,44] Only one study investigated the association between *CES* gene polymorphism with pharmacokinetics.[45] The most studied rifamycins are rifampicin (thirteen studies) and rifapentine (two studies). No study is available that reported the pharmacokinetic-pharmacogenetic association for rifabutin and rifaximin.

There was variation among studies in sample size, the type of study participants, and the pharmacokinetics parameter compared with gene polymorphism. The smallest sample size was 34,[39] while the largest was 256.[34] The study participants were TB patients from 13 different countries and races. The majority of the studies were done on adults, but one study data were obtained from children.[42] In some studies, participants were TB-HIV co-infected patients. The pharmacokinetics parameters commonly compared with gene polymorphism were maximum concentration (Cmax), AUC (area under the curve), and clearance. However, methods for blood sample collection and pharmacokinetic parameter determination varied among studies.

Association Between Drug Transporter and Rifamycins Pharmacokinetics

Association Between Polymorphism of *SLCO1B1* and Rifamycins Pharmacokinetics

SLCO1B1 gene encodes for an Organic Anion Transport Proteins 1B1 (OATP1B1). It is located on chromosome 12. OATP1B1 is a transmembrane protein involved in the uptake of various drugs including rifamycins from the blood into the hepatocyte.[46] Currently, 191 clinical variants have been reported. *SLCO1B1*c.521T>C (rs4149056), where the valine amino acid changed to alanine at position 174, was reported to affect drug response.[47] Eight studies assessed the effect of rs4149056 SNPs on rifamycin pharmacokinetic parameters. Among these studies, only Huerta-García et al reported increased AUC among heterozygous CT for *SLCO1B1* 521T>C than the other genotypes. However, the observed increase in AUC was not statistically significant.[39] A summary of specific transporters influence on pharmacokinetics is presented in Table 1.

SLCO1B1 g.38664C>T (rs4149032) was reported in twelve studies. rs4149032 is an intronic SNP most common in the African population.[48,49] Gengiah et al reported high frequency in the *SLCO1B1* (rs4149032) gene polymorphism and its association with low median rifampicin C2.5hr in the heterozygous and homozygous variant carriers.[32] Similarly, Chigutsa et al reported high allelic frequency of the *SLCO1B1* rs4149032 polymorphism and 28% reductions in the bioavailability of rifampin for homozygous variants.[40] No statistically significant increase in the rifampicin exposure for the homozygous TT of *g.38664 C > T* (rs4149032) was observed in the study of Kim et al.[37] However, the large number of studies reviewed here did not report any observed significant effect of *SLCO1B1* rs4149032 SNP polymorphism with rifamycin pharmacokinetic variation.

SLCO1B1 c.388A>G (rs2306283) is another SNP in the *SLCO1B1* gene. This SNP causes a change of asparagine amino acid to aspartic at 130, but the effect of this change on the transporter function is not clear yet. Huerta-García et al reported the AG genotype derived from SNP *SLCO1B1 c.388A>G* was associated with lower rifampicin AUC0–24 h values compared to those with AA genotype.[39] In post hoc analysis, Dompreh et al observed that the *SLCO1B1 c.388AA* genotype was associated with low rifampin concentrations compared to those with *c.388GG*.[42] The five remaining studies did not report any association between rs2306283 SNP and rifamycin pharmacokinetics. The SNP *SLCO1B1 c.463 C>A* (rs11045819) is another variant allele of the *SLCO1B1* gene reported to affect rifamycin pharmacokinetics. According to Weiner et al, patients with *SLCO1B1c.463C>A* variant allele had 42% lower rifampin exposure, 34% lower peak concentration levels, and 63% greater apparent oral clearance compared with *SLCO1B1 c.463CC*.[36] However, the remaining five studies did not report any association between rs11045819 SNPs and rifamycin pharmacokinetics.

Association Between Polymorphism of *ABCB1* and Pharmacokinetics

ABCB1 (ATP-binding cassette sub-family B member 1) genes encode for P-gp also known as multidrug resistance protein 1 (MDR1). P-gp is a transmembrane protein, which acts as an energy-dependent drug efflux pump. It decreases intracellular drug accumulation, thereby decreasing the effectiveness of many drugs.[50] The *ABCB1*c.3435 C>T

Table 1 Summary of the Studies Reported the Drug Transporter (*SLCO11* and *ABC1B*) Gene Polymorphisms Association with Rifamycins Pharmacokinetics Variation

Reference	Gene	SNPs	Characteristics of Study Participant	Rifamycins PK Change Observed
[31]	*SLCO1B1*	rs2306283 rs4149032 rs4149056 rs4149015	Tuberculosis recurrent black South African of which 127 (73.8) are HIV positive	No significant association between rifampicin pharmacokinetic and all variants of *SLCO1B1* gene SNPs studied was observed
[43]	*SLCO1B1*	rs11045819 rs4149032	174 Malawian adults with pulmonary TB of which 98 are HIV-infected patients	No association was reported for both variants of *SLCO1B1* gene SNPs studied and the pharmacokinetics of rifampicin
[32]	*SLCO1B1*	rs4149032	57 newly diagnosed TB-HIV co-infected South African patients	Lower median concentration of rifampicin at 2.5hr; 3.7 μg/mL in heterozygous and 3.4μg/mL in homozygous variants
[38]	*SLCO1B1*	rs4149056 rs2306283	Adult tuberculosis patients 57 study group of 30% are diabetics and 27 validation group of 27% are diabetics	No variation of rifampicin volume of distribution or clearance was observed for both *SLCO1B1* gene *A388T* (rs2306283) and *T521C* (rs4149056).
	ABCB 1	rs1045642		No effect of rs1045642 SNP on rifampicin pharmacokinetics was observed
[33]	*SLCO1B1*	rs4149032	100 tuberculosis patients where 50 are HIV positive	No effect of SLCO1B1 rs4149032 genotype on rifampin Median Cmax and Median AUC0–24 was observed
[34]	*SLCO1B1*	rs4149032 rs4149033 rs11045819	256 adult tuberculosis patients from India	No significant difference in 2 hr rifampicin plasma concentration for all SNPs studied was observed
[39]	*SLCO1B1*	rs4149056	34 tuberculosis patients of which 41.2% are diabetics and some are taking other drugs	AG genotype of SLCO1B1 388A>G had lower rifampicin AUC0–24 h compared to AA genotype (83.42 mcg.h/mL versus 108.31 mcg.h/mL) respectively
	ABCB 1	rs1045642 *(3435C>T)*		Patients with CC or CT genotypes showed lower values in Cmax, and AUC0–24 h compared to those with a TT genotype (Cmax = 9.1 6 mcg/mL versus 15.8 6 mcg/mL; AUC0–24 h = 72.83mcg.h/mL versus 130.35 6 29.5 mcg.h/mL respectively)
[42]	*SLCO1B1*	rs2306283 rs11045819 rs4149056 rs4149032	113 children aged 3 months to 14 years and 59 (52.2%) were HIV co-infected	In post hoc analysis, the rare SLCO1B1 c.388AA genotype was associated with lower rifampicin Cmax (1.81μg/mL versus 7.11 μg/mL) and AUC0–8h (9.33 μg.h/mL versus 29.50 μg.h/mL) and higher CL/F and V/F compared to those with c.388GG
[40]	*SLCO1B1*	rs4149032 rs4149056 rs11045819	60 adult tuberculosis patients aged from 18 to 55 years and 16% were HIV infected.	Patients heterozygous and mutant homozygous for rs4149032 had 18% and 28% reductions in the bioavailability of rifampicin respectively.
	ABCB 1	rs1045642 rs2032582 rs1128503 rs3842		The *ABCB1 G2677T* (rs2032582) showed no statistically significant increase (19%) in the CL/F and a 19% increase in the mean transit time
[41]	*SLCO1B1*	rs2306283 rs4149032	162 pulmonary tuberculosis from two clinical studies receiving rifapentine in South Africa	No effect on oral clearance, apparent volume of distribution, and F was detected

(*Continued*)

Table 1 (Continued).

Reference	Gene	SNPs	Characteristics of Study Participant	Rifamycins PK Change Observed
[37]	*SLCO1B1*	rs2306283 rs11045819 rs4149056 rs4149032	105 adult patients were newly diagnosed with active pulmonary TB, and Twenty (19%) patients had diabetes mellitus	rs4149032 wild type (TT) had lower oral clearance and higher AUC but no statistically significant differences were detected
[35]	*SLCO1B1*	rs4149032 rs2306283	A cohort of 50 HIV negative patients 25 with rifampicin sensitive pulmonary TB and 25 patients with rifampicin-resistant	When adjusted for all covariates no significant effect of the two SLCO1B1 genotypes on rifampicin pharmacokinetics parameters was identified
[36]	*SLCO1B1*	rs11045819 rs4149056 rs59502379 rs2306283 rs4149015	72 TB patients (37 from Africa and 35 from the United States and Spain) and 16 healthy controls from USA	Patients with the SLCO1B1 c.463C>A (rs11045819) polymorphism had 42% lower rifampicin AUC0–24, 34% lower Cmax, and 63% CL/F
[44]	*SLCO1B1*	rs2239751 rs2306283 rs11045819 rs4149014 rs4149032 rs4149056	173 adults of different races and countries of origin of which 12 are HIV positive	None of the *SLCO1B* gene polymorphism investigated were associated with rifapentine exposure (AUC 24hour)

Abbreviations: AUC, area under curve; PK, pharmacokinetic; SNP, single nucleotide polymorphism; Cl, clearance; F, bioavailability; Cmax, maximum concentration; CL/F, apparent oral clearance; V/F, apparent predicted volume of distribution.

(rs1045642), *ABCB1c.G2677 T/A* (rs2032582) and *ABCB1c*.1236C>T (rs1128503) SNPs are the most common non-synonymous and synonymous SNPs studied.[51] Rifamycins are a substrate and inducer of the *ABCB1* gene.[52] The decrease in rifampicin exposure with the time of treatment is partly explained by the induction of the *ABCB1* gene. Three studies assessed the effect of four *ABCB1*, rs1045642 rs2032582, rs1128503, and rs3842 (*ABCB1c.4036A>G*) SNPs. Huerta-García et al demonstrated that the rs1045642 TT genotype is a predictor that explains 34.8% of the variability in rifampicin Cmax and 48.5% of the variability in AUC0–24 h.[39] However, the other two studies did not replicate this observed result of Huerta-García et al.[38,40]

Association Between Drug-Metabolizing Enzyme and Pharmacokinetics

Rifamycins are metabolized by esterase enzymes. The esterase enzymes implicated in the metabolism of rifamycins are hepatic carboxylesterases (CES), and serine esterase arylacetamide deacetylase (AADAC). Two carboxylesterases, CES1 and CES2, are recognized to play major roles in drug metabolism. These enzymes metabolize rifamycins to their respective deacetylrifamycins.[14,15,53] Polymorphism of the *CES1* and *CES2* genes have been shown to influence the metabolism of several drugs.[54] However, few studies investigated the effect of *CES1* and *CES2* gene variants on rifamycin metabolism (Table 2).

Sloan et al investigated *CES1* rs12149368 SNP effect on rifampicin pharmacokinetics in Malawian tuberculosis patients. The rs12149368 variant does not affect the plasma rifampicin concentration[43] (Table 2). Song et al identified 10 variations in *CES2* in Korean TB patients. Among the ten variants three closely linked SNPs, *c.-2263A>G* (rs3759994, *g.738A>G), c.269–965A>G* (rs4783745, *g.4629A>G*), and *c.1612+136G>A (g.10748G>A)*, may alter the metabolism of rifampicin by affecting the efficiency of transcription of the gene. In particular, the *CES2 c.-2263A>G* variant, which is found in the promoter region is associated with increased plasma concentrations of rifampicin.[45]

Shimazu et al reported that microsomes from a liver sample genotyped as *AADAC*3/AADAC*3* showed decreased enzyme activities, compared with others. However, the allelic frequency is low, 1.3% European American, and 2.0% African American. The *AADAC*2* (rs1803155) allele, which has a higher frequency has also shown reduced enzyme

Table 2 Summary of the Studies Reported the Drug-Metabolizing Enzyme (*AADAC* and *CES*) Gene Polymorphisms Association with Rifamycins Pharmacokinetics Variation

Reference	Gene	SNPs	Characteristics of Study Participant	Rifamycins Pharmacokinetics
[43]	*CES 1*	rs12149368	174 Malawian adults with pulmonary tuberculosis of which 98 are HIV-infected patients	No associations between rifampicin AUC, Cmax, (CL/F), or V/F and AADAC or CES-1 SNPs polymorphism were identified
	AADAC	rs1803155 rs61733692		
[42]	*CES2*	rs3759994	113 children aged 3 months to 14 years and 59 (52.2%) were HIV co-infected	No significant effect of studied CES2 SNPs on rifampicin Cmax, AUC, and CL/F was observed
[41]	*AADAC*	rs1803155	162 pulmonary tuberculosis patients from two clinical studies receiving rifapentine in South Africa	Patients carrying the AA variant of *AADAC* rs1803155 were found to have a 10.4% lower rifapentine clearance
[45]	*CES2*	*c.-2548C>T* *c.-2263A>G* *c.269–965A>G* *c.474–152T>C* *c.615+120G>A* *c.1612+136G>A* *c.1613–87G>A* *c.1872*69A>G* *c.1872*302_304delGAA* *c.1872*445C>T*	35 patients with tuberculosis receiving a first-line antituberculosis treatment and 100 healthy individuals for analysis of the frequency of genetic variations in CES2 in the general population	The plasma rifampicin concentration increased with the number of risk alleles at c.2263A>G, c.269–965A>G and c.1612+136G>A, for example for c.2263A>G 8.9 mg/L versus 13.9mg/L for GG and AA respectively, while the plasma concentration decreased along with an increase in the number of risk alleles at c.1872*302_304delGAA
[44]	*AADAC*	rs1803155	173 adults of different races and countries of origin of which 12 are HIV positive	Rifapentine exposure (AUC 24) decreased by 10.2% in black participants for AADAC rs1803155 G versus A allele
	CES2	rs8045523 rs8192925 rs4783745		17.2% increase in rifapentine AUC0-24 for rs8192925 G versus A was observed

Abbreviations: AUC, area under the curve; CES, carboxylesterases; AADAC, arylacetamide deacetylase; Cmax, maximum concentration; CL/F, apparent oral clearance; V/F, apparent predicted volume of distribution.

activity. The recent report of Francis et al and Weiner et al revealed that rs1803155 SNP has a significant effect on rifapentine exposure in tuberculosis patients. The mean AUC-24 of rifapentine decreased by 10.2% in black tuberculosis patient carriers of *AADAC* rs1803155 G versus A allele.[44] The odds increase for GG allele carriers. A similar result was reported by Francis et al. Patients carrying the AA variant of *AADAC* rs1803155 were found to have a 10.4% lower clearance of rifapentine.[41] However, another study from Malawi showed that *AADAC* rs1803155 SNP did not affect rifampicin pharmacokinetics.[43]

Discussion

This systematic review provides current updates on the impact of genetic polymorphisms of drug transporters and drug-metabolizing enzymes on the pharmacokinetics of rifamycins. The overall finding suggests that the polymorphism in the drug transporter *SLCO1B1* rs4149032, rs2306283, rs11045819, and *ABCB1* rs1045642 and metabolizing enzyme AADACrs1803155 and CES2 c.-22263A>G (g.738A>G) of rifamycins partly contributes to the variability of pharmacokinetic parameters in tuberculosis patients.

The *SLCO1B1* gene is located on chromosome 12. Fifteen exons and many variants have been identified in the *SLCO1B1* gene. The missense mutation of rs4149056 (*c.521T>C*) where the wild type T is substituted with variant C causes a change in amino acid of OATP1B1 protein from valine to alanine at 174 positions. This change has been

implicated in reduced OATP1B1 protein function and is associated with an increased risk for statin-induced muscle toxicity.[55] However, an increase in the exposure to rifamycins was not reported in seven studies, and the one study, which reported an increase in AUC for the heterogeneous variant is also statistically non-significant. Lower frequency of rs4149056 CC variant in African populations[56] where the majority of studies were done and small sample size may contribute to no difference in the pharmacokinetics. rs2306283 (*388A>G*) SNP causes a change of asparagine amino acid to aspartic at 130 positions. The consequence of this change on the transporter function is not well elucidated. The patients who were homozygous wild type (AA)[42] and heterozygous (AG)[39] were reported to have lower rifampicin exposure. Similarly, no myopathy was observed with rs2306283 polymorphism which was observed in other *SLCO1B1* genes in patients taking statins suggesting no effect or increased activity of the mutant variant.[57]

rs11045819, which is located on exon 4, is another missense variant known in *SLCO1B1*gene. Of the four studies that assessed the impact of rs11045819 SNPs on rifampicin pharmacokinetics, only Weiner et al reported lower rifampicin exposure, lower peak concentration levels and greater apparent oral clearance with the *SLCO1B1* rs11045819 variant allele (CA) compared to the wild-type allele (CC).[36] This is consistent with a previous report that rs11045819 polymorphism increases OATP1B1 transporter activity and decreases systemic exposure of the OATP1B1 substrate.[58,59]

The well-studied *SLCO1B1* gene SNPs believed to affect rifamycin pharmacokinetics is rs4149032. The rs4149032 is an intron-located SNP and is reported to have a high allelic frequency. The effect of *SLCO1B1* rs4149032 on gene expression and OATP1B1 protein transporter function is not clear yet. Nevertheless, *SLCO1B1* rs4149032 polymorphism was found to be associated with lower rifampicin exposures. Emmanuel et al and Gengiah et al reported that patients who are homozygous mutant and heterozygous for rs4149032 polymorphism have lower bioavailability and Cmax respectively of rifampicin.[32,40] In addition, Kim et al observed lower oral clearance and higher rifampicin exposure for rs4149032 homozygous wild type (TT).[37]

Rifampicin significantly increases gene expression, protein levels, and efflux activity of *ABCB1*.[25,60] It is also a substrate for P-glycoprotein.[61] Huerta-García et al demonstrated that the rs1045642 SNPs, which is a silent mutation, is associated with rifampicin pharmacokinetics. Patients with CC or CT genotypes showed lower values of Cmax and AUC 24 compared to those with a TT genotype.[39] Although the rs1045642 SNPs is a silent mutation, previous studies have shown that rs1045642 affects the P-gp protein either by being in linkage disequilibrium with other functional SNPs or by allele-specific differences in the codon usage affecting the protein folding and function.[62,63] The observed change in the rifampicin pharmacokinetics with rs1045642 SNPs may be attributed to the above explanation.

Rifamycins are metabolized by the esterase enzyme family; microsomal hepatic carboxylesterases (CES), and serine esterase arylacetamide deacetylase (AADAC) to 25-deacetylrifamycins.[14] Three esterase enzymes AADAC, CES1, and CES2 have been reported as enzymes responsible for rifamycin deacetylation. Several genetic polymorphisms of the *CES1* and *CES2* genes have been shown to affect drug metabolism. For example, variations of the *CES1* gene have been reported to affect the metabolism of dabigatran oseltamivir, imidapril, and clopidogrel. Similarly, CES2 gene polymorphisms have been found to affect aspirin and irinotecan.[54] Few studies are available that report the association of *CES1* and *CES2* variants and rifamycin pharmacokinetics. Song et al evaluated 10 SNPs of *CES2* and found increased plasma rifampicin concentrations with the *CES2 c.-22263A>G (g.738A>G)* variants.[45] Although Dompreh et al did not report similar results,[42] the higher frequency of this variant allele warrants further investigation.

AADAC is primarily expressed in the liver and metabolizes clinically important drugs including rifamycins. Three, namely, *AADAC*1* (wild-type), *AADAC*2*, and *AADAC*3,* where the latter two have decreased enzymatic activity, were reported so far.[14,15] Recently, Francis et al and Weiner et al reported *AADAC* rs1803155 SNPs to have a significant effect on rifapentine metabolism. Shortly, a mutant variant of rs1803155 (AA) has decreased activity and decreased clearance of rifapentine. On the other hand, patients who have the wild type (GG) have shown decreased rifapentine exposure.[41,44] Furthermore, Gabriele et al discovered the presence and inter-individual variation of AADAC in the human lung.[64] These findings suggest the important role of *AADAC* pharmacogenetics in tuberculosis drug therapy.

Exposure to rifamycins in particular rifampicin is a crucial variable for successful tuberculosis treatment outcomes. The high inter-individual variability in rifamycins pharmacokinetics have been associated with various factors such as

diabetes mellitus[65] and partly HIV co-infection.[66,67] The majority of studies included in this review included patients with co-morbid conditions. The sample size is also inadequate for some studies.

In conclusion, the genetic polymorphism of drug transporters and drug-metabolizing enzymes has an impact on rifamycin pharmacokinetics. However, based on the available data, it is difficult to identify candidate SNPs in the drug transporters SLCO1B1 and ABCB1 for therapeutic drug monitoring. On the other hand, the effect of drug-metabolizing enzyme SNPs on the rifamycin pharmacokinetics is promising but needs more studies. In general, further controlled clinical studies with adequate sample size are required to characterize the genetic variation influence on the pharmacokinetics of rifamycins for tuberculosis chemotherapy optimization.

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Disclosure

The authors declare no conflicts of interest.

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Correlation of N-acetyltransferase 2 genotype and acetylation status with plasma isoniazid concentration and its metabolic ratio in ethiopian tuberculosis patients

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Unfavorable treatment outcomes for tuberculosis (TB) treatment might result from altered plasma exposure to antitubercular drugs in TB patients. The present study investigated the distribution of the N-Acetyltransferase 2 (*NAT2*) genotype, isoniazid acetylation status, genotype–phenotype concordance of NAT2, and isoniazid plasma exposure among Ethiopian tuberculosis patients. Blood samples were collected from newly diagnosed TB patients receiving a fixed dose combination of first-line antitubercular drugs daily. Genotyping of *NAT2* was done using TaqMan drug metabolism assay. Isoniazid and its metabolite concentration were determined using validated liquid chromatography-tandem mass spectrometry (LC–MS/MS). A total of 120 patients (63 male and 57 female) were enrolled in this study. The mean daily dose of isoniazid was 4.71 mg/kg. The frequency of slow, intermediate, and fast *NAT2* acetylators genotypes were 74.2%, 22.4%, and 3.3% respectively. The overall median isoniazid maximum plasma concentration (C_{max}) was 4.77 µg/mL and the $AUC_{0-7\,h}$ was 11.21 µg.h/mL. The median C_{max} in slow, intermediate, and fast acetylators were 5.65, 3.44, and 2.47 µg/mL, respectively. The median $AUC_{0-7\,h}$ hour in slow, intermediate, and fast acetylators were 13.1, 6.086, and 3.73 mg•h/L, respectively. The majority (87.5%) of the study participants achieved isoniazid C_{max} of above 3 µg/mL, which is considered a lower limit for a favorable treatment outcome. There is 85% concordance between the *NAT2* genotype and acetylation phenotypes. *NAT2* genotype, female sex, and dose were independent predictors of C_{max} and $AUC_{0-7\,h}$ ($p < 0.001$). Our finding revealed that there is a high frequency of slow *NAT2* genotypes. The plasma C_{max} of isoniazid was higher in the female and slow acetylators genotype group. The overall target plasma isoniazid concentrations in Ethiopian tuberculosis patients were achieved in the majority of the patients. Therefore, it is important to monitor adverse drug reactions and the use of a higher dose of isoniazid should be closely monitored.

Tuberculosis (TB) remains the major cause of death from infectious diseases. About 1.6 million people died from TB. This makes TB the second leading killer infectious disease after COVID-19 in 2021[1]. Although the six-month treatments for tuberculosis with the first-line antitubercular drugs (rifampicin, isoniazid, pyrazinamide, ethambutol) are effective, the emergence of drug resistance (DR-TB) impacts TB control success achieved[2,3]. DR-TB emerges because of inadequate or interrupted drug use, and the infecting mycobacteria are partially drug-resistant[4]. An increased drug exposure improved treatment outcomes; showing a positive relationship between anti-tuberculosis drug exposure and treatment outcome[5,6].

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The introduction of isoniazid, which is relatively inexpensive, and well tolerated in 1952, for tuberculosis care opened the modern era of tuberculosis treatment[7]. Isoniazid has high early bactericidal activity (EBA) and it can reduce bacterial load by 90–95% in the first 2 days of treatment[8]. EBA activity of isoniazid depends on the concentration that reaches the bacilli[9]. Several pharmacokinetic studies suggest a target of 3–6 µg/mL for the peak concentration (C_{max}) following a 300 mg once-daily dose of isoniazid. The C_{max} of isoniazid occurs 1–2 h post-dose[10].

Isoniazid is primarily metabolized to N-acetyl- isoniazid by the arylamine N-acetyltransferase 2 (NAT2) enzyme[11]. The *NAT2* gene is located on chromosome 8p22 and encodes the NAT2 enzyme[12]. *NAT2* gene is highly polymorphic displaying wide between-patient and between-population variations in its expression and enzyme activity. *NAT2*4* is a wild-type allele which is a fast acetylator genotype, while the common defective variant alleles *(NAT2*5, *6, *7,* and **14)* result in decreased acetylation activity and slow acetylation status. Intermediate acetylators carry one copy of *NAT2*4*[13]. Identification of *NAT2* polymorphism is useful to predict the effective therapeutic doses and adverse effects of isoniazid in different acetylators groups[14,15]. Slow acetylators are at increased risk of toxicity from isoniazid[16,17] while fast acetylators are at increased risk of treatment failure[6].

Isoniazid is metabolized to N-acetylisoniazid (AcINH) by the NAT2 enzyme, isonicotinic acid (INA), and hydrazine (Hz) by the amidase enzyme. NAT2 also catalyzes the acetylation of acetyl hydrazine (AcHz), which is a metabolite of AcINH, to non-toxic diacetylhydrazine. It also undergoes non-enzymatic conjugation with various endogenous substrates such as vitamin B6[18]. The mechanism by which isoniazid induces liver injury is not well established but believed that the metabolism of isoniazid produces a reactive metabolite that causes liver damage. Nevertheless, several recent studies showed that slow acetylators are at increased risk of hepatotoxicity[14,16–18].

The distribution of slow acetylator and *NAT-2* defective variant allele frequency varies across regions and populations within the region[19,20]. Black Africans display wide variations in *NAT2* genotype frequencies and slow acetylator phenotypes than non-Africans. Similarly, previous studies reported a high frequency of slow acetylator genotypes and phenotypes in the Ethiopian population[21,22]. Higher plasma isoniazid concentration was observed in Ethiopian pediatric patients[21]. On another hand, sub-clinical hepatotoxicity was observed in 17.3% of the patients who received the first-line antitubercular drugs[23].

Ethiopia is listed among the top 20 high TB and TB/HIV burden countries globally[24]. Isoniazid is part of the first-line anti-TB regimen in the country. Variations in the isoniazid acetylation rate, partly due to *NAT2* genetic variation, may influence TB treatment outcomes. But data is lacking on the distribution of the NAT2 acetylation status and its relationship with plasma isoniazid concentrations among Ethiopian TB patients. Therefore, this study investigated the distribution of the *NAT2* genotype-based acetylation status and its correlation with the C_{max} and plasma exposure ($AUC_{0-7\,h}$) of isoniazid in Ethiopian tuberculosis patients.

Materials and methods

Study participants. The study population comprised adult TB patients aged 18–65 years, receiving standard first-line drugs for TB treatment according to the Ethiopian treatment guidelines[25]. Newly diagnosed patients with drug-susceptible *Mycobacterium* TB were recruited from the TB clinics of the health center found in Addis Ababa (Beletshachew, Teklehymanote, Kazanchis, Woreda 2, and Areda Health Centre) from October 2019 to November 2021. Patients with either pulmonary or extrapulmonary forms of TB were included in the study. Patients received a daily dose of fixed-dose combination tablets containing 150, 75, 400, and 275 mg of rifampicin, isoniazid, pyrazinamide, and ethambutol respectively. The number of tablets received daily was based on the patient's body weight. Patients with a body weight greater than 55 kg received four fixed-dose combinations (FDC) tablets daily. Patients with a body weight between 40 and 55 kg received three FDC tablets daily and those under 40 received two FDC tablets. Treatment was provided under directly observed therapy (DOTs) at a primary health care facility found in Addis Ababa.

The study received ethical approval from the Institutional Review Board of the College of Health Science, Addis Ababa University (Ref number 080/17/IM), and the national research ethics review committee (Ref. Number MoSHE/RD/401/10,975/20). The study was conducted following the ethical principle of the Helsinki Declaration. All participants received a detailed explanation of the study protocol and provided written informed consent.

Blood sample collection. Blood samples were collected after observing drug intake in an EDTA tube. The sample was collected 2 weeks post-treatment initiation and only during the intensive phases of treatment. Blood samples were drawn at three-time points ranging from 1 to 7 h post-drug intake. But for a few patients, blood samples were drawn at two-time points. Plasma was separated immediately and stored at − 80 °C at the Department of Pharmacology and Clinical Pharmacy at Addis Ababa University until being transported to Karolinska Institutet in Stockholm, Sweden for analysis on dry ice.

DNA extraction and SNP genotyping. Genomic DNA was extracted from whole blood samples using the QIAmp DNA Blood Midi Kit (QIAGEN GmbH, Hilden, Germany) following the manufacturer's protocol. DNA was quantified using a NanoDrop spectrophotometer (Thermo Scientific) and stored at −20 °C until genotyping assay analysis. The recommended 4-SNP genotype panel of *NAT2*5* (c.341 T > C), *NAT2*6* (c.590G > A), *NAT2*7* (c.857G > A), *NAT2*14* (191G > A, rs1801279) for reliable estimation of rapid, intermediate, and slow acetylator phenotypes were selected[13,26]. Genotyping was performed using TaqMan drug metabolism assay reagents for allelic discrimination (Applied Biosystems Genotyping Assays) with the following ID numbers for each SNP: C___1204093_20 for *NAT2*5* (c.341 T > C, rs1801280), C___1204091_10 for *NAT2*6* (c.590G > A, rs1799930), C____572770_20 for *NAT2*7* (c.857G > A, rs1799931), C____572770_20 for *NAT2*14* (191G > A, rs1801279). The final volume for each reaction was 10 µL, consisting of 9 µL TaqMan® fast advanced master

mix (Applied Biosystems, Waltham, MA, United States), DNA/RNA free water, TaqMan 40X for all *NAT2*, drug metabolism genotyping assays mix (Applied Biosystems) and 1 µL genomic DNA.

Genotyping was performed by real-time Q-PCR (Applied Biosystems) equipped with 7500 software V2.3 (life technologies corporation) for allelic discrimination. The PCR conditions consisted of an initial step at 60 °C for 30 s, hold stage at 95 °C for 10 min and PCR stage for 40 cycles, step 1 at 95 °C for 15 min and step 2 at 60 °C for 1 min, and after reading stage with 60 °C for 30 s.

Quantification of plasma isoniazid and its metabolite concentration. For the determination of plasma concentration of isoniazid 4 mL venous blood was collected 2 weeks post-treatment initiation in the morning after an overnight fast in an EDTA tube. Plasma was separated immediately and stored at − 80 °C at the department of pharmacology and clinical pharmacy, Addis Ababa University until transported to Karolinska Institutet, Stockholm, Sweden for analysis. Quantification of isoniazid and acetyl-isoniazid were done at the therapeutic drug monitoring laboratory, Department of Clinical Pharmacology, Karolinska University Hospital. In brief, the concentration of isoniazid and acetyl-isoniazid were determined simultaneously using a liquid chromatography-tandem mass spectrometry (LC–MS/MS) system consisting of an Acquity Ultra Performance LC-system coupled to a Xevo TQ-S Micro (Waters, Milford, MA, USA). The chromatographic column consisted of YMC-ultraHT hydrosphere C18, 2 µm, 100 × 2 mm, reversed-phase column (Waters). And the mobile phase gradient of 0.1% formic acid in Milli-Q pure water, 100% methanol: methanol/Milli-Q pure water: Formic acid (10:90:0.1), methanol: Milli-Q pure water: isopropanol: Formic acid (70: 20: 10: 0.1), Methanol: Milli-Q pure water (10:90). The plasma sample preparation was based on protein precipitation with acetonitrile containing Isoniazid-d4, and Acetylisoniazid-d4 as an internal standard. The lower limit of quantification for isoniazid and acetyl-isoniazid were 0.05 µg/mL and 0.05 µg/mL respectively and the quantification ranges were 0.05–20 µg/mL and 0.05–10 µg/mL respectively. The method was validated according to the European Medicines Agency Guideline on bioanalytical criteria[27].

Statistical analysis. For each patient, the C_{max} was defined as the highest concentration measured, and the Tmax was the time point at which the C_{max} occurred. $AUC_{0-7\,h}$ calculation was performed using the trapezoidal rule. Graphpad prism was used to calculate $AUC_{0-7\,h}$. Continuous data were presented as median (interquartile range) for non-normal distributed data and mean standard deviation for normally distributed data. The Chi-square test was used to assess the Hardy–Weinberg equilibrium and genotype–phenotype concordance. Kruskal–Wallis tests were performed to see differences in C_{max} of isoniazid and acetyl isoniazid concentrations among the different genotypes. Univariate followed by stepwise multivariate linear regression analysis was performed to identify a predictive factor of isoniazid C_{max} and $AUC_{0-7\,h}$. Statistical analyses were performed using SPSS, version 27. P value < 0.05 was considered statistically significant.

Results

Patient characteristics. A total of 120 newly diagnosed tuberculosis patients who were non-diabetic and HIV-negative (63 males and 57 females) were included in this study. The detailed patient characteristic is described in Table 1. Nearly two-thirds of the patients had pulmonary tuberculosis. The median age of the patients was 28 years (IQR, 22–35). The mean dose of isoniazid received was 4.7 mg/kg/day (4.6–4.78, 95% CI). The documented rate of substance use was 13.33%, 17.5%, and 16.67% for cigarettes, khat, and alcohol respectively. Overall, 96.7% (N = 116) of participants completed treatment; 2 (1.67%) were lost to follow-up, and 2 (1.67%) were transferred to another health facility. Only one patient showed treatment failure. None of the study participants discontinued treatment because of the medication's adverse effects.

NAT2 variant allele and genotype frequencies. The frequency distribution of *NAT2*4*, **5*, **6*, **7*, and **14* alleles in Ethiopian tuberculosis patients were 14.6%, 47.1%, 31.3%, 5.4%, and 1.7%, respectively. There was no significant variation between observed and expected genotype frequencies according to Hardy–Weinberg equilibrium. Genotyping for the four most common functional variant alleles of *NAT2* rs1801280 (c.341 T > C), rs1799930 (c.590G > A), rs1799931 (c.857G > A), and rs1801279 (c.191G > A) was done for all the 120 TB patients enrolled in this study. The four SNP panels reliably estimate acetylator genotype groups[13]. There were twelve *NAT2* genotype groups observed among the study participants. The frequency distribution of the *NAT2* genotype and inferred phenotype is presented in Table 2. The most frequent genotype was *NAT2 *5/*5* followed by *NAT2 *5/*6*, and *NAT2 *6/*6*. All three were slow acetylators. The frequency of the homozygous wild type (*NAT2 *4/*4* genotype) was rare. Among 120 patients enrolled in the study, 4, 27, and 89 patients were fast, intermediate, and slow acetylator genotypes, respectively. The overall frequencies of genotype-predicted slow, intermediate, and fast acetylators were 74.2%, 22.4%, and 3.3% respectively.

Isoniazid plasma exposure. Spare pharmacokinetic sampling during the intensive phase of the therapy was done (median sampling point = 20 days after anti-TB treatment initiation, range = 11 to 46 days). Plasma sampling took place three times for 112 (92.5%) patients, two times for 7 (5.8%) patients, and one time for 1 (0.8%) patient. Plasma sampling time ranges from 1 to 7 h post-drug intake on an empty stomach. C_{max} was determined by taking the highest of the measured isoniazid plasma concentration. The time at which Cmax was observed is shown in Fig. 1. The regression line in Fig. 1 shows that the highest Cmax is achieved when the plasma is sampled earlier and gradually decreases as the time of sampling increases.

Isoniazid plasma exposure displayed wide between-patient variability, with the median C_{max} being 4.77 µg/mL (IQR 3.78–5.96). A comparison of the median C_{max} of isoniazid (Fig. 2), isoniazid $AUC_{0-7\,h}$, and acetyl isoniazid between fast, intermediate, and slow acetylators is shown in Table 3. There was a significant difference in

Characteristics	Pulmonary TB	Extrapulmonary TB	Total
Sex (n)			
Male	46 (38.3%)	17 (14.2%)	63 (52.5%)
Female	33 (27.5%)	24 (20%)	57 (47.5%)
Age (years), Median (IQR)	26 (21–35)	28 (24.5–36)	28 (22–35)
Body weight (Kg), Median (IQR)	53 (45–60)	58 (52.5–68.5)	54.75 (48–61.75)
Drug dose (mg/kg), Mean (95% CI)	4.73 (4.62–4.84)	4.63 (4.47–4.78)	4.7 (4.62–4.78)
Marital status (n)			
Single	54 (45%)	14 (11.67%)	68 (56.7%)
Divorced	2 (1.67%)	1 (0.8%)	3 (2.5%)
Married	22 (18.3%)	24 (20%)	46 (38.3%)
widowed	1 (0.8%)	2 (1.67%)	3 (2.5%)
Educational level (n)			
Illiterate	10 (8.3%)	7 (5.8%)	17 (14.17%)
Primary	29 (24.17%)	13 (10.8%)	42 (35%)
Secondary	27 (22.5%)	16 (13.3%)	42 (35%)
Tertiary	13 (10.8%)	5 (4.17%)	18 (15%)
Smoking (n)			
Yes	15 (12.5%)	1 (0.83%)	16 (13.3%)
No	64 (53.3%)	40 (33.3%)	104 (86.67%)
Khat Chewer (n)			
Yes	19 (15.83%)	2 (1.67%)	21 (17.5%)
No	60 (50%)	39 (32.5%)	99 (82.5%)
Alcohol (n)			
Yes	17 (14.17%)	3 (2.5%)	20 (16.67%)
No	62 (51.67%)	38 (31.67%)	100 (83.3%)

Table 1. Study participants' sociodemographic characteristics stratified by type of tuberculosis infection (n = 120). *n* number, *CI* Confidence interval, *IQR i*nterquartile range.

NAT2 genotype	*NAT-2* genotype frequency (n = 120)	*NAT-2* genotype (%)	Acetylator type	Acetylator (%)
*NAT-2 *4/*4*	4	3.3	Fast	3.3
*NAT-2 *4/*14*	1	.8	Intermediate	22.4
*NAT-2 *4/*5*	18	15.0		
*NAT-2 *4/*6*	7	5.8		
*NAT-2 *4/*7*	1	.8		
*NAT-2 *5/*14*	2	1.7	Slow	74.2
*NAT-2 *5/*5*	29	24.2		
*NAT-2 *5/*6*	26	21.7		
*NAT-2 *5/*7*	9	7.5		
*NAT-2 *6/*14*	1	.8		
*NAT-2 *6/*6*	19	15.8		
*NAT-2 *6/*7*	3	2.5		

Table 2. Frequency and percentage distribution of *NAT2* genotype acetylators in Ethiopian tuberculosis patients (N = 120).

median values of C_{max} of isoniazid and acetyl isoniazid and isoniazid $AUC_{0-7\,h}$ among the three *NAT2* acetylators groups. Of the 120 study participants, 15 (12.5%) had an isoniazid C_{max} of < 3 µg/mL (low C_{max}), and 28 (23.3%) had a C_{max} of > 6 µg/mL (high) compared to published data. There was no significant difference in isoniazid C_{max} with fast acetylators compared to those with intermediate acetylators ($p = 0.81$). However, the difference in the C_{max} value of isoniazid was significant between fast and slow acetylators ($p = 0.04$) and intermediate and slow acetylators ($p < 0.001$).

There was a significant difference in isoniazid $AUC_{0-7\,h}$ between acetylator groups. The overall median isoniazid $AUC_{0-7\,h}$ for slow, intermediate, and fast acetylators was 13.09 µg.h/mL, 6.09 µg.h/mL, and 3.73 µg.h/mL, respectively. The variation of $AUC_{0-7\,h}$ between the slow genotype group and the other two groups is high ($p < 0.001$). Similarly, acetyl-isoniazid C_{max} concentration varies among the three *NAT2* genotypes. A significant difference in acetyl-isoniazid concentration was observed between slow and intermediate ($p < 0.001$) and slow

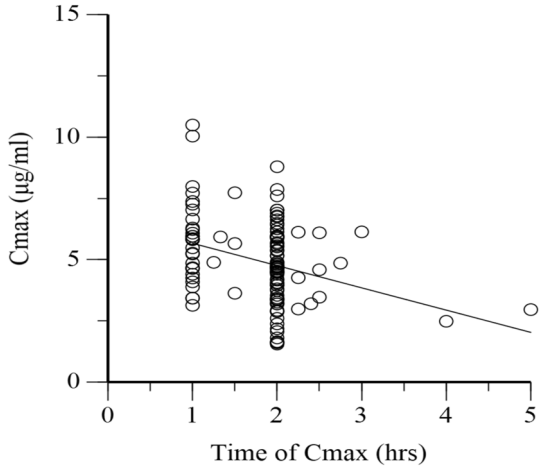

Figure 1. C_{max} of isoniazid compared to the time at which C_{max} achieved.

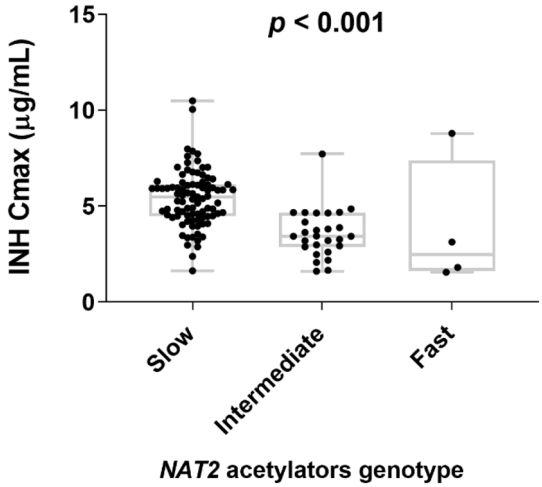

Figure 2. Comparison of isoniazid C_{max} among fast (n = 4), intermediate (n = 27), and slow (n = 89) *NAT2* acetylator genotype groups. The box plots show the median ± interquartile range, while the whiskers denote the minimum and maximum values.

Variable		Slow	Intermediate	Fast	*p* value
Isoniazid	C_{max}	5.48 (4.49–6.13)	3.43 (2.9–4.4)	2.47 (1.68–5.96)	< 0.001
	$AUC_{0-7\,h}$*	13.1 (10.48–15.03)	6.086 (5.21–7.44)	3.73 (2.22–21.87)	< 0.001
Actylisoniazid	C_{max}°	0.67 (0.51–0.83)	1.57 (1.1–2.1)	2.21 (1.16–3.44)	< 0.001
Ac-INH/INH		0.12 (0.09–0.167)	0.38 (0.27–0.69)	0.95 (0.3–1.94)	< 0.001

Table 3. Comparison of isoniazid C_{max} and $AUC_{0-7\,h}$, actyl-isoniazid C_{max}, and actylisoniazid /isoniazid ratio (Ac-INH/INH) among the three *NAT2* genotypes groups. Values are presented as median (interquartile range), $AUC_{0-7\,h}$ -area under the time-concentration curve, C_{max} -maximum concentration, °data is available for 108 patients, *data is available for 112 patients.

and fast ($p = 0.001$) acetylators. The difference in AcINH/INH metabolic ratio among the three genotype groups had high variation ($p < 0.001$). A significant difference in AcINH/INH metabolic ratio was observed between slow, intermediate, and slow and fast acetylators. On the other hand, the difference in AcINH/INH metabolic ratio between fast and intermediate metabolizers was statistically non-significant ($p = 0.17$). The pattern of C_{max} and AcINH/INH of the three metabolizer groups is shown in Figs. 2 and 3. At the time of C_{max}, ten slow acetylators and two intermediate acetylators had undetectable acetyl isoniazid concentrations. So that the metabolic ratio was available only for 108 patients.

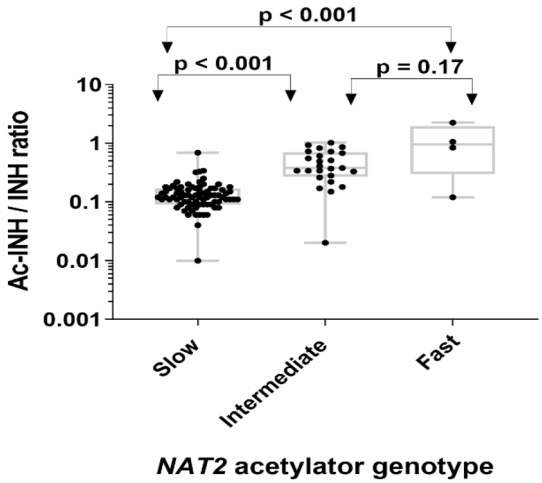

Figure 3. Comparison of acetyl-isoniazid to isoniazid metabolic ratio (Ac-INH/INH) among fast (n = 4), intermediate (n = 25), and slow acetylators (n = 79) genotype groups. The box plots show the median ± interquartile range, while whiskers denote the minimum and maximum values.

Predictors of isoniazid plasma exposure. A univariate and stepwise multivariate linear regression model including age, cigarette smoking, khat chewing, alcohol use, gender, drug dose, and the three *NAT2* acetylators genetic variants was done to identify a predictor of isoniazid plasma exposure (Table 4). In the univariate analysis, sex ($p = 0.001$) and *NAT2* acetylator genotypes ($p < 0.001$) were significant variables to predict isoniazid C_{max}. In stepwise regression analysis, *NAT2* acetylator genotypes alone, sex and *NAT2* acetylator genotypes, sex, *NAT2* acetylator genotypes, and drug dose (mg) explained 18.9%, 27.6%, and 33% variability in isoniazid C_{max} respectively. Similarly, age, cigarette smoking, khat chewing, and alcohol use, did not predict isoniazid $AUC_{0-7\,h}$. Sex ($p = 0.004$), drug dose (mg) ($p = 0.036$), and *NAT2* acetylators genotypes ($p < 0.001$) were significantly associated with variations in isoniazid $AUC_{0-7\,h}$. In the multivariate stepwise analysis, Sex, *NAT2* acetylator genotypes and drug dose (mg) were responsible for 35.7% variation in $AUC_{0-7\,h}$ of isoniazid. *NAT2* acetylator genotypes alone are responsible for 25.9% of isoniazid $AUC_{0-7\,h}$ variation and 29.9% with sex.

Isoniazid metabolic ratio. Plasma acetyl-isoniazid to isoniazid ratio (AcINH/INH) ranged between 0.01 to 2.24 (median = 0.145, IQR = 0.106–0.295). The classification of acetylator phenotypes as slow and fast was done as described by Varshney E et al.[28] and Aklillu et al.[22]. In brief, a probit plot and regression analysis were used to identify the anti-mode cut-off value to classify slow and rapid acetylators. The cut-off value identified for AcINH/INH ratio was 0.473 and according to this cut-off value, 86.3% of study participants were classified phenotypically as slow acetylators and the remaining 12.76% as fast acetylators.

PK parameter	Predictor	Univariate analysis		Multivariate analysis	
		Coefficient (β)	*P*	Coefficient (β)	*p*
C_{max}	Sex (male vs. female)	1.16	0.001	1.16	< 0.001
	Age	0.01	0.57	–	–
	Smoking (smoker vs. non-smoker)	0.39	0.4	–	–
	Khat (chewer vs. non-chewer)	0.03	0.95	–	–
	Alcohol (user vs. non-user)	0.26	0.53	–	–
	Dose (mg)	0.007	0.066	0.009	0.003
	Acetylator genotypes (fast and intermediate vs. slow)	1.41	< 0.001	1.28	< 0.001
$AUC_{0-7\,h}$	Sex (male vs. female)	2.45	0.004	2.36	0.001
	Age	− 0.04	0.32	–	–
	Smoking (smoker vs. non-smoker)	0.05	0.97	–	–
	Khat (chewer vs. non-chewer)	− 0.29	0.8	–	–
	Alcohol (user vs. non-user)	− 0.51	0.66	–	–
	Dose (mg)	0.021	0.036	0.026	0.002
	Acetylator genotypes (fast and intermediate vs. slow)	4.53	0.00	4.26	< 0.001

Table 4. Univariate and multivariate analysis showing factors associated with C_{max} and $AUC_{0-7\,h}$ of isoniazid.

NAT2 genotype—phenotype concordance. The phenotype-genotype concordance was described using traditional phenotype classification. Genotype inferred acetylations status described above. Similarly, using AcINH/INH ratio phenotypic acetylation status as slow and fast acetylators was done as described above. The overall *NAT2* genotype–phenotype concordance was 85%. Concordance between genotype inferred acetylator status and measured NAT2 acetylator phenotype is presented in Table 5. *NAT 2* genotype predicted acetylator phenotype in 92 patients accurately. Almost all slow acetylator genotypes (98.3%) were accurately predicted, whereas 13.88% of fast acetylators genotypes were predicted as slow acetylator phenotypes. Only one *NAT2*5/*5* slow acetylator genotype was predicted as a fast acetylator phenotypically. Heterogeneity was observed for *NAT2*4/*5* and **4/*6* on the acetylation status. More than half of *NAT2*4/*5* (62.5%) and *NAT2*4/*6* (57%) genotype carriers were slow acetylator phenotypically.

Discussion

The effect of *NAT2* genotype on the pharmacokinetics of isoniazid in TB patients is well explored in various Asian and Caucasian populations but data is scarce from sub-Sharan Africa, including Ethiopia, the seventh top high-TB burden country globally and the 2nd most populous nation in Africa. Ethiopians display wide pharmacogenetics variations compared to other populations within and outside of Africa[29,30]. In this study, we investigated the profile and predictors of isoniazid plasma exposure and the effect of the *NAT2* genotype on isoniazid and its metabolite acetyl isoniazid pharmacokinetics in a cohort of newly diagnosed Ethiopian tuberculosis patients. Our main findings include i) a significant association of *NAT2* acetylator genotype with between-patient variability in isoniazid pharmacokinetics (C_{max}, $AUC_{0-7\,h}$, metabolic ratio), ii) a high concordance rate (85%) between *NAT2* genotype and acetylation rate of isoniazid, iii) high prevalence of slow acetylators in Ethiopian TB patients and the majority of (85%) achieved therapeutic isoniazid plasma concentration. iv) *NAT2* genotype and sex are significant predictors of isoniazid plasma exposure.

Interestingly, we found a high prevalence of genotypic (74.2%) and phenotypic (86%) slow acetylators in Ethiopian TB patients. Genotypically, 22.4% were intermediate acetylators, and only 3.3% were fast acetylators. Our finding is in line with a previous study among healthy Ethiopians, reporting the frequency of slow, intermediate, and fast acetylators being 73.6%, 24.6%, and 1.8%, respectively[22]. The frequency distribution of the slow acetylators genotype varies between populations. About 10–20% of Asians and 40–70% of Caucasians are slow acetylators[31]. Black Africans, the most genetically diverse population on earth, display the highest level of within-population diversity of *NAT2* genotype and outside of the region[19,20]. The fast acetylators are predominant in West Africa. Compared to the Ethiopians, a lower frequency of slow acetylators in Senegalese (44.3%)[32], South African (52.5%)[33], and Tanzanians (48%) TB patients[34] is reported. This confirms the wide heterogeneity of black Africans and results from one population may not apply to others within the region.

Various levels of concordance between the *NAT2* genotype and acetylation phenotype are reported. Our study revealed high concordance (85%) between *NAT 2* genotype and NAT2 acetylation phenotype. Aklillu et.al[22] reported a lower (75%) but significant *NAT2* genotype–phenotype concordance in healthy Ethiopians using caffeine as a probe drug for NAT2 enzyme activity. Unlike our finding in Ethiopian TB patients, a recent study in Zulu-speaking South Africans reported a lower percentage (55%) of slow acetylators and poor or no significant concordance between the *NAT2* genotype and isoniazid phenotype concordance[33].

Low isoniazid concentrations have been postulated to result in unfavorable treatment outcomes[35,36]. A target of 3–6 µg/mL for the peak concentration (C_{max}) following a 300-mg daily dose of isoniazid is considered vital for a favorable treatment outcome[35]. Studies also reported that anti-TB drug-induced hepatotoxicity was associated with slow acetylation[37]. In this study, the C_{max} of isoniazid was greater than 3 µg/mL in 87.5% of patients and the $AUC_{0-7\,h}$ of isoniazid was high suggesting high isoniazid exposure in Ethiopian tuberculosis patients. A similar pattern of isoniazid plasma concentration was observed in Ethiopian pediatric TB patients[21]. The large proportion of slow acetylators in our cohort means isoniazid plasma exposure is sufficiently high to provide clinical benefit.

In univariate and multivariate analysis, sex, and *NAT2* acetylator genotype status were predictors of isoniazid C_{max}. Females had higher C_{max} compared to males, which is in agreement with those of previous studies[38,39]. This may explain the previous finding of an increased risk of isoniazid-induced drug toxicity[40] and a lower risk of unfavorable treatment outcomes in females[41]. Plasma isoniazid C_{max} increased as the isoniazid dose increased. This suggests that dose is also a predictor of C_{max}. Nonsmokers had higher isoniazid C_{max} than smokers; inversely khat chewer had higher isoniazid $AUC_{0-7\,h}$ than nonchewers though the differences in both were not significant.

	Phenotype inferred NAT 2 acetylator status (AcINH to INH ratio)		
Genotype inferred *NAT 2* acetylator status	Fast	Slow	Total
Rapid	3 (21.4%)	1 (1.1%)	4 (3.7%)
Intermediate	11 (78.5%)	14 (15%)	25 (23.15%)
Slow	1 (7.1%)	78 (83.87%)	79 (73.15%)
Total	15 (12.96%)	93 (86.11%)	108

Table 5. Concordance between genotype inferred acetylator status and measured NAT2 acetylator phenotypes in Ethiopian tuberculosis patients ($\chi 2$ test $p < 0.001$).

NAT2 enzyme activity is the rate-limiting step in acetylating isoniazid to acetyl isoniazid. A high interindividual variation was observed in the clinical efficacy, elimination, and side effects of isoniazid. These variations were related to the difference in the NAT2 enzyme which metabolizes isoniazid. We observed a bimodal isoniazid C_{max}, unlike other studies which reported trimodal C_{max} and AUC based on *NAT2* genotype[42,43]. A significant variation of isoniazid C_{max} and AUC_{0-7} were observed between the slow and the other two acetylators groups while there is no significant variation between fast and intermediate acetylators. The low number of fast acetylators in our study population might be attributed to the absence of difference between fast and intermediate acetylators groups. Fast isoniazid acetylators showed lower C_{max} and exposure to the drug than slow acetylators. Several authors reported an increased risk of toxicity in slow acetylators[37,44] and an increased risk of therapeutic failure in fast acetylators[34,45] patients. *NAT2* genotype-guided isoniazid administration reduced toxicity and improved treatment outcomes in Japanese trials[46]. Thus, owing to high exposure to isoniazid, Ethiopians are at increased risk of toxicity from isoniazid. Indeed high rates of anti-TB and antiretroviral treatment-induced liver toxicity in Ethiopian TB-HIV coinfected patients, particularly in slow acetylators is reported previously[47].

We evaluated the plasma isoniazid C_{max} and drug exposure following standard laboratory recommendations like collecting plasma from patients who have received anti-drug after fasting overnight. Several studies reported that food decreased absolute bioavailability and maximum concentration of isoniazid[48,49]. Plasma was immediately separated and kept at –20 °C until transported for storage at – 80 °C on the same day. The cold chain was kept during sample transportation. The concentration of isoniazid after a week of storage at -20 °C was about 80% of the initial amount and no significant change in the initial concentration was observed if stored at -80 °C for longer than six months[50]. Study participants were patients receiving a standard dose of isoniazid in a fixed dose combination with rifampicin, ethambutol, and pyrazinamide. Patients had no prior exposure to the drugs and had no reported comorbidities of liver, kidney, HIV infection, or diabetes. Low isoniazid concentration was observed in TB-HIV co-infected patients[51].

Our study has some limitations. Although the spare sampling strategy is evolving in recent years and found to be useful to capture AUC0-24 h[52], the time point at which we collected the plasma sample varied from patient to patient. The sparse sampling strategy may not fully define the individual C_{max} and AUC. We enrolled 120 patients in both the pharmacokinetics and pharmacogenetics studies. Because of the low frequency of fast acetylators in our study participants, we did not observe significant pharmacokinetics variation between fast and intermediate acetylators.

In conclusion, we report a high prevalence of the slow *NAT2* acetylator genotype in Ethiopian tuberculosis patients. NAT2 acetylation status and the female sex are strong predictors of isoniazid plasma concentrations. The majority of the patients attain therapeutic plasma isoniazid exposure for a favorable treatment outcome. On the other hand, slow acetylators and females are at a higher risk of concentration-dependent isoniazid toxicity. Therefore, close safety monitoring, particularly for patients on high-dose isoniazid short-course MDR-TB therapy is recommended for early identification and management of treatment-associated adverse events.

Data availability

All data generated or analyzed in this study are included in this article. The datasets used and/or analyzed during the study are available from the corresponding author upon reasonable request.

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Author contributions

Conceptualization and study design T.S, E.M, and E.A; experimental analysis T.S, N.F.T, V.B, and E.A; data analysis T.S, N.F.T, and E.A; draft manuscript preparation T.S; review and editing of the manuscript N.F.T, E, M, and E.A. All authors read and approved the manuscript for submission.

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Competing interests

The authors declare no competing interests.

Additional information

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RESEARCH ARTICLE

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Variability in plasma rifampicin concentrations and role of *SLCO1B1*, *ABCB1*, *AADAC2* and *CES2* genotypes in Ethiopian patients with tuberculosis

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ABSTRACT

Background: Rifampicin, a key drug against tuberculosis (TB), displays wide between-patient pharmacokinetics variability and concentration-dependent antimicrobial effect. We investigated variability in plasma rifampicin concentrations and the role of *SLCO1B1*, *ABCB1*, arylacetamide deacetylase (*AADAC*) and carboxylesterase 2 (*CES-2*) genotypes in Ethiopian patients with TB.

Methods: We enrolled adult patients with newly diagnosed TB ($n = 119$) who had received 2 weeks of rifampicin-based anti-TB therapy. Venous blood samples were obtained at three time points post-dose. Genotypes for *SLCO1B1* (*c.388A > G*, *c.521T > C*), *ABCB1* (*c.3435C > T*, c.4036A > G), *AADACc.841G > A* and *CES-2* (*c.269-965A > G*) were determined. Rifampicin plasma concentration was quantified using LC-MS/MS. Predictors of rifampicin C_{max} and $AUC_{0-7\,h}$ were analysed.

Results: The median rifampicin C_{max} and AUC_{0-7} were 6.76 μg/mL (IQR 5.37–8.48) and 17.05 μg·h/mL (IQR 13.87–22.26), respectively. Only 30.3% of patients achieved the therapeutic efficacy threshold (C_{max}>8 μg/mL). The allele frequency for *SLCO1B1*1B* (*c.388A > G*), *SLCO1B1*5* (*c.521T > C*), *ABCB1 c.3435C > T*, *ABCB1c.4036A > G*, *AADAC c.841G > A* and *CES-2 c.269-965A > G* were 2.2%, 20.2%, 24.4%, 14.6%, 86.1% and 30.6%, respectively. Sex, rifampicin dose and *ABCB1c.4036A > G*, genotypes were significant predictors of rifampicin C_{max} and AUC_{0-7}. *AADACc.841G > A* genotypes were significant predictors of rifampicin C_{max}. There was no significant influence of *SLCO1B1* (*c.388A > G*, *c.521T > C*), *ABCB1c.3435C > T* and *CES-2 c.269-965A > G* on rifampicin plasma exposure variability.

Conclusions: Subtherapeutic rifampicin plasma concentrations occurred in two-thirds of Ethiopian TB patients. Rifampicin exposure varied with sex, dose and genotypes. *AADACc.841G/G* and *ABCB1c.4036A/A* genotypes and male patients are at higher risk of lower rifampicin plasma exposure. The impact on TB treatment outcomes and whether high-dose rifampicin is required to improve therapeutic efficacy requires further investigation.

KEYWORDS
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Introduction

Whilst effective tuberculosis (TB) treatment has been available for the past seven decades, the latest 2022 WHO Annual Global Tuberculosis Report highlights that TB remains a leading cause of death from an infectious disease worldwide [1]. Considerable success have been achieved in treatment outcomes since the introduction of rifampicin in 1970. However, the global increase in HIV incidence, poor adherence to 6-month therapy and suboptimal drug concentrations due to interindividual pharmacokinetic variations of first-line antitubercular drugs have contributed to the emergence of resistance to TB drugs [2–4]. Drug-resistant TB is a concern in East African countries [5]. Ethiopia is among the top 30 countries with the highest TB and TB-HIV burden with an incidence of 119 cases per 100,000 people in 2021 [1].

A combination of rifampicin with isoniazid is the backbone of modern anti-TB therapy. Rifampicin has concentration-dependent bactericidal activity [6]. The microbial killing of rifampicin was linked to the ratio of the area under the concentration-time curve and the minimum inhibitory concentration (AUC/MIC) and the maximum concentration (C_{max})/MIC (C_{max}/MIC) ratio. Rifampicin prevents resistance to itself and attains sufficient bactericidal effect at a free C_{max}/MIC ratio of $\geq$175 [7,8]. A rifampicin C_{max} between 8 and 24 μg/mL is considered optimal and C_{max} below 4 μg/mL is a risk factor for treatment failure [9].

Rifampicin undergoes hepatic metabolism by genetically polymorphic carboxylesterases (CES) and arylacetamide deacetylase (AADAC), a serine esterase to 25-deacetylrifampicin [10]. Rifampicin pharmacokinetics and treatment outcomes display wide between-patient variations [11,12]. Genetic variation in enzymes and transporter proteins relevant to rifampicin disposition may influence the variability of plasma rifampicin exposure. Previous studies in various populations investigated the impact of genetic variation in *AADAC* and *CES* on rifampicin plasma exposure with varying results [13–16]. Rifampicin is a substrate and inducer of the organic anion transporter polypeptide 1B1 (OAT1B1) encoded by the *SLCO1B1* gene [17] and P-glycoprotein (P-gp) encoded by the *ABCB1* gene [18]. OAT1B1 mediates hepatocellular uptake of rifampicin while P-gp mediates drug efflux. Both *SLCO1B1* and *ABCB1* genes are genetically polymorphic displaying wide between-population variation in enzyme activity and variant allele frequency distributions. In the few published studies investigating the effect of the *SLCO1B1* and *ABCB1* gene polymorphism on rifampicin pharmacokinetics, the result is inconclusive [16,19–22].

The pharmacokinetics and pharmacogenetics of rifampicin display wide between-race and between-population variations, highlighting the need for investigation in different geographic locations where the burden of TB is high. The effect of pharmacogenetic variability in rifampicin pharmacokinetics using a targeted candidate gene approach has been explored in various Asian and Caucasian populations [22–24], but data from sub-Saharan Africa remain scarce. Ethiopia is the seventh top high-TB burden country globally [1]and the second most populous nation in Africa. The pharmacogenetics of *SLCO1B1* and *ABCB1* in Ethiopians differs from that of other black African populations and inhabitants of European origin [23,25,26]. In this study, we examined the variability in rifampicin C_{max} and AUC_{0-7} in Ethiopian TB patients in relation to the recommended target concentration for optimal therapeutic efficacy and the impact of common functional genetic variants in *SLCO1B1* (rs2306283 and rs4149056), *ABCB1* (rs1045642 and rs3842), *CES 2* (rs4783745) and *AADAC* (rs1803155) on between-patient variability in rifampicin plasma concentration.

Methods

Study participants and settings

The study participants were newly diagnosed adults aged 18–65 years with either pulmonary or extrapulmonary drug-sensitive *Mycobacterium tuberculosis* attending TB clinics in Addis Ababa (Beletshachew, Teklehymanote, Kazanchis, Woreda 2 and Areda Health Centre). The study was conducted from October 2019 to November 2021.

Blood sample collection

Blood samples were obtained 2 weeks after treatment initiation during the intensive phase of TB therapy. Following overnight fasting, participants received drugs under direct observation in the morning. A total of 351 venous blood samples were collected in EDTA tubes, with three samples taken at different times from 113 subjects and two times from 6 subjects. The blood sampling points ranged from 1 to 7 h post-dose, with the majority of subjects sampled at 1, 2, 4, or 2, 4, or 6 h post-dose. Plasma was separated immediately and stored at −80 °C at the Department of Pharmacology and Clinical Pharmacy, Addis Ababa University until

transported to Karolinska Institutet, Stockholm, Sweden for analysis.

Ethical approval

Ethical approval was obtained from the Institutional Review Board of the College of Health Sciences at Addis Ababa University and the National Research Ethics Review Committee. All patients were informed about the purpose of the study and those willing to participate and who provided written informed consent were enrolled. The study was conducted following the ethical principle of the Helsinki Declaration.

Treatment

Study participants received a standard daily dose of rifampicin in combination with isoniazid, pyrazinamide and ethambutol according to the Ethiopian treatment guidelines [27]. Patients with a body weight above 55kg received four fixed-dose combinations (FDC) tablets daily. Patients with a body weight between 40 and 55 kg received three FDC tablets daily and those below 40 kg received two FDC tablets daily. Each FDC tablet contains 150, 75, 400 and 275 mg of rifampicin, isoniazid, pyrazinamide and ethambutol, respectively. Treatment was given as directly observed therapy at a primary health care facility in Addis Ababa, Ethiopia.

DNA extraction and genotyping

Genomic DNA was extracted from whole blood samples using the QIAmp DNA Blood Midi Kit (QIAGEN GmbH, Hilden, Germany) following the manufacturer's instructions. Common functional variant alleles in the black African population relevant to rifampicin disposition were selected for genotyping. Genotyping was performed using TaqMan® drug metabolism assay reagents for allelic discrimination (Applied Biosystems Genotyping Assays) as described previously [28] with the following ID numbers: C___8911003_1 for *AADAC2* (c.841G > A, rs1803155), C__31760486_10 for *CES2* (c.269-965A > G, rs4783745), C___7586657_20 for *ABCB1* (3435 C > T, rs1045642), C__11711730_20 for *ABCB1* (c.193A > G, rs3842), C___1901697_20 for *SLCO1B1* (c.388A > G, rs2306283) and C__30633906_10 for *SLCO1B1* (c.521T > C, rs4149056).

The final volume for each reaction was 10 μL, consisting of 1 μL genomic DNA and 9 μL of TaqMan® fast advanced master mix (Applied Biosystems, Waltham, MA, United States), DNA/RNA free water, TaqMan 40X for *SLCO1B1*, *ABCB1* and TaqMan 20× for *AADAC2* and *CES2* drug metabolism genotyping assays mix (Applied Biosystems). Genotyping was performed by real-time qPCR (Applied Biosystems) equipped with 7500 software V2.3 (Life Technologies Corporation) for allelic discrimination.

Quantification of rifampicin plasma concentrations

To determine rifampicin plasma concentrations, blood samples were collected 2 weeks after treatment initiation during the intensive phase of TB therapy. After overnight fasting, study participants received drugs under direct observation in the morning. Venous blood was taken in EDTA tubes at three time points from 1 to 7 h post-dose. Plasma was separated immediately and stored at −80 °C at the Department of Pharmacology and Clinical Pharmacy, Addis Ababa University until transported to Karolinska Institutet, Stockholm, Sweden for analysis.

Rifampicin plasma concentrations were determined using a liquid chromatography-tandem mass spectrometry (LC-MS/MS) as described previously [11]. The method was validated according to the European Medicines Agency guidelines [29]. The LC-MS/MS system consisted of an Acquity Ultra Performance LC-system coupled to a Xevo TQ-S Micro (Waters, Milford, MA, USA) and aYMC-ultraHT hydrosphere C18, 2 μm, 100 × 2 mm, reversed-phase column (Waters) was used. Sample preparation consisted of protein precipitation with acetonitrile containing deuterated rifampicin as an internal standard. In brief, 100 μL plasma samples were diluted with a 300 μL solution containing the internal standards dissolved in acetonitrile. After shaking for 30 s and 5 min of centrifugation, 150 μL of the supernatant was transferred to another plate. The supernatant dried for 30 min at 35 °C and the dried sample was re-suspended with 15 μL methanol and 275 μL 0.1% formic acid for injection. The mobile phase gradient of 0.1% formic acid in Milli-Q pure water, 100% methanol:methanol/Milli-Q pure water:formic acid (10:90:0.1), methanol:Milli-Q pure water:isopropanol:formic acid (70:20:10:0.1), methanol:Milli-Q pure water (10:90). Rifampicin concentrations were calculated by linear regression from a six-point calibration curve. The limits of the quantitation range for rifampicin were 0.1 and 40 μg/mL.

Pharmacokinetic and statistical analyses

Study participants' sociodemographic and baseline clinical parameters are summarised as the median and interquartile range (IQR) or as frequency and percentages. The rifampicin C_{max} was determined from the available plasma concentrations. The highest concentration observed was taken as C_{max}. $AUC_{0-7\,h}$ calculation was performed using the trapezoidal rule. GraphPad Prism was used to calculate $AUC_{0-7\,h}$.

The Shapiro–Wilk test was used to determine the normality of pharmacokinetics data. Non-normally distributed data are presented as median (IQR) and normally distributed as mean (standard deviations [*SD*]). The chi-square test was used to assess correlations between the observed and expected genotype frequencies according to the Hardy–Weinberg equilibrium. All plasma concentration data were log 10 transformed before conducting statistical analyses [29]. The association of each genotype with between-patient variability in C_{max} and AUC_{0-7} was analyzed using a one-way analysis of variance, comparing the geometric mean of log-transformed concentration data [30]. Predictors of C_{max} and $AUC_{0-7\,h}$ of rifampicin were subjected to further analysis through univariate followed by multivariate regression analysis, incorporating study participant characteristics and genotypes as potential predictors. Variables with p value <0.2 from the univariate analysis were included in the multivariate regression analysis. Data were analyzed using SPSS version 25 and a p value ≤ 0.05 was considered to indicate statistical significance.

Results

Study participants characteristics

Of the 119 study participants, consisting of 62 males and 57 females, 78 were diagnosed with pulmonary TB and 41 had extrapulmonary TB. The median body weight was 54.8 kg (IQR, 48.0–61.7), and the median age was 28 years (IQR, 22 – 35). The mean dose of rifampicin was 9.39 mg/kg ($SD = 0.98$). The prevalence of cigarette, khat and alcohol use was 13.4%, 18.5% and 16.8%, respectively. Notably, a lower percentage of patients with extrapulmonary TB reported cigarette, khat and alcohol use compared to those with pulmonary TB. Furthermore, patients with extrapulmonary TB showed higher rifampicin C_{max} ($p = 0.07$) and AUC_{0-7} ($p = 0.23$) values but the differences were not statistically significant. The sociodemographic characteristics of the participants are presented in Table 1.

Genotype and allele frequency

Study participants were genotyped for *SLCO1B1c.388A > G*, *SLCO1B1 c.521T > C*, *ABCB1 c.3435C > T*, *ABCB1 c.4036A > G*, *AADAC c.841G > A* and *CES-2 c.269-965A > G*. The observed genotype and allele frequency distributions among patients are shown in Table 2. There were no significant differences between observed and expected genotype frequencies according to Hardy–Weinberg equilibrium. The variant allele *SLCO1B1c.388A > G* was frequent (62.2%), while the defective variant allele *SLCO1B1c.521T > C* (*5) was less frequent (20.2%). The minor variant allele frequency for *ABCB1 c.3435T* and *ABCB1 c.4036G* were 24, 4%, and 14.6%, respectively. The variant *AADAC c.841A* variant allele had a much higher frequency (86.1%), whereas the CES-2 c.269-965G allele occurred in 30.6%.

Rifampicin pharmacokinetics

There was high between-patient variability in rifampicin C_{max} (range 1.90–18.57 μg/mL) and AUC_{0-7} (range 3.61–47.1 μg × h/mL). The median rifampicin C_{max} was 6.76 μg/mL (IQR 5.33–8.49). Only 30.3% ($n = 36$) of participants achieved the target plasma concentration (> 8 μg/mL) for optimal therapeutic efficacy [31]. C_{max} <4 μg/mL, which is associated with risk for treatment failure, was observed in 5 (4.2%) patients. The median $AUC_{0-7\,h}$ was 17.1 μg × h/mL (IQR 13.9–22.3).

Effect of genotype on rifampicin pharmacokinetics

A comparison of the median and geometric mean of rifampicin C_{max} and $AUC_{0-7\,h}$ between the different genotypes using one-way analysis of variance is presented in Table 3. Although no significant influence of *SLCO1B1*1B* and *SLCO1B1*5* genotype on variation in rifampicin C_{max} and $AUC_{0-7\,h}$ was found, patients homozygous for *SLCO1B*5/*5* (*C/C*) had a C_{max} below the target concentration. No significant difference in C_{max} and AUC_{0-7} was observed in *ABCB1 c.3435C > T* and CES 2 c.269-965A > G genotype groups.

Significant variability in rifampicin C_{max} ($p = 0.018$) and $AUC_{0-7\,h}$ (0.02) between the *ABCB1 c.4036A > G* genotype groups was observed. The geometric mean of C_{max} and $AUC_{0-7\,h}$ was significantly higher among patients homozygous for the variant allele *ABCB1c.4036G/G* than heterozygous A/G or homozygous wild type (A/A)(Table 3). A further post hoc analysis using Bonferroni correction indicated significant differences in C_{max} ($p = 0.036$) and $AUC_{0-7\,h}$ ($p = 0.023$) between homozygous *ABCB1 c.4036 A/A* and

Table 1. Sociodemographic and clinical characteristics of 119 Ethiopian tuberculosis patients.

Variables		Pulmonary TB ($n = 78$)	Extrapulmonary TB ($n = 41$)	All patients ($n = 119$)
Sex (*n*)	Male	45	17	62 (52.5%)
	Female	33	24	57 (47.5%)
Smoking (*n*)	Yes	15	1	16 (13.4%)
	No	63	40	103 (86.6%)
Khat chewer (*n*)	Yes	20	2	22 (18.5%)
	No	58	39	97 (81.5%)
Alcohol (*n*)	Yes	17	3	20 (16.8%)
	No	61	38	99 (83.2%)
Age (years), median (IQR)		26 (21–35)	28 (24.5–36)	28 (22–35)
Median body weight in kg (IQR)		53 (45–60)	58 (52.5–68.5)	54.75 (48–61.75)
Drug dose (mg/kg, *SD*)		9.46 (0.99)	9.26 (0.98)	9.39 (0.98)
Median C_{max}, µg/mL (IQR)		6.45 (5.13–8.54)	7.46 (6.02–8.72)	6.75 (5.39–8.58)
Median AUC_{0-7} µg.h/mL (IQR)		16.52 (13.81–21.98)	17.55 (14.3–22.59)	17.05 (13.87–22.26)

$AUC_{0-7\,h}$: area under the time-concentration curve; C_{max}: maximum concentration; *n*: number; IQR: interquartile range; *SD*: standard deviation.

Table 2. Genotype and variant allele frequency of *SLCO1B1*, *ABCB1*, *AADAC* and *CES-2*.

Variant allele	Protein	Genotype frequency (*n*, %)			Allele frequency (%)		χ^2	*p* value
*SLCO1B1*1B (c.388A > G)*	Asn130Asp	*A/A* (15, 12.6)	*A/G* (60, 50.4)	*G/G* (37, 44)	*A* (37.7)	*G* (62.2)	0.618	0.43
*SLCO1B1*5 (c.521T > C)*	Val174Ala	*T/T* (76, 63.9)	*T/C* (38, 31.9)	*C/C* (5, 4.2)	*T* (79.8)	*C* (20.2)	0.008	0.99
ABCB1 c.3435C > T	Ile1145Ile	*C/C* (67, 56.3)	*C/T* (46, 38.7)	*T/T* (6, 5.0)	*C* (75.6)	*T* (24.4)	0.28	0.59
ABCB1c.4036A > G	Located in 3′-UTR	*A/A* (88, 73.8)	A/G (28, 23.5)	*G/G* (3, 2.5)	*A* (85.4)	*G* (14.6)	0.183	0.67
*AADAC*2 (c.841G > A)*	Val281Ile	*G/G* (3, 2.5)	*G/A* (26, 21.7)	*A/A* (90, 75)	*G* (13.9)	*A* (86.1)	0.447	0.5
CES-2 c.269-965A > G	Located in intron 1	*A/A* (55, 46.2)	*A/G* (55, 46.2)	*G/G* (9, 7.6)	*A* (69.4)	*G* (30.6)	0.896	0.34

n: number; UTR: untranslated region. The chi-square test and *p* value show correlations between the observed and expected genotype frequencies according to the Hardy–Weinberg equilibrium.

Table 3. Effects of *SLCO1B1*, *ABCB1*, *AADAC* and *CES-2* genotype on rifampicin C_{max} and $AUC_{0-7\,h}$ in Ethiopian TB patients ($n = 119$).

			C_{max} (µg/mL)			AUC_{0-7} (µg h/mL)		
Genotype		*N*	Median (IQR)	Geometric mean ± *SE*	*p* Value*	Median (IQR)	Geometric mean ± *SE*	*p* value*
SLCO1B1*1B (c.388A > G)	A/A	15	6.88 (5.83–9.36)	7.08 ± 1.1	0.87	17.95 (16.59–20.93)	17.78 ± 1.12	0.67
	A/G	60	6.62 (6.23–7.75)	6.76 ± 1.05		16.35 (14.36–18.22)	16.6 ± 1.05	
	G/G	44	6.82 (6.1–7.4)	6.92 ± 1.05		17.14 (15.35–18.78)	17.78 ± 1.07	
SLCO1B1*5 (c.521T > C)	T/T	76	6.59 (6.18–7.02)	6.76 ± .05	0.15	16.65 (15.08–18.12)	16.98 ± 1.05	0.18
	T/C	38	7.62 (6.76–8.12)	7.24 ± 1.07		18.49 (17.03–22.4)	18.2 ± 1.07	
	C/C	5	5.1 (4.76–7.41)	5.37 ± 1.1		12.5 (12.44–19.61)	13.18 ± 1.12	
ABCB1 c.3435C > T	C/C	67	6.42 (6.1–7.2)	6.61 ± 1.05	0.70	16.7 (15.08–18.22)	16.98 ± 1.05	0.87
	C/T	46	7.23 (6.63–7.95)	6.92 ± 1.07		17.85 (16.48–19.73)	17.78 ± 1.07	
	T/T	6	6.76 (6.1–10.43)	7.24 ± 1.15		14.68 (13.4–23.99)	16.6 ± 1.12	
ABCB1 c.4036A > G	A/A	88	6.53 (6.10–7.18)	6.61 ± 1.05	0.018	16.7 (15.08–17.77)	16.6 ± 1.05	0.02
	A/G	28	7.29 (6.59–8.85)	7.41 ± 1.07		18.31 (15.5–23.09)	18.2 ± 1.07	
	G/G	3	9.35 (7.07–18.57)	10.72 ± 1.32		32.13 (17.3–47.05)	29.51 ± 1.35	
AADAC2 c.841G > A	A/A	90	7.045 (6.49–7.82)	7.08 ± 1.05	0.047	17.56 (16.48–18.58)	17.78 ± 1.05	0.16
	G/A	26	6.21 (4.85–6.79)	6.03 ± 1.07		15.06 (14.11–20.29)	15.49 ± 1.07	
	G/G	3	4.69 (4.27–6.63)	5.13 ± 1.15		13.99 (9.92–16.59)	13.18 ± 1.17	
CES-2 c.269-965A > G	A/A	55	7.18 (6.59–8.04)	7.08 ± 1.05	0.08	17.85 (15.5–19.06)	17.38 ± 1.05	0.19
	A/G	55	6.42 (6.10–7.2)	6.31 ± 1.05		16.58 (14.82–17.3)	16.22 ± 1.05	
	G/G	9	6.87 (6.18–13.94)	8.13 ± 1.15		18.32 (15.08–29.74)	21.38 ± 1.15	

$AUC_{0-7\,h}$: area under the time-concentration curve; C_{max}: maximum concentration; *n*: number; IQR: interquartile range; *SE*: standard error; GM: geometric mean; TB: tuberculosis. *p value from analysis of variances using $\log_{10}$ transformed C_{max} and $AUC_{0-7\,h}$ data.

homozygous wild-type (*G/G*) groups. The comparison of C_{max} and $AUC_{0-7\,h}$ between the different *ABCB1 c.4036A > G* genotype groups is presented in Figure 1(A). No significant difference in C_{max} and AUC_{0-7} was observed in the different *ABCB1 c.3435C > T* genotype groups.

Furthermore, a significant association of *AADAC c.841G > A* genotype with rifampicin C_{max} ($p = 0.047$) and a similar trend for AUC_{0-7} ($p = 0.16$) was observed and was lower in the wild type (*G/G*) genotype than heterozygous (*A/G*) or homozygous for *A* variant allele (*A/A*) (Figure 1(B)). However, a post hoc test showed no significant variation for AUC_{0-7} among the pairs of all three genotypes of *AADAC c.841G > A*. There was no significant association of *CES 2 c.269-965A > G* genotype with rifampicin C_{max} and $AUC_{0-7\,h}$.

Predictors of rifampicin pharmacokinetics

A univariate followed by a multivariate analysis was conducted to identify predictors of C_{max} and $AUC_{0-7\,h}$ using log10 transformed concertation data. Table 4 shows the results of univariate and multivariate analyses of

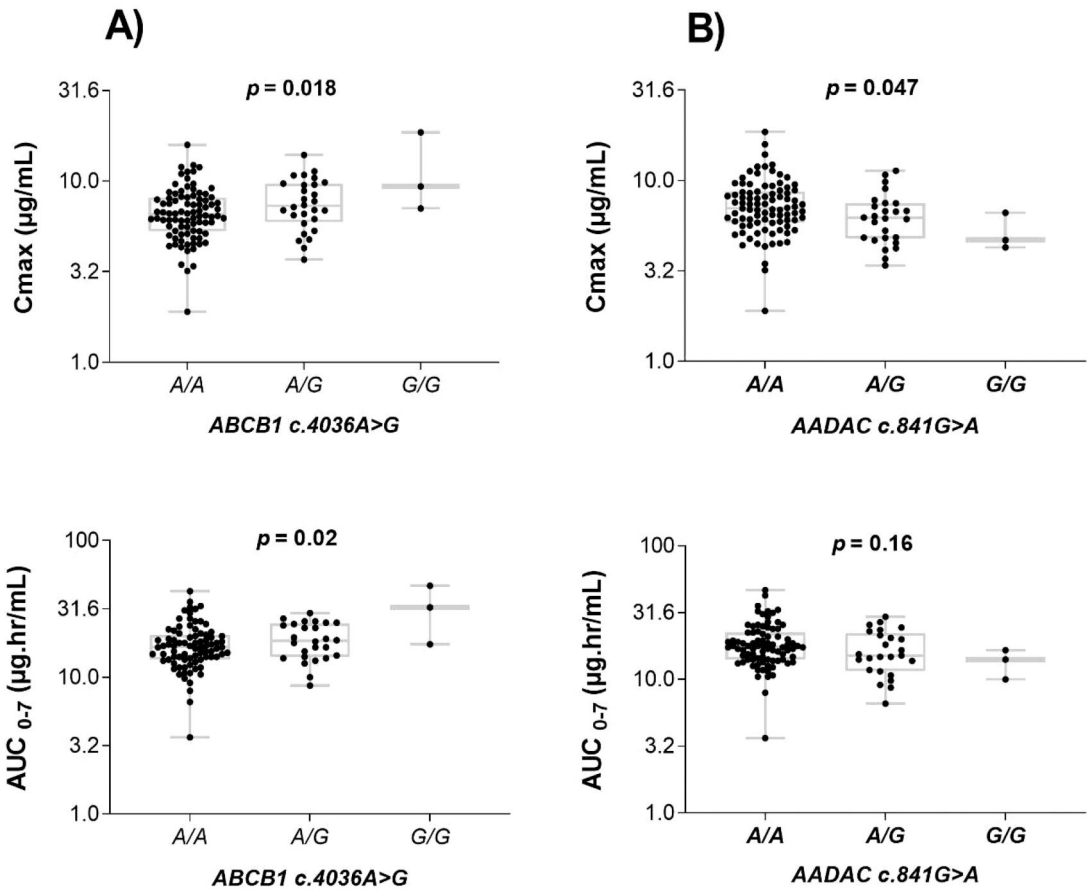

Figure 1. Comparison of rifampicin C_{max} and $AUC_{0-7\,h}$ in the *ABCB1 c.4036A > G* (right) and *AADAC2 c.841G > A* (left) genotypes. The box plots show the median ± interquartile range, whereas whiskers denote the minimum and maximum values.

associations between variables and rifampicin C_{max} and $AUC_{0-7\,h}$. In univariate analysis, *ABCB1 c.4036A > G*, *AADAC c.841G > A* genotypes and rifampicin dose were significant predictors of rifampicin C_{max} ($p \leq 0.05$), and a nearly significant effect was observed for sex ($p = 0.06$). All variables with p value <0.2 were further tested in the multivariate regression model. In multivariate analysis, sex, rifampicin dose, *ABCB1 c.4036A > G* and *AADAC c.841G > A* genotypes remained independent predictors of rifampicin C_{max}.

ABCB1 c.4036A > G and drug dose were significant predictors for rifampicin $AUC_{0-7\,h}$ in both univariate and multivariate analysis. In multivariate analysis, sex was also a predictor of rifampicin $AUC_{0-7\,h}$. Overall, females had higher exposure to rifampicin compared to males. Age, alcohol, cigarette and khat use, *SLCO1B1c.388A > G*, *SLCO1B1c.521T > C*, *ABCB1 c.3435C > T*, *CES-2 c.269-965A > G* genotypes and days on drug therapy did not predict rifampicin exposure (C_{max} and $AUC_{0-7\,h}$).

The stepwise multivariate regression analysis demonstrated that *ABCB1 c.4036A > G* genotypes independently accounted for 5.8% of the variability in rifampicin C_{max}. Combining *AADAC c.841G > A* and *ABCB1 c.4036A > G* genotypes increased the explained variability to 10.8%. Additionally, 14% variability in rifampicin C_{max} was observed when the drug dose (mg) was added to the two genotypes. The overall variability in rifampicin C_{max} explained by the two genotypes, drug dose and sex was 17.2%. Similarly, *ABCB1 c.4036A > G* genotypes explained 6.1% of the variability in rifampicin AUC_{0-7} explained by. With the sequential addition of sex, drug dose and *AADAC c.841G > A* to the model, the variability in rifampicin AUC_{0-7} increased to 10.1%, 15.8%, and 19.3%, respectively. These findings underscore the significant roles of *AADAC c.841G > A* and *ABCB1 c.4036A > G* genotype, along with sex and drug dose in predicting rifampicin C_{max} and AUC_{0-7} among the variables examined.

Discussion

The study is the first to examine the relationship between genetic polymorphism and rifampicin pharmacokinetics in the Ethiopian population. We investigated the between-patient variability of rifampicin pharmacokinetics parameters (C_{max} and $AUC_{0-7\,h}$) in Ethiopian adults commencing TB treatment and the role of

pharmacogenetic variations in drug transporter proteins (*SLCO1B1* and *ABCB1*) and metabolising enzymes relevant for rifampicin disposition (*AADAC2* and *CES2*). There were several notable findings. First, there was substantial between-patient variability in rifampicin plasma concentrations. Second, a majority (70%) of patients had rifampicin plasma concentrations below the recommended target (≥8 μg/mL). Third, rifampicin dose, *ABCB1c.4036A > G* and *AADACc.841G > A* genotypes and to some extent, sex were independent predictors of rifampicin C_{max} and $AUC_{0-7\,h}$.

Two weeks after treatment initiation, a 2-h post-dose plasma sample is recommended for therapeutic drug monitoring to predict TB treatment outcomes. Rifampicin C_{max} should exceed 8 mg/L for optimal therapeutic efficacy [32–34]. This peak concentration was not attained in about 70% of our patients who received the standard rifampicin dose. Our finding is in line with previous studies reporting that many patients receiving first-line anti-TB therapy do not achieve the rifampicin C_{max} target concentration, but the proportion varies between populations [16,34–36]. To the best of our knowledge, the proportion of TB patients below the target 8 mg/mL in this study is one of the highest. This finding is of concern since subtherapeutic levels are associated with unfavorable outcomes and risk for development of drug resistance [9,37]. Indeed, drug-resistant TB is an increasing concern in Ethiopia [5,38,39]. A higher dose of rifampicin or therapeutic drug monitoring in selected patients could be beneficial as suggested previously [9,40]. Whether high doses of rifampicin are safe and more effective than the standard dose is studied in clinical trials to shorten treatment duration and increase efficacy. The trial results indicated that a higher dose of rifampicin led to faster sputum sterilisation while maintaining a comparable level of toxicity to the standard dose [41–44]. Therefore, an increase in the dose of rifampicin in Ethiopian population may be warranted.

Several factors could contribute to the observed low rifampicin plasma concentrations in Ethiopian patients including genetic variations, malnutrition and HIV infection, which are quite prevalent in East Africa including Ethiopia [1,45,46]. However, compared to the 70% observed in this study, only 35% of Tanzanian TB patients had a rifampicin C_{max} below 8mg/L [47]. The low rifampicin concentrations in Ethiopian TB patients could be due to either higher rifampicin metabolising enzyme activities or increased autoinduction due to pharmacogenetic variations [23,25,26,48,49]. Lower plasma drug concentrations have been reported in earlier studies of antiretrovirals due to higher drug-metabolising enzyme activity and unique pharmacogenetic variation in Ethiopians compared to other populations, including Tanzanians [23,25,26,50]. Our study highlights the existence of substantial differences in rifampicin pharmacokinetics between populations in sub-Saharan Africa and findings from one population may not be directly extrapolated to others on the continent. Recently we reported high plasma isoniazid concentrations and a high prevalence of slow N-acetyltransferase 2 (NAT2) acetylators in Ethiopian TB patients [51].

There have been inconsistent results about the effects of *SLCO1B1* genetic variation on rifampicin exposure. Previous studies in South African and Ugandan patients reported an association of the *SLCO1B1* genotype with variability in rifampicin pharmacokinetics [21,22,52]. However, this finding was not replicated in many studies [11,15,53,54]. Likewise, we found no significant impact of *SLCO1B1 c.388A > G* and *SLCO1B1 c.521T > C* on rifampicin C_{max} and $AUC_{0-7\,h}$. *SLCO1B1*1B* and *SLCO1B1*5* are missense mutations, involving the change of asparagine to aspartic acid at position130 and valine to alanine at position 174, respectively (Table 2). The variant alleles *SLCO1B1*1B* and *SLCO1B1*5* were associated with increased and decreased transporter activity of OATP1B1, respectively. *SLCO1B1*1B,* which is associated with higher transporter activity, occurs at a higher frequency (62.2%) in Ethiopians and Tanzanians (86.8%) than in Europeans (34.2%) [23]. On the other hand, the defective *SLCO1B1c.521T > C* variant allele caused reduced enzyme activity occurs at a lower frequency among Ethiopians (2.8%) than Tanzanians (4.7%) or Europeans (8%) [23,25,26].

Rifampicin is a substrate and inducer of P-gp which is a product of the *ABCB1* gene [40,55]. Few studies have evaluated the effect of *ABCB1* gene polymorphism on rifampicin pharmacokinetics. Huerta-García et al. reported that patients with *CC* or *CT* genotypes of *ABCB1* (*c.3435C > T*) had lower C_{max} and AUC_{24} than those with a *TT* genotype [56]. The *TT* homozygous genotype had significantly lower P-gp expression in the small intestine and showed the highest plasma concentrations of some drugs after oral administration [24]. However, we found no significant variation in rifampicin C_{max} and $AUC_{0-7\,h}$ for *ABCB1 c.3435C > T*. The *ABCB1c.4036A > G* genotype, which is in linkage disequilibrium with *c.3435C > T,* significantly influenced between-patient variability of rifampicin C_{max} and $AUC_{0-7\,h}$. Rifampicin $AUC_{0-7\,h}$ was significantly higher in homozygous variant genotype (*GG*) carriers compared to

Table 4. Univariate and multivariate linear regression analysis of factors associated with rifampicin $\log_{10}C_{max}$ and $\log_{10}AUC_{0-7\,h}$ in Ethiopian adult tuberculosis patients.

	C_{max}				AUC			
	Univariate		Multivariate		Univariate		Multivariate	
Variable	Beta coefficients (95% CI)	*p* value	Adjusted beta coefficients (95% CI)	*p* value	Beta coefficients (95% CI)	*p* value	Adjusted beta coefficients (95% CI)	*p* value
Age	0.002 (−0.001 to 0.006)	0.12	0.002 (−0.048 to 0.004)	0.24	0.002 (−0.001 to 0.006)	0.19	0.002 (−0.002 to 0.005)	0.31
Sex (female vs. male)	−0.051 (−0.11 to 0.003)	0.06	−0.056 (−0.11 to 0.004)	0.03	−0.057 (−0.12 to 0.04)	0.07	−0.063 (−0.12 to 0.03)	0.04
Drug dose (mg)	0.00 (0.00 to 0.001)	0.05	0.000 (0.00 to 0.001)	0.03	0.000 (0.00 to 0.01)	0.05	0.000 (0.00 to 0.01)	0.03
Alcohol use (no vs. yes)	0.00 (−0.066 to 0.8)	0.84			0.002 (−0.08 to 0.083)	0.97		
Khat chewing (no vs. yes)	0.00 (−0.074 to 0.067)	0.91			0.013 (−0.066 to 0.093)	0.74		
Smoking (no vs. yes)	−0.012 (−0.093 to 0.067)	0.75			0.016 (−0.076 to 0.11)	0.73		
Days on drug therapy	−0.001 (−0.004 to 0.0012)	0.43			0.001 (−0.005 to 0.003)	0.59		
SLCO1B1c.388A > G	−001 (−0.043 to 0.04)	0.96			0.007 (−0.04 to 0.054)	0.76		
SLCO1B1c.521T > C	0.002 (−0.05 to 0.046)	0.94			0.002 (−0.052 to 0.056)	0.95		
ABCB1 c.3435C > T	0.02 (−0.026 to 0.066)	0.4			0.006 (−0.046 to 0.059)	0.81		
ABCB1 c.4036A > G	0.071 (0.018 to 0.124)	0.009	0.063 (0.013 to 0.114)	0.015	0.071 (0.011 to 0.13)	0.02	0.059 (0.001 to 0.13)	0.048
*AADAC*2c.841G > A*	−0.068 (−0.122 to −0.014)	0.01	−0.065 (−0.12 to −0.013)	0.015	−0.059 (−0.12 to 0.001)	0.06	−0.059 (−0.12 to 0.001)	0.053
CES-2 c.269-965A > G	−0.004 (−0.048 to0.04)	0.86			0.008 (−0.043 to0.058)	0.76		

$AUC_{0-7\,h}$: area under the time-concentration curve; C_{max}: maximum concentration; CI: confidence interval.

the homozygous wild-type A/A (Figure 1). Nevertheless, the homozygous variant genotype (*GG*) occurs at a low frequency in our study population, consistent with findings from a previous report [49].

Few studies have investigated the impact of *AADAC* and *CES* genetic polymorphism on rifampicin pharmacokinetics. The association of *CES-2 c.-2263A > G* (rs3759994) in the promotor region and closely linked to *c.269-965A > G* (rs4783745) and c.1612 + *136 G > A* with increased rifampicin exposure is reported [13]. Patients who carry the *CES2* (rs8192925) *G* versus *A* allele had a 17.2% increase in rifapentine AUC_{0-24} (14). In our study, there was no significant association of *CES2 c.269-965A > G* genotypes with rifampicin C_{max} and $AUC_{0-7\,h}$. Likewise, no significant effect of *CES-2* on rifampicin exposure variability was observed in Ghanaian children [16]. *AADAC* and *CES-1* genotypes were not associated with rifampicin pharmacokinetics in Malawian TB patients [15].

We found a significant association between *AADAC c.841G > A* genotype and rifampicin C_{max}, which was significantly higher in carriers of the mutant variant allele (A/A, G/A) than in those with wild-type G/G genotype (Figure 1). Our result is consistent with previous reports [3,14]. Francis et al. reported that patients with *A/A* genotype had a lower rifapentine clearance. Similarly, a previous study found an association of *AADAC c.841G* variant allele with low rifapentine AUC, particularly in black patients [14]. However, this finding was not observed in Malawian adult TB patients [15]. The low frequency of a wild-type (*GG*) genotype in Malawians may have contributed to the differing results. Indeed, the frequency distribution of *AADAC*2* (c.841G > A) exhibits considerable variability across races and populations. Notably, the reported allele frequencies of *AADAC*2* among European American, African American, Japanese and Korean populations were around 60%, contrasting with the 99.9% prevalence in Peruvian TB patients [57] where the wild-type variant is almost missing. Our study among Ethiopian TB patients reveals *AADAC*2* allele frequencies of 86%, and the wild-type *G* variant was less prevalent with only three individuals exhibiting homozygosity for *G/G* genotype. This underscores the need for further investigation in populations where the *AADAC c.841G* variant occurs at higher frequencies to replicate and confirm our findings.

In addition to genetic polymorphism, other predictors such as age, sex, duration of therapy with rifampicin, drug dose and substance use were tested in univariate followed by multivariate analyses. Sex and drug dose

were significantly associated with rifampicin C_{max} and $AUC_{0-7\,h}$ in multivariate analysis. Females had higher rifampicin exposure (higher C_{max} and $AUC_{0-7\,h}$) than males. This is consistent with previous studies where male sex was associated with lower rifampicin exposure [35,36,52,58].

Our study presents the first insight into the extent of variability in rifampicin exposure (C_{max} and AUC_{0-7}) and the impact of genetic variation in drug transporters and metabolising enzymes in Ethiopian TB patients. However, it is imperative to acknowledge certain limitations in our study. The estimation of rifampicin pharmacokinetics in our study relied on three sampling time points within 7 h post-dose, adhering to the recommended approach for therapeutic drug monitoring [31]. A 2-h post-dose sample approximates the C_{max} for most TB drugs and adding a 6-h sample allows the clinician to distinguish between delayed absorption and malabsorption [31,32,34]. Nevertheless, although the spare sampling strategy is useful for capturing the $AUC_{0-24\,h}$ [59], the three time point concentration dataset in our study may not entirely capture the AUC accurately. Nevertheless, it is crucial to underscore that obtaining multiple blood samples solely for the study's objectives from newly diagnosed TB-infected patients undergoing an intensive phase of treatment is impractical and raises ethical concerns.

Furthermore, in our study population, the occurrence of the wild-type *AADAC c.841 G/G* and the variant *ABCB1 c.4036 G/G* genotype occurred at a lower frequency, potentially influencing the association of rifampicin C_{max} and $AUC_{0-7\,h}$ with the investigated genotypes. It is noteworthy that globally, and particularly within Africa, G variant alleles exhibit lower frequencies for both *AADAC c.841G > A* and *ABCB1 c.4036A > G*. The frequency of *ABCB1 c.4036A > G* varies among black Africans, ranging from 29% in Tanzanians [60] to 18% in Ethiopians [28]. Considering these variations, future large-sample studies across diverse populations in high TB-burden areas, including Africa, where rifampicin is a cornerstone of TB therapy, are recommended to validate and replicate our findings.

In conclusion, we report low rifampicin exposure and high variability in rifampicin C_{max} and AUC_{0-7} in about two-thirds of Ethiopian TB patients. Rifampicin exposure varied with sex, dose, *ABCB1 c.4036A > G* and *ADAC c.841G > A* genotypes. *AADAC c.841GG* and *ABCB1 c.4036A > GAA* genotype groups and male patients had a higher risk of low rifampicin plasma exposure than females. *SLCO1B1 c.388A>*, *SLCO1B1 c.521T > C*, *ABCB1 c.3435C > T* and *CES2 c.269-965A > G* genotypes did not affect rifampicin exposure. The impact of low rifampicin exposure on treatment outcomes needs further investigation in Ethiopian TB patients. Our findings may have important clinical implications and warrant studies on whether high-dose rifampicin improves therapeutic efficacy.

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Disclosure statement

The authors declare that there is no conflict of interest.

Transparency declarations

None to declare.

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Population Pharmacokinetics of Rifampicin in Ethiopian Adults undergoing treatment of Tuberculosis

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Abstract

Background: Rifampicin is a key component of first-line drugs in tuberculosis (TB) chemotherapy. However, it exhibits large pharmacokinetic (PK) variability. We have developed a population PK model to evaluate the role of sociodemographic and genetic polymorphism on rifampicin pharmacokinetic variability.

Methods: Newly diagnosed TB patients (n=145) who received first-line anti-TB drugs were selected from primary healthcare facilities in Addis Ababa, Ethiopia. Serial blood samples were obtained at three time points post-dose. Genotypes for two *SLCO1B1*, two *ABCB1*, *AADACc.841G>A*, and *CES-2 c.269-965A>G* were determined. Rifampicin plasma concentration was quantified using LC-MS/MS. Data was analysed using established methods for Population PK (POPK) modeling in the NONMEM software.

Results: The dataset contained a total of 427 PK samples collected between day 10 to 54 and between 1 to 7 hours postdose. The observed RIF PK data was described by a two-compartment model with well-stirred hepatic clearance model, saturable clearance, hepatic autoinduction and transit absorption kinetics. The estimated parameters of the final POPPK model were absorption rate constant (1.863/h), number of transit compartments (5.786), central volume of distribution (5.823 L), peripheral volume of distribution (26.2 L), and intrinsic hepatic clearance (46.44 L/h). Genetic polymorphisms in *ABCB1*, namely *c.4036A>G* and *3435C>T* were identified as covariates on clearance and absorption rate constant, respectively. Subjects with *ABCB1 c.4036A>G GG* genotype are estimated to have 41% lower intrinsic clearance compared to subjects with *AA* or *AG* genotypes and subjects with *ABCB1 3435C>T TT* genotype were estimated to have 100% higher absorption rate constant than those with *CC* or *CT* genotypes.

Conclusion: The final model describe pharmacokinetics of rifampicin in the studied subjects. Genetic polymorphisms in *ABCB1, c.4036A>G* and *3435C>T* were identified as covariates partly accounting for inter-individual variability in clearance and absorption rate constant, respectively.

Keywords: rifampicin, tuberculosis, pharmacokinetic, Ethiopia

Introduction

Rifampicin, a semisynthetic derivative of rifamycin discovered in 1963, demonstrates potent bactericidal activity against many Gram-positive and Gram-negative bacteria [1]. Rifampicin today is a key component of first-line drugs in tuberculosis (TB) chemotherapy. The bactericidal action and sterilizing effect of rifampicin extend to *Mycobacterium tuberculosis* residing in both cellular and extracellular environments. Rifampicin kills bacteria by inhibiting of DNA-dependent RNA polymerase enzyme, thereby blocking the synthesis of mRNA and inhibiting protein synthesis [2].

The antimycobacterial activity of rifampicin is concentration-dependent and it correlates with AUC/MIC and C_{max}/MIC ratio [3]. The target Cmax of 8-24μg/mL was reached at approximately 1 to 3 h following oral administration of 10mg/kg. A few studies associated reduced rifampicin plasma level with relapse and increased the risk of drug resistance [4, 5].

Rifampicin is nearly completely absorbed following oral administration and exhibits widespread body distribution. The process of absorption, distribution, and excretion of a wide range of drugs are facilitated by plasma membrane-bound drug transporters, specifically solute carrier (SLC) transporters and the adenosine triphosphate (ATP)-binding cassette (ABC) transporters [6]. The absorption, distribution, and excretion of rifampicin are also mediated by these drug transporters [7, 8]. The metabolic transformation of rifampicin includes deacetylation, a process catalyzed by microsomal hepatic carboxylesterases (CES), and serine esterase aryl acetamide deacetylase (AADAC), to the formation of 25-deacetylrifampicin [9, 10]. Rifampicin also induces several enzymes which also induce its metabolism leading to saturation kinetics up on increasing the dose and treatment duration [11].

Several studies reported high inter-patient pharmacokinetic variability of rifampicin. Variations in the plasma concentration of rifampicin among the population have been attributed, in part, to sex [12, 13], age [14], human immunodeficiency virus (HIV) co-infection [15], and diabetes [16]. In addition, concomitant food [17, 18] and drug administration [19] have been recognized as contributing factors for rifampicin pharmacokinetics variation. Food impairs the absorption of rifampicin and reduces absolute bioavailability and maximum concentration [20].

Genetic polymorphism in genes encoding drug-metabolizing enzymes or drug transporters may also be responsible for between-subject variability in rifampicin pharmacokinetics. The impact of genetic diversity on the pharmacokinetics of rifampicin, however, varies greatly. Huerta-García *et al.*, for instance, found that the *TT* genotype of *ABCB1* rs1045642 predicts approximately 34.8% variability in rifampicin C_{max} and 48.5% variability in $AUC_{0–24}$ [21]. However, others did not find a similar influence of *ABCB1 rs1045642* on rifampicin pharmacokinetics [22, 23]. For the *SLCO1B1* gene, *rs4149032, rs2306283,* and *rs11045819* [21, 24] were linked to variation in rifampicin exposure. Among the rifampicin metabolizing enzyme, patients with the *G* allele of *CES2 (rs8192925)* have demonstrated a 17.2% increase in rifapentine AUC_{0-24} [25] while lower rifapentine clearance and decreased exposure to rifapentine observed for *AA* genotype [26] and *GG* genotype of *AADAC rs1803155* [25] respectively.

Several pharmacokinetic studies which proposed a rifampicin pharmacokinetic model have been reported. The results of these studies were recently summarized in a systematic review by Muda et al [16]. We also recently reported high variability in rifampicin C_{max} and AUC_{0-7} in Ethiopian adult tuberculosis patients [27]. Numerous potential covariates are suggested to be responsible for the variability of rifampicin pharmacokinetics parameters. However, there is an inconsistency in the types of covariates reported, resulting in our inability to describe the potential covariates in rifampicin pharmacokinetic variability among the population.

Ethiopia is listed among the top twenty countries with the highest TB and TB-HIV burden [28] and 2nd in terms of population size in Africa. Ethiopians exhibit significant genetic variation compared to other black African populations and European origins [29]. Furthermore, Ethiopian populations are relatively underexplored in the context of drug exposure, with limited population pharmacokinetic studies available. This study aims to characterize the population pharmacokinetics of rifampicin in Ethiopian adult TB patients. Furthermore, we assess the influence of sociodemographic, and genetic covariates on rifampicin pharmacokinetics parameters.

Methods

Study design and participants

The details of this study including, study setting, ethical considerations, drug treatment, genotyping, and quantification of plasma concentration of rifampicin have been previously reported [27]. Briefly, newly diagnosed and treatment naïve TB patients were selected from primary healthcare facilities in Addis Ababa, Ethiopia. Treatment adhered to Ethiopian treatment guidelines [30]. Genomic DNA was extracted from whole blood samples using QIAmp DNA Blood Midi Kit (QIAGEN GmbH, Hilden, Germany) following manufacturer's instructions. Genotyping was conducted using real-time Q-PCR (Applied Biosystems) equipped with 7500 software V2.3 (Life Technologies Corporation) for allelic discrimination. The determination of rifampicin plasma concentration was achieved using liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Population Pharmacokinetic Analysis

Population pharmacokinetic (POPK) modeling was done using NONMEM (Version 7.5.0, Icon Development Solutions, http://www.iconplc.com, Ellicott City, MD, USA) with PsN (version 5.2.6; https://uupharmacometrics.github.io/PsN/) and Pirana (version 2.9.9; https://www.certara.com/software/pirana-modeling-workbench/) as modeling and simulation workbench. Data preparation and NONMEM results post-processing and visualization were conducted using the R statistical software (version 4.2.0; www.r-project.org).

The POPPK model was developed by adapting a reference model from literature; following the recommended best practices for using the PRIOR subroutine in NONMEM [31]. The maximum likelihood estimation method (FOCE-I in NONMEM) was used for parameter estimation. Structural model parameters for individuals (P_i) were derived from equation $P_i=P_{TV}\times e^{\eta}$, where P_{TV} represents the typical population parameter value and η (ETA) is randomly sampled from a normal distribution with a mean of 0, and variance of ω^2, i.e., $\eta_i=N(0,\omega^2)$.

Proportional (prop), additive (add), or combined (add+prop) residual error models were tested to account for within-subject variability and any experimental errors. Models were evaluated using log-likelihood ratio test, goodness of fit (GOF) plots, parameter uncertainty, and visual predictive checks. The difference in OFV > 3.84 units (equivalent to p-value $\leq = 0.05$ for χ^2 distribution) between nested models was considered to be a statistically significant improvement

in model fit. Uncertainties of model parameters were measured by relative standard errors (RSE) or bootstrap confidence intervals for the final model.

Inter-individual variability (IIV) of model parameters, as measured by the coefficient of variation (CV %), was calculated from ω^2 using the formula: $CV\% = 100 \times \sqrt{\left(e^{(\omega^2)} - 1\right)}$. Potential covariates were identified by visual inspection of covariates versus η plots. However, only biologically plausible covariates were added to the model by stepwise forward inclusion and backward elimination. First, all plausible parameter-covariate relations were separately included in the model. Then, all relations resulting in ≥ 3.84 decrease in OFV (i.e., p-value ≤ 0.05) were jointly included in the model. At this step, some covariate coefficients shrunk toward zero. In the next iterations of the model, covariates with the greatest decrease in coefficient values or with relative standard error (RSE) > 50% were eliminated in a step-wise manner. The tested covariates are presented in the table of subject characteristics.

The final model was qualified by goodness of fit (GOF) plots, visual predictive check (PcVPC), and non-parametric confidence intervals (CI) obtained by fitting the final model to bootstraped samples of the current dataset (Bootstrap confidence intervals).

Results

Participants demographic characteristics

A total of 145 newly diagnosed TB patients participated in the study with a mean age of 31.26 years and a mean body weight of 55.87kg. The majority of the patients did not have co-morbid conditions; however, 11 patients (7.6%) had diabetes and 10 (6.8%) patients had HIV comorbidity. Of the total 145 patients, 101 patients (69.7%) had pulmonary TB, while 44 (30.3%) had extrapulmonary TB. Among the participants, males account for a higher proportion (55.2%) compared to females (44.8%). Cigarette smoke, khat use, and alcohol use were reported by 14.38%, 19.17%, and 19.17% respectively.

The mean dose of rifampicin received by participants was 9.41 mg/kg (SD, 0.97). The median C_{max} and $AUC_{0\text{-}7h}$ of rifampicin vary based on the dose of rifampicin received. Higher C_{max} and

$AUC_{0\text{-}7h}$ were observed in those who received 600mg. The genotype of study participants for whom genetic data is available, including *SLCO1B1c.388A>G, SLCO1B1 c.521T>C, ABCB1 c.3435C>T, ABCB1 c.4036A>G, AADAC c.841G>A*, and *CES-2 c.269-965A>G* are presented in **Table 1**.

Table 1 Socio-demographic, clinical, and genotype characteristics of 145 Ethiopian tuberculosis patients stratified by dose received.

Variables		**Dose (mg)**			**Total**
		300	450	600	
Sex (n, %)	Male	0	32	48	80(55.5)
	Female	5	34	26	65(44.5)
Smoking (n, %)	Yes	0	9	12	21 (14.38)
	No	5	57	62	124 (85.6)
Khat Chewer (n, %)	Yes	0	12	16	28 (19.17)
	No	5	54	58	117 (80.8)
Alcohol (n, %)	Yes	0	9	19	28 (19.18)
	No	5	57	55	117 (80.8)
Age (years), (mean, range)		31(20-600)	29(18-55)	33 (18-65)	31 (18-65)
Body weight in Kg, (mean, range)		36 (34-38.5)	48.2(40-54)	63.9(55-82)	55.9 (34-82)
TB-HIV (n, %)		0	5	5	10 (6.7)
TB-DM (n, %)		0	4	7	11(7.5)
Median rifampicin C_{max} µg/mL (IQR)		5.3 (4.9- 6)	6.5 (5.1-8.1)	7.34 (5.7-8.9)	6.79 (5.46-8.46)
Median AUC_{0-7h} mg. h/mL (IQR)		15.15 (13.14-17)	15.74 (13.67-20.4)	18.67 (14.66-24)	17.18 (13.85-22.46)
*SLCO1B1*1B (c.388A>G) (n, %)*	*AA*	2	3	10	15 (12.6)
	AG	1	34	25	60(50.4)
	GG	2	18	24	44 (37)
*SLCO1B1*5 (c.521T>C)*	*TT*	3	33	41	77 (63.9)

(n, %)	*TC*	0	21		37 (31.9)
	CC	2	1	2	5 (4.2)
ABCB1 c.3435C>T (n, %)	*CC*	4	30	33	67 (56.3)
	CT	0	21	25	46 (38.7)
	TT	1	3	1	5 (4.42)
ABCB1 c.4036A>G (n, %)	*AA*	4	43	41	88 (73.8)
	AG	1	12	15	28 (23.5)
	GG	0	0	3	3 (2.5)
AADAC c.841G>A (n, %)	*GG*	0	1	2	3 (2.5)
	GA	0	11	15	26 (21.7)
	AA	5	43	42	90 (75)
CES-2 c.269-965A>G (n, %)	*AA*	2	28	25	55 (46.2)
	AG	3	22	30	55 (46.2)
	GG	0	5	4	9 (7.6)
Total		5	66	74	145

Population pharmacokinetic model

The dataset for POPPK modeling consisted of 145 subjects with a total of 427 PK samples collected between days 10 to 54 and between 1 to 7 hours postdose. The number of subjects who underwent PK sample collection at 10 to 14-, 15 to 21-, 22 to 28-, and greater than 28 days following the initiation of tuberculosis treatment were 27, 70, 25, and 23, respectively. Most subjects had the samples taken at 1, 2, 4, and 6 hours after dose.

We evaluated 3 POPPK models of rifampicin from literature two of which reported 1-compartment disposition kinetics for rifampicin. The adequacy of the models to describe the observed PK in the current dataset was assessed using GOF plots, OFV, and AIC (Akaike information criterion) obtained with MAXEVAL = 0 in NONMEM, and NPDE (normalized prediction distribution error) statistics. We selected Svensson's 2019 model as the reference model for our modeling [32]. In addition to its relatively lower OFV/AIC compared to other

models, Svensson's model had better GOF, and good precision of parameter estimates (See **Table 2** and Error! Reference source not found.).

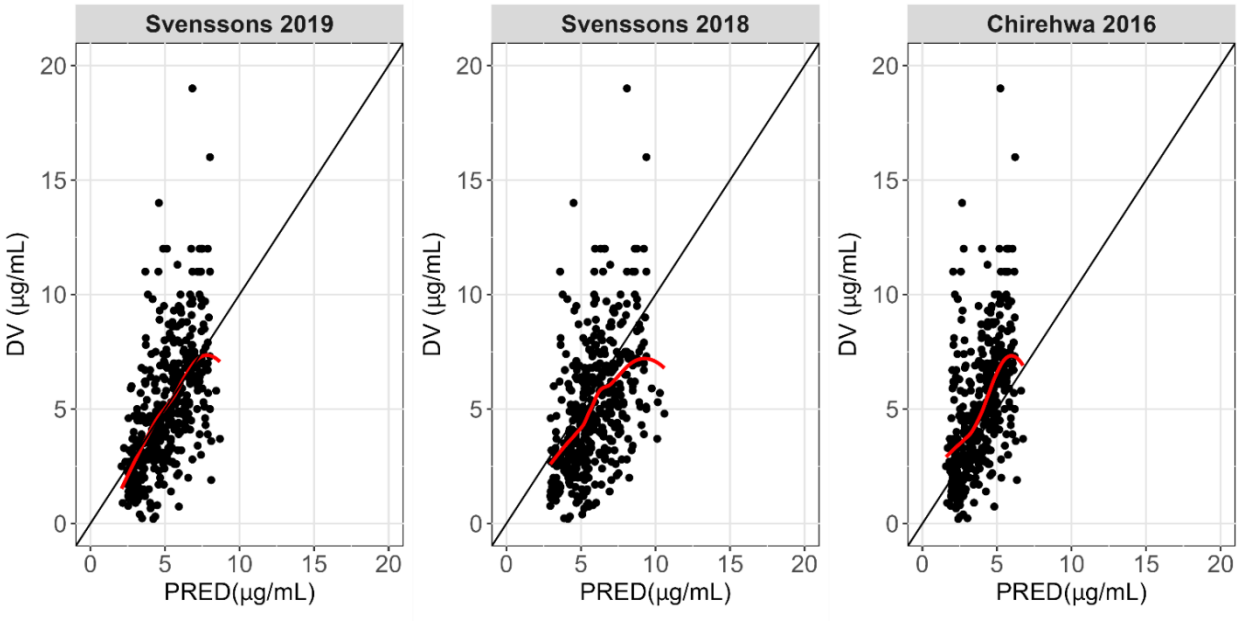

Figure 1: Goodness of fit plots for external validation of the population PK models of rifampicin published by Svenson in 2018 [33] and 2019 [32] and Chirehwa in 2016 [34]. Svensson's 2019 model appears to describe the data better than the other models since the red LOESS line is on top of the identity line for Svensson's 2019 model but deviates from the identity line for the other model.

Table 2 Comparisons of the goodness of fit statistics of the population PK models of rifampicin published by Svensson in 2018 and 2019 and Chirehwa in 2016. Svensson's 2019 model appears to have better goodness of fit statistics (lower OFV/AIC and Mean NPDE that is relatively closer to 0) compared to the other models.

Model	OFV	AIC	Mean	SE	Variance	Shapiro-Wilk normality Test	GlobalTest
Svensson et al., 2018	1,095	1,127	0.517	0.068	1.993	0.04	$< 1.0 \times 10^{-13}$
Svensson et al., 2019	858	894	0.191	0.044	0.827	0.00016	5.4×10^{-05}
Chirehwa et al., 2016	1,001	1,041	-0.299	0.054	1.229	0.00016	1.3×10^{-07}

OFV Objective functional value, AIC Akaike information criterion, SE standard error

Svensson's 2019 population PK model described 2-compartment disposition kinetics coupled with transit absorption kinetics. Clearance was modeled using a well-stirred model with saturable intrinsic clearance described by Michaelis Menten kinetics. The volume of distribution appeared to decrease after day 4 on treatment. The parameters of the model were oral bioavailability (F), saturable intrinsic clearance (CLi), Michaelis–Menten constant (Km), percent increase in CLi after day 4 (IND), central volume (Vc), percent decrease in Vc after day 4 (DTV), inter-compartment clearance (Q), peripheral volume (Vp), absorption rate constant (Ka), mean transit time (MTT), number of transit compartments (NN). Allometric scaling was applied to clearance (CL, Q) and volume (Vc, Vp) parameters with fixed allometric exponents (e.g.,$(CL)_i = (CL)_{pop} \times \left(\frac{WT}{70}\right)^{0.75}$, and $(Vc)_i = (Vc)_{pop} \times \left(\frac{WT}{70}\right)$)

Refitting the model with PK data from adult Ethiopian subjects (after supplying priors and weights of the priors) led to a decrease in OFV ($\Delta OFV = -113$). The weights of the priors were

calculated from the RSE reported in the reference model. The resulting model with priors converged successfully but the NONMEM covariance step was aborted because the covariance matrix was non-positive semi-definite indicating over parameterization of the model. To remove over-parameterization, 5 parameters including Q, Vp, IND, DTV, and F were fixed to prior values while the OMEGA degree of freedom was recalculated based on half of RSE from the reference model. The new model converged and the NONMEM covariance step was successful, therefore, it was used as a base model for further development.

The next step involved identifying parameters that could be stably estimated without supplying priors. This was achieved by comparing RSE from the base model with those reported in the reference model, followed by sensitivity analysis. Comparisons of RSE indicated a RSE ratio of ≤ 0.5 for CLi, V, Ka, and NN, therefore we conducted a sensitivity analysis for these parameters. The sensitivity analysis showed that despite a 50% change of the prior values, the estimates of CLi, V, and Ka changed by $\leq 10\%$ indicating that these parameters were less sensitive to their priors. Therefore, priors for these parameters were removed in the subsequent covariate model development. However, although the resulting model converged, the covariance step failed indicating that the model was still over-parameterized. Therefore IIV of Q and IOV of MTT were fixed to prior values. The resulting model was used for covariate model development. Except for η of CL and F1, η for other parameters indicated high shrinkage and correlation. Therefore, although covariates versus η plots indicated parameter-covariate relations, only biologically plausible relations were tested in the model as described in the methods section. Relations resulting in a significant decrease in OFV when added individually to the model were: Sex on CL ($\Delta OFV = -11$), HIV on CL ($\Delta OFV = -4$), ABCB1 rs3842 on CL ($\Delta OFV = -10$), AADAC on F ($\Delta OFV = -6$), and ABCB1 CT3434 on KA ($\Delta OFV = -8$). When all these covariates were jointly added, the model did not converge due to rounding errors and OFV decreased by 20 units. Stepwise backward elimination removed sex on CL and AADAC on F without a significant increase in OFV.

The final POPPK parameter estimates and bootstrap confidence intervals are presented in **Table 3**. The RSE is consistent with the narrow bootstrap confidence intervals and indicates that the parameters were estimated with good precision. Genetic polymorphisms in *ABCB1*, namely *rs3842A>G* and *3435C>T* were identified as covariates on CL and KA, respectively. According

to the final model, subjects with *ABCB1 rs3842 GG* genotype are estimated to have 41% lower CLi compared to subjects with *ABCB1 rs3842 AA* or AG genotypes. Similarly, subjects with a *3435C TT* genotype were estimated to have 100% higher KA than those with *ABCB1 3435C>T CC* or *CT* genotypes. Based on the VPC (Error! Reference source not found.), the developed model provides an adequate description of the observed data from the present study.

Table 3: Parameter estimates and model conditional number of the final population PK model

Parameters	Estimates (RSE)	95% Confidence Interval (Bootstrap)
Model conditional number	1.1	
KM (mg/L)	15.05(16%)	14.91 (10.26 - 17.33)
Mean transit time (h)	0.6704(7%)	0.68 (0.57 - 0.76)
Number of transit compartments	5.786(9%)	5.79 (4.82 - 6.77)
Intrinsic clearance (L/h)	46.44(5%)	46.7 (43.38 - 52.08)
Volume of distribution - central (L)	5.823(15%)	5.83 (4.38 - 7.53)
Absorption rate constant (/h)	1.863(11%)	1.84 (1.44 - 2.5)
Induction (%)	0.48 (Fixed to this value)	0.48
Difference in volume after day 4 (%)	0.19 (Fixed to this value)	0.19
Intercompartimental clearance (L/h)	93.1 (Fixed to this value)	93.1
Volume of distribution - peripheral (L)	26.2 (Fixed to this value)	26.2
Bioavailability (%)	0.776 (Fixed to this value)	0.78
Additive residual error	0.2493 (13%)	0.26 (0.15 - 0.3)
Proportional residual error	0.112 (8%)	0.1 (0.05 - 0.14)

Parameters	Estimates (RSE)	95% Confidence Interval (Bootstrap)
Proportional decrease in intrinsic clearance for the *ABCB1 rs3842 GG* genotype	-0.4114 (27%)	-0.43 (-0.69 - 0.08)
Proportional increase in the absorption rate constant for the *3435C>T TT* genotype	1.01 (42%)	1.06 (0.52 - 3.24)
Inter-individual variability for Vc (%CV)	1.34 (9%)	1.34 (1.16 - 1.5)
Inter-individual variability for KA (%CV)	0.74 (8%)	0.74 (0.67 - 0.81)
Inter-individual variability for CL (%CV)	0.25 (12%)	0.24 (0.18 - 0.3)
Inter-individual variability for F1 (%CV)	1.03 (10%)	1.01 (0.74 - 1.31)
Inter-individual variability for Q (%CV)	0.95 (Fixed to this value)	0.95
Inter-individual variability for MTT (%CV)	0.49 (Fixed to this value)	0.49

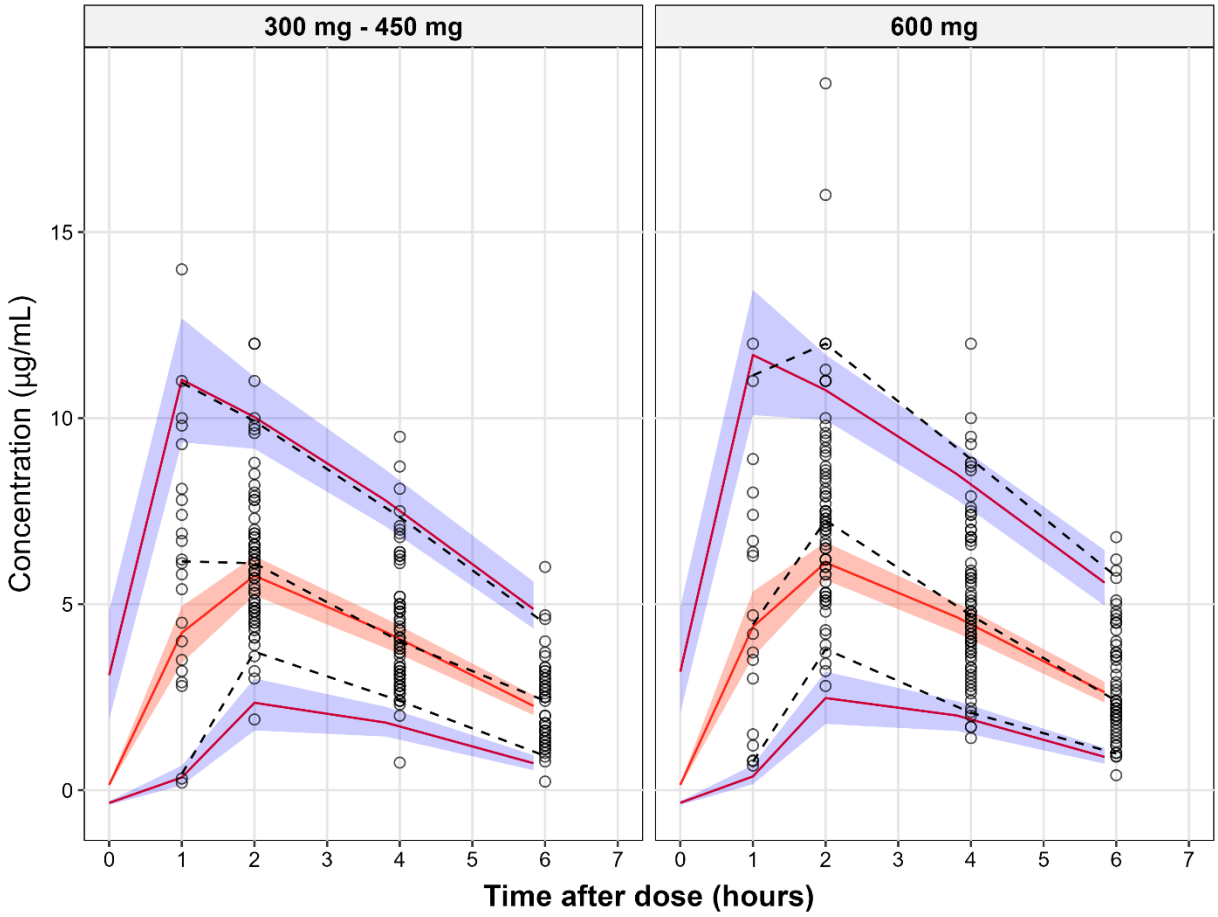

Figure 2: Visual Predictive Check of the final POPPK model. The dashed and solid lines represent the 5th, 50th, and 95th percentiles of the observed and simulated data respectively. The shaded areas represent a 95% confidence interval of the 5th, 50th, and 95th percentiles of the simulated data

Discussion

Rifampicin has been used for more than five decades based on body weight-adjusted dose. However, high inter-individual variability in rifampicin pharmacokinetics parameters is well recognized across different populations. We developed a population pharmacokinetics and analyzed covariates that could affect rifampicin pharmacokinetics in Ethiopian TB patients on treatment with a standard dose (10mg/kg) of first-line anti-TB drugs. A two-compartment model coupled with transit absorption adequately fitted the rifampicin plasma concentration data. Genetic polymorphisms in *ABCB1*, namely *c.4036A>G* and *3435C>T* were identified as covariates on CL and KA, respectively. Subjects with *ABCB1 rs3842 GG* genotype are estimated

to have 41% lower CL compared to subjects with *ABCB1 c.4036A>G AA* or *AG* genotypes. Similarly, subjects with an *ABCB1 (c.3435C>T) TT* genotype were estimated to have a 100% higher KA than those with *ABCB1 (3435C>T) CC* or *CT* genotypes.

The population pharmacokinetic model was developed to describe the time course of rifampicin exposure. Due to the sparseness of our pharmacokinetic data, the prior from the previous model [31] was used to stabilize the estimation of the plasma model's parameter estimates. In our final model, the datasets are best described by a two-compartment model coupled with transit absorption and saturable clearance. In contrast, several previous models described the population pharmacokinetics of rifampicin using a one-compartmental model [13, 33, 35, 36]. First-order [37, 38], lag time [39, 40], and transit compartment [35, 41-43] absorption models were reported in previous studies to describe the absorption phases of rifampicin. The transit absorption kinetics with 5.786 transit compartments and a mean transit time of 0.6704h best describe the present rifampicin pharmacokinetic model. Various levels of the number of transit compartments and mean transit time were indicated. For example, in the Willkins *et al* model, the mean transit time was 0.424 h and the number of transit compartments was 7.13, which is nearly similar to ours. A longer mean transit time of 1.5 h, 0.71 h, 1.31 h and a larger number of transit compartments 27.6, 19.3, 54.6 were reported by Jeremiah *et al*, [44], Chirehwa *et al* [34] *and* Denti *et al* [45] respectively.

The model predicted KA is 1.863/h with an inter-individual variability of 74%. Several previous studies reported a range of 0.236 to 2.15/h KA for rifampicin [16]. The inter-individual variability in the KA is lower in several previous reports [46, 47], but studies reporting a higher inter-individual variability in KA also exist [23, 44]. The rate and extent of drug absorption are affected by various factors. Rifampicin oral absorption is modified by co-administration with food [48], nutritional status of the patients [44], co-morbidities [47], and drug formation [13]. Recent reports also implicate genetic polymorphism as a factor for inter-individual variability for rifampicin pharmacokinetic parameters [49, 50]. The effect of food and drug formulation can be discounted from these factors in our study because a similar formation of the rifampicin was administrated on empty stomachs for all subjects. Genetic polymorphisms in *ABCB1 3435C>T* in part may explain the observed inter-individual variability in the KA where *TT* genotypes were estimated to have a 100% higher KA than those with *ABCB1 3435C>T CC* or *CT* genotypes.

Interestingly, the *TT* genotype is associated with 80–100% lower P-gp activity compared with wild type [51], and associated with higher exposure to statins and increased risk of toxicity from statins [52]. Similarly, for rifampicin patients with *CC* or *CT* genotypes showed lower values in C_{max}, and $AUC_{0-24\ h}$ compared to those with a *TT* genotype [21] indicating higher efflux capacity of these genotypes compared to *TT* genotype. .

The estimated CL of rifampicin was 46.44L/h with an inter-individual variability of 25%. A maximum of steady state intrinsic clearance of 176L/h was reported by Chirehwa et al. [34] and 34.8L/h intrinsic clearance was observed in South African children [53]. Intrinsic clearance was well described by Michaelis Menten kinetics with a K_m of 15.05mg/L. A previous model-based estimate of K_m of 3.35 mg/L [34], which is lower than our estimate, and an estimate of 35.3 mg/L, which is higher than ours were reported [33]. Numerous pharmacokinetics studies showed changes in the clearance of rifampicin over time. The autoinduction of rifampicin has been well-recognized from the early days of its introduction into clinical use. Autoinduction accelerates clearance and reduces rifampicin concentration after multiple doses of therapy. A 48% induction was observed in our model. Previous reports vary on when the induction starts and ends. Smythe *et al* described the need for 8 days and 40 days for the first half-life of induction and to full induction respectively [54]. However, Chirehwa *et al* indicated that 90% of the induction could be reached after 2 weeks of oral therapy, consistent with a half-life of 4.5 days. The results of our model incline towards Chirehwa *et al* because our study subjects received rifampicin daily in contrast to Smythe *et al* where the rifampicin dosing was intermittent.

Many drugs have been reported to show faster clearance in males compared to females because males have greater metabolic activity [55, 56]. The influence of sex on rifampicin clearance was also reported previously [13]. However, the covariates modeling did not show any influence of sex on clearance in our present work. Other population pharmacokinetic studies also did not report the influence of sex on rifampicin clearance [23]. Among the evaluated covariates in the final model, only *ABCB1 c.4036A>G* was associated with CL. Generally, subjects with *ABCB1 c.4036A>G GG* genotype are estimated to have 41% lower CL compared to subjects with *ABCB1 c.4036A>G AA* or *AG* genotypes. We previously reported the significant influence of *ABCB1c.4036A>G* genotype on rifampicin C_{max} and $AUC_{0-\ 7h.}$ Rifampicin AUC_{0-7h} was significantly higher in homozygous variant genotype (*GG*) carriers compared to the homozygous

wild-type *AA* [27]. The consistency of these findings suggests that the genetic polymorphism in *ABCB1c.4036A>G* might have a role in the high inter-individual variability of rifampicin pharmacokinetics.

HIV and diabetes mellitus are well-known risk factors for developing TB. Reports linking the lower plasma concentrations of anti-TB drugs in patients with TB and diabetes [57] and TB and HIV [15] than those with TB only are available. In Korean TB patients' diabetes affected the KA and the volume of distribution of rifampin [47]. In the population pharmacokinetic of Ethiopian TB patients we did observe the effect of HIV and diabetes on rifampicin pharmacokinetic parameters.

Several studies evaluated the effect of *SLCO1B1* gene polymorphisms on rifampicin pharmacokinetics. In our present work, we evaluated the effect of *SLCO1B1 (c.388A>G)* and *SLCO1B1 (c.521T>C)* on the rifampicin pharmacokinetics. We did not observe differences in pharmacokinetic parameters based on the genetic polymorphism of these genes. Our current result is consistent with the various previous reports [23, 35, 37, 44, 46] suggesting a non-significant impact of these two gene polymorphisms in rifampicin pharmacokinetics. We did not find an association between *AADAC* or *CES-2* genotypes and rifampicin pharmacokinetics parameters in the model. A similar observation was noted in Malawian adult TB patients [58]. However, patients carrying the *AA* variant of *AADAC* rs1803155 were found to have a 10.4% lower clearance of rifapentine [26].

This is the first population pharmacokinetic study of rifampicin in the Ethiopian population. We evaluated multiple covariates' effects on the pharmacokinetic parameters of rifampicin. In model development, we did external validation and the sample size was large. However, blood sampling takes place only on three occasions and up to 7 hours post-drug intake. As a result, the estimation of clearance, especially for those with slower absorption may not be precise. The use of population modeling which is very useful for spare pharmacokinetic data can help in mitigating the above limitations.

In conclusion, many of the covariates included in the population pharmacokinetics analysis did not describe the inter-individual variability of rifampicin pharmacokinetic parameters. Only genetic polymorphisms in *ABCB1*, namely *c.4036A>G* and *3435C>T* were identified as

covariates on clearance and absorption rate constant, respectively. Further study may required to assess for further covariates and to characterize the pharmacokinetic/pharmacodynamics relationship in this population.

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