GENETIC POLYMORPHISMS OF N-ACETYL TRANSFERASE 2 AND THE RISK OF HEPATOTOXICITY IN PATIENTS ON ISONIAZID PREVENTIVE THERAPY AT KENYATTA NATIONAL HOSPITAL

AKUMU REVESZT MAUGO

U52/88146/2016

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN MOLECULAR PHARMACOLOGY

DEPARTMENT OF PHARMACY
UNIVERSITY OF NAIROBI

NOVEMBER 2022

UNIVERSITY OF NAIROBI DECLARATION OF ORIGINALITY FORM

Name of Student:Akumu Reveszt Maugo

Registration Number:U52/88146/2016

Faculty: Health Sciences

Department: Pharmacy

Course Name: Msc. Molecular Pharmacology

Title of the work: Genetic Polymorphisms of N-Acetyl Transferase 2 and the risk of hepatotoxicity in patients on isoniazid preventive therapy at Kenyatta national hospital

DECLARATION

1. I understand what plagiarism is and I am aware of the university's policy in this regard.
2. I declare that this thesis is my original work and has not been submitted elsewhere for examination, award of a degree or publication. Where other people's work or my own work has been used it has been properly acknowledged and referenced in accordance with the University of Nairobi's requirements
3. I have not sought or used the services of any professional agencies to produce this work.
4. I have not allowed and shall not allow anyone to copy my work with the intention of passing it off as his or her own work.
5. I understand that any false claim in respect of this work shall result in disciplinary action, in accordance with University Plagiarism Policy

Signature……………….. **Date**…28 November 2022…

AKUMU REVESZT MAUGO
U52/88146/2016

APPROVAL BY SUPERVISORS

This is to certify that this proposal has been submitted for examination with our approval as the University supervisors.
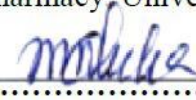
1. **Dr. Eric M. Guantai, PhD**

   Department of Pharmacology and Pharmacognosy,

   School of Pharmacy, University of Nairobi

   Signature.................................... Date. 28th November 2022

2. **Dr. Margaret N. Oluka, PhD**

   Department of Pharmacology and pharmacognosy

   School of Pharmacy, University of Nairobi

   Signature.................................... Date. 28-11-2022 ....

3. **Dr. James Kimotho, PhD**

   Innovation & Technology Transfer Division

   Kenya Medical Research Institute

   Signature.................................... Date. 28/11/2022 ....

DEDICATION

I dedicate this thesis to my mother my father and my sisters.

ACKNOWLEDGEMENTS

I would like to express my gratitude to the University of Nairobi, Faculty of Health Sciences School of Pharmacy Department of Pharmacy, for giving me the opportunity to be a student here. I would also like to offer my thanks to the University of Nairobi through Mapronano for having awarded me the scholarship to pursue this research.

My special thanks first and foremost go to my supervisors Dr Eric M.Guantai, Dr Margaret N Oluka and Dr James Kimotho for having sacrificed their precious time insight and resources to guide me till I successfully completed this thesis. I would also like to sincerely thank Prof. George O. Osanjo, Prof Faith A. Okalebo and Dr David wata for their valuable expertise and guidance.

I am also deeply grateful to Mr. Jeremiah Omari, Mr. Brian Khasimwa and Mr. Erickson Angiro of the Immunology Department, University of Nairobi; for their immense technical support and guidance throughout the DNA assays. I would also like to thank Mr. Paul Ngugi and Mr. David Mutabari of KNH CCC for their support during the data collection.

I am also grateful to my lecturers for their guidance throughout the coursework; their help, professionalism, guidance and academic support are well appreciated.

Finally, I must express my boundless gratitude to God for his grace, mercy and favour that have enabled me to reach this far and my family, for their encouragement, moral support and prayers. May God bless them.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS AND ACRONYMS

ACP	Acyl Carrier Protein
ADR	Adverse drug Reaction
AIDS	Acquired immune deficiency syndrome
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
ART	Anti-Retroviral Therapy
ATDILI	Anti-Tuberculosis Drug Induced Liver Injury
Anti-TB	Anti-Tuberculosis
BTACH	BTB and CNC homology 1
CCC	Comprehensive Care Clinic
CDC	Centre for Disease control
CD4	Cluster of Differentiation 4
CES1	Carboxylesterase 1
COA	Coenzyme A
CNS	Central nervous system
CYP2E1	Cytochrome P450 2E1
CYP3A4	Cytochrome P450 3A4
DILI	Drug Induced Liver Injury
DNA	Deoxyribonucleic Acid
GST	Glutathione S-transferase
GSTM1	Glutathione S-transferase Mu 1
GSTT1	Glutathione S-transferase theta 1
FDA	Food Drug Administration
HIV	Human immunodeficiency virus
HLA	Human leukocyte Antigen
ICF	Intensive case finding tool
IgG3	Immunoglobulin G3
INH	Isonicotinic acid hydrazide
IPT	Isoniazid Preventive Therapy
KatG	Catalase peroxidase gene
KNH	Kenyatta National Hospital
LTBI	Latent TB Infection
MAFK	Maf Bzip transcription factor K
MAH	Mono Acetyl Hydrazine
MDR-TB	Multi drug resistant TB
MNSOD SOD2	Manganese -dependent superoxide dismutase
$MnCL_2$	Manganese chloride

MTB	Mycobacterium tuberculosis
NADH	Nicotinamide Adenine Di nucleotide
NATP	N-Acetyltransferase pseudogene
Nat	Mouse N-acetyl Transferase
NAT2	Human N-Acetyltransferase 2
NASCOP	National AIDs and STI control Program
NCI	National Cancer Institute
NOS	Nitric oxide synthase
pABA	Para-aminobenzoic acid
pABG	Para-aminobenzoylglutamate
PLHIV	People Living with HIV
RCT	Randomized control trial
SNPS	Single Nucleotide Polymorphism

OPERATIONAL DEFINITIONS

Allele   One of two or more possible forms of a gene that arise by mutation and are found at the same place on a chromosome.

Baseline characteristics   Important Attributes of the participants when enrolled at the start of a study.

Covariates   Variables that are likely to determine the outcome of a study.

Gene The basic physical and functional unit of heredity which is passed from parent to offspring.

Genotype The set ofgenes in the DNA that is responsible for a particular trait.

Genotyping   The process of determining the genetic variants in an individual.

Mutation   The permanent alteration in the nucleotide sequences in the genome of an organism.

MTB/Rif test MTB/RIF is a cartridge based nucleic acid amplification test (NAAT), automated diagnostic test that can identify Mycobacterium tuberculosis (MTB) DNA and resistance to rifampicin (RIF).

Pharmacodynamics   A branch of pharmacology concerned with the effects of drugs and their mechanism of action.

Pharmacokinetics Branch of Pharmacology concerned with absorption, bioavailability, distribution metabolism and excretion of drugs.

Pharmacogenetics The branch of Pharmacology which examines the relation of genetic factors to variations in response to drugs.

Phenotype   The set of observable characteristics of an individual resulting from the interaction of its genotype with the environment.

Polymorphism Natural variation in a gene, DNA sequence or chromosomes that have no adverse reactions on the individual and occur with high frequency in the general population.

Single Nucleotide Polymorphism (SNP): Variation at one position on the DNA sequence in at least 1% of individuals in a population.

ABSTRACT

BACKGROUND

Isoniazid preventive therapy (IPT) is an important intervention to prevent activation of latent tuberculosis (TB) infection. Genetic variations associated with N-acetyl transferase (*NAT2*) are closely associated with isoniazid-induced hepatotoxicity in IPT. This occurs as a result of the patient's unique acetylation phenotype, which can affect clinical outcomes of patients on IPT therapy.

OBJECTIVES

The main objective was to characterize the distribution of selected single nucleotide polymorphisms (SNPs) of *NAT2* in patients on IPT at the Comprehensive Care Centre (CCC) of Kenyatta National Hospital (KNH) and to investigate for a relationship between acetylator status and isoniazid induced hepatotoxicity as indicated by elevated alanine amino transferase (ALT) levels.

METHODS

This was a cross-sectional study of human immunodeficiency virus (HIV) patients on IPT at KNH CCC. Convenient sampling was employed during routine patient visits at the CCC, following which inclusion and exclusion criteria were applied to recruit patients. Data was extracted from patient records using a data collection form. Blood samples were taken from patients. The QIAamp ® DNA Mini-Kit was used at the University of Nairobi's Institute of Tropical Infectious Diseases (UNITID) for DNA extraction and purification of genomic DNA, followed by DNA sequencing. Data analysis included both descriptive and inferential statistical methods which were used to summarize the data, this was done in SPSS version 25.

RESULTS

Output data were summarized as follows: Mean, BMI (basal metabolic index) and age were 25.75 kg/m² and 46.7 years, respectively. As a proxy indicator for hepatotoxicity, the cut-off for ALT levels was 40 UI/L (units per liter). The median ALT level was 22 UI/L and the mean CD4 level was 492 cells/mm³. The prevalence of the homozygous *NAT2* genotype was 19% and that of the heterozygous genotype was 50%. The proportion of the population with slow acetylator alleles was 56% and the proportion of fast acetylator alleles was 44%. Fisher's exact test showed no significant association between ALT levels and *NAT2* genotype (P = 0.330). Similarly, *NAT2* genotype and ethnolinguistic group (P = 1.0), alcohol consumption (P = 0.577), smoking status

(P = 0.751), comorbidities (P = 0.127), and gender (P = 0.346). *NAT2*5* and *NAT2*6* alleles were more frequent in this population compared to *NAT2*7* and *NAT2*14*. On multivariate regression analysis, the independent predictors. Smoking status, alcohol status, comorbidities, and gender were not significant in predicting elevated ALT levels (P > 0.05).

CONCLUSION

The study found no significant association between *NAT2* genotypes and ALT levels (P = 0.33). However it found that there was a high frequency of *NAT2*5* and *NAT2*6* alleles compared to *NAT2*7* and *NAT2*14* alleles among the 41 genotyped sequences in this study population. In addition, the study also found that, there was no significant differences in the distribution of *NAT2* genotypes and the Ethno-linguistic groups (P = 1.00). Similarly, there was no association between ALT levels and factors such as age, BMI, smoking status, alcohol status, gender and comorbidities. More studies on the effect of *NAT2* genotypes on Anti-tuberculosis drug induced hepatoxicity in the Kenyan population with a larger sample size is recommended.

CHAPTER 1: INTRODUCTION

1.1 Background

Tuberculosis (TB) is an infectious disease caused by bacilli of the genus Mycobacterium and species Mycobacterium tuberculosis (MTB) complex (Mwangi, 2016). In 2020 alone, there were 5.8 million TB related deaths worldwide (World Health Organization, 2022). In Kenya, according to the Global list for high burden TB, TB/HIV and MDR/RR-TB (multidrug/rifampicin-resistant TB) report published in 2021, Kenya was listed as a Multi-drug resistant TB (MDR-TB) high burden country (WHO, 2022). Estimates show that as of 2017, TB incidences occurred at a rate of 319 per 100,000 population, while TB mortality was estimated at 50 per 100,000 (Gichuki & Mategula, 2021).

TB is the most common presenting illness among people living with Human Immunodeficiency Virus (PLHIV), including those on Anti-Retroviral Therapy (ART) treatment (WHO, 2015a). As an opportunistic infection, it is the leading cause of death among PLHIV and accounted for 370,000 deaths with Africa contributing to 86% of the deaths in 2016 (WHO, 2017a). In addition, PLHIV are 21 times more likely to fall ill with TB than those without HIV, and face the highest risk of drug resistant TB (WHO, 2017a). In Kenya, about 1.6 million people were living with HIV infection at the end of 2016 (UNAIDS, 2017).

To prevent TB in PLHIV, World Health Organization (WHO), recommends Isoniazid Preventive Therapy (IPT). According to WHO, IPT for a span of three years is prescribed in settings with a high transmission of TB (World Health Organization, 2015). According to guidelines on the treatment of TB and leprosy in Kenya, a 6 month course of Isoniazid , 5mg/kg bodyweight daily, prevents the development of Active TB in HIV infected people (Guidelines for Management of Tuberculosis and Leprosy in kenya, 2013). To corroborate this, a recent study on the benefits of IPT in ART in Ethiopia, found that IPT was effective in reducing TB incidence independently and with concomitant ART (Anti-Retro-viral Therapy) (Yirdaw et al., 2014).

Unfortunately, isoniazid which is essential in Isoniazid Preventive Therapy, has been associated with hepatotoxicity (Chan *et al.*, 2017) Since its introduction in 1952, cases of isoniazid-induced hepatotoxicity have been reported (Kabbara et al, 2016). Notwithstanding.the beneficial effects of isoniazid, severe adverse effects especially peripheral neuropathy and hepatotoxicity have been linked to therapy (Wang et al., 2016).

Around 10–20% of patients on isoniazid have a transient elevation of serum alanine aminotransferase (ALT) levels. Most of the patients can adapt to it and their serum ALT levels come back to normal without discontinuation, while a few patients (1– 3%) develop severe liver injury and even liver failure(wang et al., 2016). An Eritrean study reported a high incidence rate of INH-related hepatotoxicity than what was reported in patients who were enrolled on IPT (Russom et al., 2019). In another study, hepatotoxicity was reported as a risk factor in Taiwanese patients on Isoniazid preventive therapy for 9 months, in the same study hepatotoxicity was exacerbated by Rheumatoid arthritis and ankylosing spondylitis (Lai et al., 2021)

Genetic factors have been reported as a risk factor for isoniazid induced hepatotoxicity; they are ascribed to genetic variability in arylamine N-acetyltransferase 2 (*NAT2* gene), a cytosolic phase II conjugation enzyme essentially responsible for the metabolic deactivation of isoniazid. Furthermore the variability in different enzymes like amidase, *CYP2E1*, glutathione S-transferase (*GST*), manganese superoxide dismutase (*MNSOD, SOD2*), *UDP* glucuronosyl transferase (*UGT*), immunogenetic factors and host factors also play a role (Ramappa and Aithal, 2013; wang et al., 2016).

The presence of different polymorphism determines the distinctive degrees of activity for *NATs* and an individual acetylation profile. The human population can be isolated into slow, intermediate and fast acetylators. The fast acetylators of isoniazid rapidly decrease the concentration of active drug and this influences the success of treatment. In slow acetylators the risk of adverse reactions due to the toxicity of high concentration of isoniazid metabolites could occur leading to hepatotoxic effects (Matei, 2015).

1.2 Problem statement

Despite many years of utilization and large numbers of patients exposed to Anti-TB drugs around the world, the pathogenesis underlying isoniazid-induced hepatotoxicity is poorly comprehended (Ramappa and Aithal, 2013). Coupled to this, there is limited pharmacogenetic data with regards to the distribution of *NAT2* single nucleotide polymorphism (SNPs) and association with isoniazid toxicity in Kenyan populations (Oluka, 2012).

Among hospitalized patients, hepatotoxicity due to first line Anti-TB drugs is reported to be common (Shu et al., 2013). The incidence of Anti-TB induced hepatotoxicity during standard multi drug TB therapy ranges from 1-36% (Guaoua et al., 2014) to 2-28%.(Ramachandran and Swaminathan, 2012) This incidence however depends on the investigators' definition of hepatotoxicity as well as the population being studied (Isa et al., 2016).

According to Metushi (2016), Isoniazid's high efficacy, undoubtedly makes it the preferred drug of choice for treatment of latent tuberculosis (TB) notwithstanding the fact that it can cause liver failure (Metushi et al, , 2016). Four substantial population based observations, showed that the incidence of isoniazid hepatotoxicity when utilized as a monotherapy in the treatment of latent infection, lies in the range of 0.1-0.56% (Ramappa and Aithal, 2013).

The use of isoniazid has also been associated with hepatotoxicity (Chan *et al.*, 2017). A review premised on data from the US Food and Drug administration (FDA), estimated 24 per 100,000 people die as a result of hepatotoxicity when receiving isoniazid based prophylactic therapy (Ramappa and Aithal, 2013). Furthermore, in a meta-analysis, it was observed that isoniazid in combination with rifampicin is associated with a higher rate of hepatotoxicity than when isoniazid is given alone (odds ratio = 2.6). (Steele, et al, 1991).

Several studies have identified the slow acetylator genotype as a predisposing factor for isoniazid induced hepatotoxicity (Santos *et al.*, 2013; Ng *et al.*, 2014; Shi *et al.*, 2015; Chan *et al.*, 2017). In Kenyan populations the genotype frequency for the *NAT2* slow acetylator genotype has been reported as *NAT2*5/*5* (13-18%) and *NAT2*5/*6* (11-19%) as the more prevalent genotypes (Oluka *et al.*, 2014).

In Kenya, clinical pharmacogenetic evaluation before treatment initiation with IPT is recommended but is not conducted (unpublished data). Furthermore, even when cases of hepatotoxicity do occur clinicians do not routinely investigate the underlying host genetic factors. As a consequence, the prevalence of Anti-TB drug related genetic polymorphisms that are associated with hepatotoxicity is unknown.

1.3 Research questions

1. What is the prevalence of the various *NAT2* variant alleles among patients who are on IPT?
2. What is the distribution of *NAT2* genotypes in patients on isoniazid preventive therapy at the Kenyatta National Hospital Comprehensive Care Center?
3. Do patients who are on IPT and who have experienced ALT elevation have a higher prevalence of the *NAT2*5, NAT2*6, NAT2*7* and NAT2*14* allelic variants, alcohol consumption, smoking, comorbidities or gender compared to those who do not have ALT elevation?

1.4 Study objectives

1.4.1 Broad objective

To investigate the relationship between acetylator status and isoniazid induced hepatotoxicity (as indicated by elevated ALT levels) in patients on isoniazid preventive therapy (IPT) at the Comprehensive Care Centre (CCC) between February and August 2019.

1.4.2 Specific objectives

1. To determine the distribution of *NAT2* allelic variants (*NAT 2*5, NAT2*6, NAT2*7* and NAT2*14*) in patients on isoniazid preventive therapy at the Kenyatta National Hospital Comprehensive Care Centre.
2. To describe the distribution of *NAT2* genotypes in patients on isoniazid preventive therapy at the Kenyatta National Hospital Comprehensive Care Center
3. To determine the genotypic and other predictors of elevated alanine aminotransferase levels in patients on isoniazid preventive therapy at the Kenyatta National Hospital Comprehensive Care Center

1.5 Study justification

Many studies have demonstrated the benefit of IPT among PLHIV. However, there are challenges associated with therapy. A qualitative study in Ethiopia, identified hepatotoxicity as one of the major challenges in IPT implementation (Teklay et al., 2016). Similarly a recent Randomized control trial (RCT) showed a high incidence of hepatotoxicity during IPT and ART (Ngongondo et al., 2018).

Patients possessing the slow acetylation *NAT2* genotypes have been reported to have a fourfold risk of developing isoniazid induced hepatotoxicity (Ramappa and Aithal, 2013). however different studies have demonstrated modest or no critical contrasts in the rate of isoniazid induced hepatotoxicity between various acetylator status (X Lv et al., 2012; Sharma et al., 2016). Therefore, the effect of NAT2 acetylator status is not well characterized. This study is expected to provide data as to whether selected *NAT2* genotypes are predictive of hepatotoxicity in HIV Kenyan patients on IPT.

Pharmacogenomics plays an important role in the metabolism of isoniazid. In an RCT, *NAT2* genotype guided regimens reduced incidences of isoniazid- drug induced liver injury (DILI) and early treatment failure compared to the conventional standardized regimen (Azuma *et al.*, 2013).

The findings of this study may be important in making a case for the importance of pharmacogenomics testing prior to IPT initiation.

According to published data, a longer duration of TB preventive therapy appears to have correlations with hepatotoxic outcomes while shorter durations may provide a lower effect for the outcome. Preventive therapy is currently practiced for 6-9 months. Premised on data on the 3-HP adverse monitoring schedule (UNITAID, 2019), 3-HP is a short course TB preventive treatment (TPT) regimen, taken once weekly for 12 weeks. According to the schedule, when 3-HP regimens are compared to TPT regimens, 6-9 months of 3-HP is associated with lower hepatotoxicity outcomes and higher rates of treatment completion. In one study, 31 study participants developed hepatotoxicity following a 24 week (IPT and ART) regimen (Ngongondo et al., 2018). As such in order to obtain an ideal representative sample, this study sought to recruit patients on IPT for at least 12 weeks, so as not to miss likely cases of hepatotoxicity as identified by patients on TB therapy.

The findings of the study, will be useful not only to the National AIDS and STI control program (NASCOP), but also the National Tuberculosis, Leprosy and Lung Disease program with regards to possibly refining guidelines on the use of IPT, thereby ensuring maximum effectiveness with minimum toxicity. The findings from this study may also support the call that clinical pharmacogenomics testing in Kenya, should be done to identify patients at risk of isoniazid induced hepatotoxicity. In addition, the study findings will possibly also increase awareness for the importance of genotyping enzymes involved in the metabolism of hepatotoxic drugs.

1.6 Conceptual framework

Age
Gender
smoking
NAT2 slow Acetylator status

Hepatotoxicity (ALT)

Alcohol consumption
BMI
Comorbidities
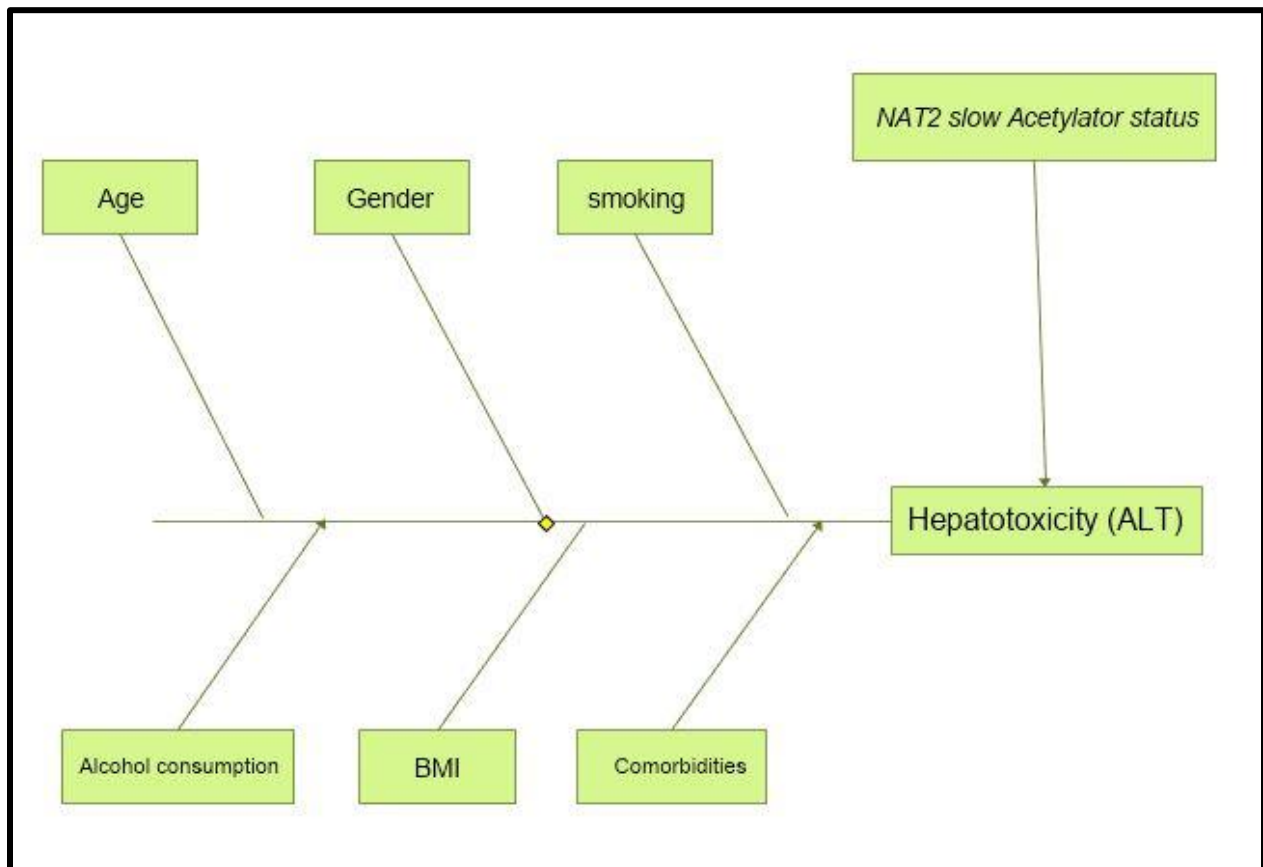
CHAPTER 2: LITERATURE REVIEW

2.1 Background of Tuberculosis

The Global Tuberculosis Report, 2021 by WHO, estimated that there were 1.3 million TB related deaths among HIV negative people and an additional 214,000 deaths among HIV positive people. An estimated 5.8 million people acquired TB in 2020 (World Health Organization, 2022)

There are currently 30 TB high burden countries in Africa; Kenya is ranked 9th (World Health Organization, 2021). In Kenya, a recent TB prevalence survey reported (558 cases per 100,000) people; in addition, there were higher frequencies among men (809 cases per 100,000) compared to women (359 per 100,000). The prevalence was highest among people aged (25-34) years at (716 per 100,000) people and also high among women over the age of 65. Surprisingly, 83% of TB cases were HIV negative compared to 17% in PLHIV (National Tuberculosis Leprosy and Lung Disease Program, 2016).

In 2021, a total of 187,000 PLHIV died due to TB (WHO, 2022). Taking isoniazid, is a simple and cost-effective measure that prevents the TB bacteria from becoming active if present. This is known as isoniazid Preventive Therapy (IPT) (Ross et al., 2021). IPT prevents TB in persons with HIV in general and in Tuberculin skin test (TST) positive individuals in particular (Ross et al., 2021).

WHO does recommend providing at the very minimum 6 months of IPT for PLHIV without active TB together with those who have successfully completed TB treatment, and conditionally recommends providing isoniazid for 36 months for PLHIV who are living in settings with high TB prevalence (Briggs et al., 2015). Pursuant to the Kenyan guidelines, IPT should be administered at a dose of 10mg/kg/day (maximum dose 300mg) for at least 6 months (NASCOP, 2016). In clinical settings isoniazid is given in line with the above mentioned NASCOP guidelines, in combination with pyridoxine at 1 to 2mg/kg/day to a maximum of 50mg daily for 6 months. IPT is contraindicated in patients with active TB and in patients with pre-existing active hepatitis. In addition, most guidelines recommend avoiding IPT in patients with existing signs and symptoms of peripheral neuropathy, this is because peripheral neuropathy is observed in 10-20% of patients. given dosages greater than 5mg/kg/day of isoniazid even though it is reversed with pyridoxine. IPT should also be differed in patients with poor adherence to Co-trimoxazole preventive therapy. and active substance abuse (Mwangi, 2016).

2.2 Indications for isoniazid preventive therapy

WHO recommends that grown-ups and young people living with HIV (PLHIV) with unknown or positive TST status and who are probably not going to have active TB ought to get no less than a half a year of IPT as part of a comprehensive package of HIV care. IPT should be given to such people regardless of the level of immunosuppression, in addition to those on ART, pregnant women and individuals who have already been treated for TB. However it also states that TST is not a requirement for initiating IPT in PLHIV (University of Cape Town, 2018). According to the operational guidelines for isoniazid preventive therapy, patients eligible for IPT are evaluated based on the standard intensive case finding tool (ICF) (National AIDS & STI Control Program, 2018)

2.3 Patient monitoring and clinical follow up for isoniazid preventive therapy.

During initiation of therapy to PLHIV, a clinical evaluation of haemoglobin levels, plasma lipid profiles, protein, glucose, creatinine and ALT levels are recommended. In addition the pregnancy status of women under reproductive age, should also be determined (National AIDS & STI Control Program, 2018)

Clinic appointments for IPT review should be harmonized with regular HIV care. Patients on IPT are reviewed after every 28 days, and are screened for TB utilizing the standard ICF tool. They are assessed to exclude hepatitis and peripheral neuropathy or some other ailment. They are likewise assessed for adherence to IPT.

2.4 Treatment failure during isoniazid preventive therapy

Treatment failure can occur during patient monitoring, in which case, patients who test positive for TB based on the ICF tool, are no longer eligible for IPT. These patients are classified as presumptive TB cases and must undergo additional testing. In all possible TB cases, the "Xpert MTB/Rif test" is the prescribed first test of TB and rifampicin (National AIDS & STI Control Program, 2018)

Failure can also occur due to underlying host genetic factors such as *NAT2* polymorphism, in which case, affected patients are susceptible to INH induced hepatotoxicity. *NAT2* is an enzymes involved in the metabolism of isoniazid (Jaramillo-Valverde et al., 2022). Several studies have shown *NAT2* polymorphisms as a predisposing risk factor for INH hepatotoxicity (Chan et al., 2017; Khan et al., 2019; wang et al., 2016; Wattanapokayakit et al., 2016)

2.5 Dose and duration of isoniazid preventive therapy

The guidelines for the application of IPT in Kenya are formulated by the National AIDS and STI control program (NASCOP). These guidelines are adapted from WHO guidelines and guide the dosage and duration of IPT. IPT dosage is based on weight. IPT should be administered at a dose of 10mg/kg/day (maximum dose 300 mg) for no less than a half year as a part of an exhaustive package of HIV care. Table 2.1 gives weight-based dosing of isoniazid.

Table 2.1: Dose of isoniazid for Isoniazid Preventive Therapy (NASCOP, 2016)

Weight (kg)	Dose in mg	Number of 100mg INH tablets
<5	50	½ tablet
5.1-9.9	100	1 tablet
10-13.9	150	1 ½ tablet (or ½ adult 300mg tablet)
14-19.9	200	2 tablets
20-24.9	250	2 ½ tablets
>25	300	3 tablets (or 1 adult 300mg tablet)

In spite of the fact that a 6 month INH regimen gives significant assurance against developing TB, it is less protective than a 9 month regimen (Hsieh et al., 2021). A 9 month regimen is the optimal INH treatment for latent TB infection (centers for disease control., 2013). The CDC guidelines recommend treating HIV positive individuals in low to medium income settings with a 9 month course of isoniazid with no option for a 6 month course (Little et al., 2018). However, treatment for LTBI for a half year instead of 9 months is more financially savvy and results in more noteworthy adherence by patients; therefore local programs may want to actualize the half year as opposed to the 9 month regimen (centers for disease control., 2013).

2.6 Bio-markers for Liver injury

When evaluating liver function tests, the most profound biomarker's include alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase (AST), gamma-glutamyl transferase, bilirubin, prothrombin time, lactate dehydrogenase, international normalized ratios, total protein albumin and globulin. AST and ALT, are markers for hepatic injury (Lala et al., 2022). These enzymes are involved in gluconeogenesis by catalyzing the transfer of amino groups from aspartic acid or alanine to ketoglutaric acid to produce oxaloacetic acid and pyruvic acid, respectively. AST is found in the liver, skeletal muscle, cardiac muscles, kidneys, brain, pancreas, lungs, white and red blood cells (Lala et al., 2022). It is present as a cytosolic and mitochondrial iso-enzyme. AST, is not as specific or sensitive for the liver as ALT, and AST elevations may be seen as arising from non hepatic causes. Elevations of ALT and AST, subject to out of proportion levels of ALP and bilirubin is indicative of hepatic injury (liver function tests visvi lala). A borderline AST and/or ALT elevation is defined as <2X ULN. A mild AST and/or ALT elevation is as <2-5X ULN, moderate AST and/or ALT elevation as <5-15X ULN, severe AST and/or ALT elevation as <15X ULN and massive AST and/or ALT elevation as >10000X IU/L (Lala et al., 2022)

2.7 Chemistry of isoniazid
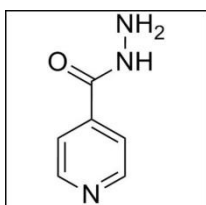
Figure 2.1 *Chemical structure of Isoniazid (Jena et al., 2014)*

Isoniazid contains 2 essential components required for the high activity against Mycobacterium tuberculosis, a pyridine ring and a hydrazine group (Saxena et al., 2019). Isoniazid is metabolized to hydrazine and acetyl hydrazine which have been both implicated in the toxicity of isoniazid (Brewer et al., 2019). Hundreds of INH derivatives have been synthesized since its original discovery but none improved on the activity of INH (Marriner et al., 2011).

In addition to emerging multi drug resistance (MDR) and extensively drug resistant (XDR) strains of tuberculosis, there has been a renewed interest in developing anti-TB drugs with minimum side effects and efficacy against MDR-TB and in patients co-infected with HIV (Asif, 2013). For instance, aconiazide is a pro drug of isoniazid which was designed to be less toxic

than the parent drug. Aconiazide is converted to isoniazid and 2-formylphenoxyacetic acid. The acid binds to isoniazid metabolites and as a result lowers toxicity. Compared to isoniazid the drug is less toxic and lacks carcinogenicity. In healthy patients It was found to produce lower levels of isoniazid than the parent molecule itself (Saxena et al., 2019).

2.8 Pharmacodynamics of isoniazid

Isoniazid is a pro-drug that enters the mycobacterial envelope through passive diffusion. It is activated by $MnCL_2$ and the catalase-peroxidase KatG, to form isonicotinoyl anions and radicals, which is able to inhibit the inhA enzyme by binding covalently to NADH, within the active site of the protein (Vögeli et al., 2018). InhA usually, catalyzes the NADH-dependent reduction of enoyl- acyl carrier protein (ACP) reductase, in the biosynthesis of fatty and mycolic acids, which are long chain β-unsaturated fatty acids that contribute to impermeability of the mycobacterial cell envelope (Unissa et al., 2016). As a result of the covalent attachment of isonicotinoyl anions and radicals to NADH at the active site of inhA, inhibition of inhA enzyme occurs (Vögeli et al., 2018). As a consequence, mycolic acids which are essential for survival, virulence and Anti-TB resistance are inhibited (Unissa et al., 2016). Some of the adverse effects of Isoniazid include hepatitis, peripheral neuropathy, psychiatric disorders, arthralgia, cutaneous, immunological and hematological ADRs. A few cases have also reported isoniazid-induced gynecomastia among patients treated with anti-TB drugs (Chiriac et al., 2015; Singh et al., 2015).

2.9 Pharmacokinetics of isoniazid

Isoniazid is promptly assimilated from the gastrointestinal tract and muscles following intramuscular injection. Peak concentrations of around 3 to 8µg for every milliliter of blood occur 1 to 2 hours after a fasting oral dose of 300mg. When isoniazid is given with nourishment lower peak concentrations are obtained. Isoniazid is not thought to be bound apparently to plasma proteins and diffuses into all body tissues and fluids, including the cerebrospinal fluid. It also appears in foetal blood if given during pregnancy and is also present in the milk of nursing mothers (Martindale & Sweetman, 2020)

The plasma half-life of isoniazid varies from around 1 to 6 hours; fast acetylator individuals have shorter half-lives. The essential metabolic course is the acetylation of isoniazid to acetylisoniazid by N-acetyltransferases found in the liver and small intestine. Acetylisoniazid is subsequently hydrolyzed to isonicotinic acid and monoacetylhydrazine. Isonicotinic acid is conjugated with glycine to isonicotinyl glycine (isonicotinuric acid) and monoacetylhydrazine is additionally

acetylated to diacetylhydrazine. Some unmetabolized isoniazid is conjugated to hydrazones. The metabolites of isoniazid do not have any tuberculostatic action and excluding presumably monoacetylhydrazine, they are additionally less toxic. The rate of acetylation of isoniazid and monoacetylhydrazine is genetically determined and there is a bimodal distribution of persons who acetylate them either slowly or rapidly. Eskimos, Japanese and Chinese are predominantly rapid acetylators whereas Caucasians, Africans and Indians, have similar proportions of slow and rapid acetylators. In patients with normal renal function, over 75% of a dose appears in the urine in 24 hours, mainly as metabolites. Small amounts of the drug are also excreted in the faeces (Martindale & Sweetman, 2020)

2.10 Pharmacogenetics of isoniazid

There are inter-individual genetic differences in the metabolism of isoniazid which are associated with individual responses to isoniazid in terms of therapeutic effects and adverse effects. An increasing number of studies suggest that genetic polymorphisms in *NAT2*, *CYP2E1*, and *GST* genes are associated with susceptibility to drug induced hepatotoxicity during TB treatment (Teixeira et al., 2013). In addition, genetic variations in the *CES* gene have been linked to INH induced hepatotoxicity(wang et al., 2016).

Isoniazid is metabolized by N-acetyl transferase 2 to form acetyl isoniazid, which is then hydrolyzed to acetyl hydrazine (Cojutti et al., 2016). Acetylation of acetyl hydrazine to non-toxic diacetyl hydrazine is also a *NAT2* dependent mechanism. Slow acetylator status results in accumulation of parent compound and monoacetyl hydrazine (Ramappa and Aithal, 2013), which are hepatotoxic.

The NAT2 diplotypes (2 haplotypes) are interpreted as acetylator phenotypes (slow, intermediate, and fast). Fast acetylators are homozygotes or heterozygotes of high activity enzymatic haplotypes (NAT2*4, NAT2*12A, and NAT2*13A), while slow acetylators are homozygotes or heterozygotes of low activity enzymatic haplotypes (NAT2*5B, NAT2*6A, and NAT2*7B). Intermediate acetylators are determined by heterozygotes of high and low enzymatic activity haplotypes.. Patients possessing the slow acetylation *NAT2* genotypes, had a fourfold risk of developing isoniazid induced hepatotoxicity (Ramappa and Aithal, 2013). However different investigations have demonstrated modest or no distinction in the frequency of isoniazid induced hepatotoxicity between various acetylator status (X Lv et al., 2012; Sharma et al., 2016) Therefore, the effect of *NAT2* acetylator status in relation to isoniazid induced hepatotoxicity is not well characterized.

The *CYP2E1 c1/c1* genotype has been associated with increased risk of isoniazid induced hepatotoxicity. *CYP2E1* oxidizes acetylhydrazine a product of isoniazid metabolism by *NAT2* to form hepatotoxic intermediates which are able to destroy hepatocytes (wang et al., 2016). Patients with the homozygous wild genotype *CYP2E1* c1/c1 are predisposed to isoniazid hepatotoxicity (Richardson et al., 2017). Genotyping of *CYP2E1* polymorphism may be useful in the prediction of isoniazid induced hepatotoxicity (wang et al., 2016). However, a lack of correlation has been described between *CYP2E1* genotypes and Anti-TB drug induced liver injury (Xiang et al., 2014).

Glutathione S-transferase (*GSTs*) detoxify the reactive metabolites created by the oxidation of acetylhydrazine and hydrazine (Boelsterli and Lee, 2014). *GSTM1* and *GSTT1*, have extensively been studied. These genotypes play a role in the susceptibility of isoniazid induced liver injury (Brito et al., 2014; wang et al., 2016); However, there exists a modest to a lack of association between *GST* polymorphism and Anti-TB induced liver injury (Cai et al., 2012; Xiang et al., 2014).

Amidase is an enzyme that hydrolyzes the amide bond of isoniazid to produce isonicotinic acid and hydrazine (Fan, 2014). Hydrazine is a hepatoxic metabolite of isoniazid (Perwitasari, Atthobari and Wilffert, 2015). As a consequence higher levels of amidase activity can result in higher incidences of INH hepatotoxicity (wang et al., 2016).

Carboxylesterases can also hydrolyze amides. since they share similar catalytic mechanisms and substrates with amidases (wang et al., 2016), however the role of carboxylesterase in isoniazid remains controversial. Tests for association in a larger sample set are necessary to determine if genetic variation in CES1 has a role in INH induced hepatotoxicity (wang et al., 2016).

2.11 Structure of N-acetyl Transferase 2

The secondary and tertiary structures of NATs are profoundly conserved from eukaryotes to prokaryotes (Zhou et al., 2013). The NAT fold is generally depicted in terms of 3 domains (Zhou et al., 2013). The human N-acetyl transferase 2 enzyme, has an N-terminal domain which comprises of 5 helices; alpha1 – 5 (α1-5), and a short beta strand (β) between helices α2 and α3. The second domain comprises of 10 β strands (β2-11) and two short helices α6 and α7

The two domains interface with the third domain, which has 4 anti-parallel β-strands (β12-15) and helix α11. The helix α11 precedes a stretch of residues that lead across the proteins molecules surface into a buried C-terminus (Zhou et al., 2013).
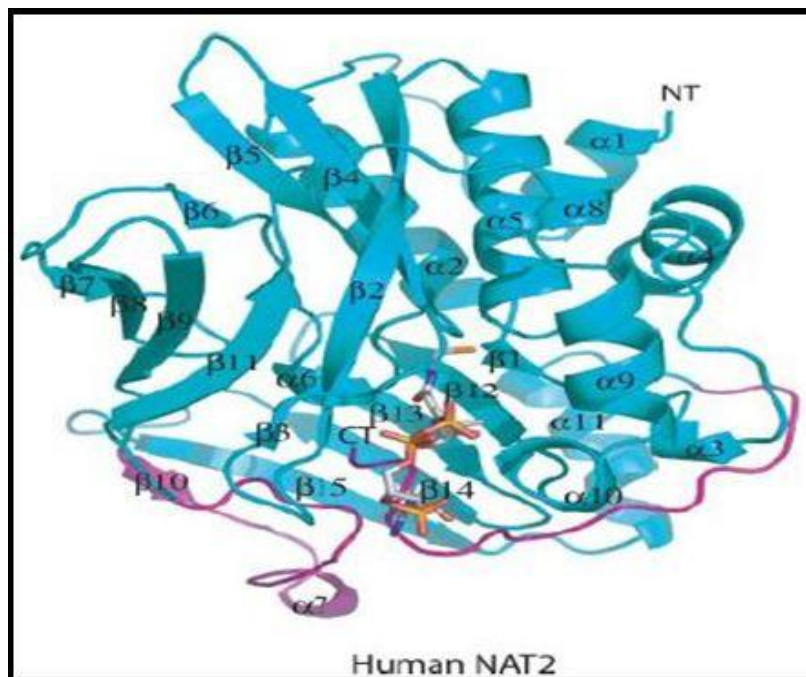
Figure 2.2 *Ribbon diagram showing the structure of human NAT2 in complex with COA, amino and carboxyl termini labeled as NT and CT respectively(Wu et al., 2007)*

2.12 Effect of N-Acetyl Transferase 2 on drug pharmacokinetics

NAT2 is a phase 2 enzyme that participates in the metabolism of numerous primary arylamines hydrazine drugs and carcinogens (wang et al., 2016). In addition to their N-acetylation catalytic activity, *NAT* enzymes have also O-acetylation activity towards arylhydroxylamines (Sim, Abuhammad and Ryan, 2014).

NAT2 acetylator status, affects the plasma levels of isoniazid and acetyl-hydrazine (Adithan and Subathra, 2016; wang et al., 2016). In an Asian cohort; the *NAT2* metabolic status contributed 72% of isoniazid pharmacokinetic variability in the subjects (Seng et al., 2015). The acetylation rate of isoniazid and acetyl-hydrazine is slow in slow acetylators (wang et al., 2016); Furthermore, the clearance rate of INH is additionally slow in slow acetylators compared to fast acetylators, (Seng et al., 2015) which contributes to the build-up of isoniazid in slow acetylators (wang et al., 2016).

The *NAT2* slow acetylator genotype affects sulfamethoxazole (SMX) plasma concentration (Kagaya et al., 2012). In renal transplant patients, the SMX area under curve (AUC) for NAT2 slow acetylator genotypes was significantly greater than the AUC for the rapid acetylators (Kagaya et al., 2012).

After oral dose, rapid acetylators, show lower hydralazine concentrations and area beneath the concentration time curve in distinction to slow acetylators (McDonagh et al., 2014). Patients with

a slow acetylator genotype showed significant reductions in blood pressure (Bp) measurements at 24 hours prior to and after hydralazine whereas significant effects were not observed in rapid and intermediate acetylators (Spinasse et al., 2014).

2.13 Genetic polymorphisms of N-Acetyl Transferase 2

Three *NAT* genes have been so far characterized in humans, specifically *NAT1*, *NAT2*, and *NATP*. These genes are located on human chromosome 8 (McDonagh et al., 2014), in the region of 8p22 (Guaoua et al., 2014). The *NAT2* gene has one non-coding exon around 8.6 kilo base pairs (Kb) and encodes proteins of 290 amino acids (McDonagh et al., 2014).

NAT2 phenotypes are assigned based on co-dominant expression of rapid and slow acetylator *NAT2* genotypes. Individuals homozygous for high activity *NAT2* alleles such as *NAT2*4*4* are assigned as rapid acetylators. Individuals homozygous for slow activity *NAT2* alleles such as *NAT2*5*5* are slow acetylators and individuals possessing one high and one low activity *NAT2* alleles such as *NAT2*4*5* are intermediate acetylators (Matejcic, 2013).

NAT2 is polymorphic and about 108 *NAT2* alleles have been listed by Arylamine N-acetyl transferase gene Nomenclature Committee (Adithan and Subathra, 2016). Most SNPs are found inside the 873 base pair (bp) intronless coding region of *NAT2* gene factor (Guaoua et al., 2014).

Among the seven most common SNPs, four lead to amino acid changes resulting in a big reduction in acetylation capacity and are related to the slow acetylator phenotype rs1801280: (c.341.T>C; *NAT2*5*), rs1799930 (c.590G>A; *NAT2*6*), rs1799931 (c.857G>A; *NAT2*7*) and rs1801279 (c.191G>A*; NAT2*14*) (Guaoua et al., 2014).

The three others, rs1041983 (c.282C>T; *NAT2*13A*), rs1799929 (c.481C>T; *NAT2*11A*), and rs1208 (c.803A>G; *NAT2*12A*), are similar SNPs and do not change the phenotype (Guaoua et al., 2014), Table 2.2 presents the common human NAT2 alleles.

*NAT2*4* is viewed as the wild type allele, and is linked with the rapid acetylator phenotype (Matejcic, 2013). A heterozygous compound genotype (*NAT2*4/*5* or *NAT2*4/*6* or *NAT2*4/*7*) is regarded as intermediate acetylator (Guaoua et al., 2014).

Table 2.2 Common human NAT2 alleles (Hein, 2006)

Common human NAT2 Alleles			
Alleles	**Nucleotide change(s)[b]**	**Amino acid change(s)[c]**	**Catalytic activity[d]**
NAT2*4	None	None	High
NAT2*5A	**341T>C**; 481C>T	1114T	Low
NAT2*5B 341T>C	**341T>C**;481C>T;803A>G	1114T; K268R	Low
NAT2*5C	**341T>C**, 803A>G	1114T; K268R	**Low**
NAT2*6A	282C>T; **590G>A**	AR197Q	Low
NAT2*6B	**590G>A**	R197Q	**Low**
NAT2*7A	**857G>A**	G286E	**Low**
NAT2*7B	282C>T; **857G>A**	G286E	**Low**
NAT2*11	481C>T	L161L[b]	High
NAT2*12A	**803G>A**	K268R	High
NAT2*12B	282C>T; **803A>G**	K268R	High
NAT2*12C	481C>T; **803A>G**	K268R	High
NAT2*13	**282C>T**	None	High
NAT2*14A	**191G>A**	R64Q	**Low**
NAT2*14C	**191G>A**; 282C>T	R64Q	**Low**

[a]Common human NAT2 alleles associated with low catalytic activity and slow acetylator are bolded. Individuals homozygous for these alleles are slow acetylators.

[b] Signature SNP for each allele cluster is bolded

[c] Amino acid substitution that confer reduced NAT2 activities are underlined

In addition human *NAT2*11, *12*, and **13* alleles confer acetylation reactions at levels comparable to the wild type allele whereas *NAT2*10* and *NAT2*17* are associated with slow acetylation (Matejcic, 2013). The slow acetylator status is associated with an increased risk of hepatotoxicity among patients receiving isoniazid; in Japanese tuberculosis patients there was a significant association between *NAT2* slow acetylators and anti-TB drug induced liver injury (DILI) OR 4.32 C.I 1.93-9.66 (Mushiroda et al., 2016). It has been recommended that the dose of isoniazid should be adjusted based on genotype and phenotype of the patient with a specific end goal of preventing hepatotoxicity (Azuma *et al.*, 2013).

2.14 Ethnic and population distribution of N-Acetyl Transferase 2 polymorphism

The frequency of occurrence of fast and slow acetylation genotypes and phenotypes differs between populations (Werely, 2012; Lakkakula *et al.*, 2014). The distribution of *NAT2* alleles has been analyzed in 3 Kenyan populations Bantus, Eastern, and Western nilotes. The prevalence

of *NAT2*5* (30-42%), *NAT*6* (20-27%), *NAT*7* (3-6%) and *NAT*14* (8-14%) was similar in the 3 populations (Oluka *et al.*, 2014).

The prevalence of *NAT2* variant alleles was similar in Kenyans and other African populations but was variable between Kenyans, Caucasians and Asians, *NAT2*5* was comparable in Kenyans (33-42%) and Caucasians (51%) but much lower in Asians (5%). *NAT2*7* was higher in Asians (12%) compared to Kenyans (3-6%) and Caucasians (1.3%). *NAT2*14* has not been reported in Caucasians and Asians and is said to be an African specific Allele (Oluka, 2012). Table 2.3 shows the interethnic comparison of *NAT2* in three Kenyan populations.

In the three Kenyan populations, *NAT2*4/*5* was the most abundant rapid acetylator genotype with a frequency of 18-20%, whereas the slow acetylator genotype of *NAT2*5/*5* (13-18%) and *NAT2*5/*6* (11-19%) was the most prevalent (Oluka *et al.*, 2014). The prevalence of the slow acetylator status is highly variable across African populations (Werely, 2012). Africans show more noteworthy allelic diversity and may contain unique variants not already elaborated in peoples of European or Asian descent (Matejcic, 2013).

NAT2*5 is more prevalent in Caucasians and Africans (40-60%) (Oluka, 2012; Werely, 2012). However the overall prevalence of *NAT2*5* (29%) in Nigerian populations is lower compared to the 40% reported for Caucasians (Ebeshi et al, 2011). Based on the Kenyan perspective *NAT2*5* (30-42%) and *NAT2*6* (20-27%) were the most common alleles reported (Oluka *et al.*, 2014).

NAT2*7 is more common in Asians (Guaoua et al., 2014). The *NAT2*14* allele is notable in African Americans and native Africans but is almost non-existent in Caucasian and Asian populations. The *NAT2*14* alleles occur at a frequency of (3.9%) in the Xhosa population in South Africa (Werely, 2012), and at a frequency of (8-14%) in Bantus, Eastern, and Western nilotes (Oluka *et al.*, 2014).

Table 2.3 Inter-ethnic comparison of NAT2 alleles and genotypes in three ethnic populations of Kenya **(Oluka, 2012)**.

	Bantu	Eastern Nilotes	Western Nilotes	χ^2	P	mean	95% CI
Prevalence (%)							
Genotypes							
NAT2 **Rapid Acetylator Genotypes**							
*4/*4	8.2	4.4	8.7	2.09	0.35	6.70	(4.59- 98)
NAT2 **Intermediate Acetylator Genotypes**							
*4/*5	18.4	20.4	19.6	0.2	0.93	19.60	(15.6-24.2)
*4/*6	5.1	7.3	10.9	2.28	0.32	7.60	(5.2-11.0)
*4/*7	2.0	0.7	1.1	0.83	0.66	1.20	(0.48-3.1)
*4/*14	2.0	1.5	5.	3.51	0.17	2.80	(1.5-5.2)
NAT2 **Slow Acetylator Genotypes**							
*5/*5	15.3	17.5	13.0	0.85	0.66	15.60	(12.1-19.9)
*5/*6	16.3	19.0	11.9	2.0	0.37	16.20	(12.6-20.6)
*5/*7	2.0	1.5	1.1	0.29	0.86	1.50	(0.7-3.5)
*5/*14	9.2	7.3	6.5	0.52	0.77	7.60	(5.2-11.0)
*6/*6	9.2	10.9	8.7	0.37	0.83	9.80	(7.0-13.5)
*6/*7	2.0	0.7	1.1	0.83	0.66	1.20	(0.48-3.1)
*6/*14	4.1	4.4	4.3	0.01	0.99	4.30	(2.6-7.1)
*7/*7	1.0	1.5	1.1	0.11	0.95	1.20	(0.48-3.1)
*7/*14	4.1	1.5	1.1	2.55	0.28	2.10	(1.0-4.4)
*14/*14	1.0	1.5	5.4	4.84	0.09	2.40	(1.2-4.8)
n	98	137	92				
Alleles							
NAT2							
*4	21.9	19.3	27.2	3.92	0.1411	22.3	(19.3-25.7)
*5	38.3	41.6	32.6	3.78	0.1508	38.1	(34.4-41.9)
*6	22.9	26.6	22.8	1.21	0.5458	24.5	(21.3-27.9)
*7	6.1	3.7	3,3	2.45	0.2943	4.3	(3.0-6.1)
*14	10.7	8.8	14.1	3.29	0.1933	10.9	(8.7-13.5)
n	196	274	184				

The mechanism of isoniazid induced liver injury remains poorly understood (metushi et al., 2014). However it is for the most part not thought to be immune mediated and has been termed to as metabolic idiosyncrasy (metushi et al., 2014).

One reason behind this view, is that as opposed to the liver injury caused by a few different medications, such as, halothane, most clinical investigations of isoniazid induced liver injury did not distinguish anti isoniazid-antibodies (Metushi et al., 2014). 79% of patients, n= 20, had antibodies against INH, CYP3A4, 2E1 and 2C9 and most patients had antibodies to a few native

or isoniazid modified proteins (Metushi et al. 2014). Mild isoniazid induced liver injury, could involve an immune mediated mechanism involving Th17 cells and induction of IL-10 (Metushi, Uetrecht and Phillips, 2016).

Extreme instances of liver injury on the other hand are associated with the IgG3 antibody which is involved with complement activation and might cause hepatocyte damage (Metushi et al., 2016). Though Anti-TB therapy with rifampicin, isoniazid, pyrazinamide and ethambutol/streptomycin is very effective, the first three drugs are hepatotoxic (Sharma et al., 2002). In an Indian study The Human leukocyte antigen (HLA), was evaluated; patients without the HLADQA1*0102 allele had a 4-fold risk of Anti-TB drug induced liver injury (ATDILI). Hence the presence of HLADQA1*0102 is protective whereas patients with the HLADQB1*0201 allele had a 2 fold risk of developing ATDILI (Sharma et al., 2002) owing to HLA variations in various ethnic populaces. The relationship amongst HLA and ATDILI warrants additional investigations (Huang, 2014).

TNF-α is a pro-inflammatory cytokine involved in the innate immune response (Olmos and Lladó, 2014). There was a significant association between anti-TB drug induced hepatotoxicity and polymorphisms in the minor variant allele A (AG or AA) genotype of the TNF-α gene (TNF-α-308 G>A) (Kim et al., 2012)

2.15 Other genetic factors that may be associated with isoniazid induced hepatotoxicity

Genetic factors could contribute fundamentally to the development of hepatotoxicity (Ramappa and Aithal, 2013). Manganese superoxide dismutase (*MnSOD, SOD2*) is a crucial phase 2 enzyme with antioxidative activity. A thymine (T)-to-cytosine (c) substitution at position 47 in the human *MnSOD* has been discovered (Huang, 2014). This entity has been linked to an increased risk of numerous cancers and alcoholic liver disease (Huang, 2014); subjects with the manganese superoxide dismutase (*MnSOD*) variant C allele had a 2.5 fold risk of Anti-TB drug induced liver injury (Ramappa and Aithal, 2013).

UDP glucuronosyl transferase (*UGT*) is an imperative phase 2 enzyme which is linked to conjugation and detoxification of many drugs, xenobiotics and bilirubin (Huang, 2014), individuals with the *UGT1A1* variant *27 and *28 allele had a high odds for ATDILI, however in view of the small sample size, the outcomes were not definitive. A large sample size with various ethnic populaces is likely to elucidate the significance of UGT genetic variations in the risk of ATDILI (Huang, 2014).

The antioxidation pathway is significant to detoxifification of numerous free radicals and harmful chemicals (Huang, 2014). This pathway requires the involvement of many enzymes and transcription factors to regulate the antioxidative process (Huang, 2014): the significant anti-oxidant and transcription factors are Nitric oxide synthase (*NOS*), broad complex tramtrack and brick a brac (BTB) and cap'n'collar (CNC) homology 1 (*BTACH)* and musculo aponeurotic fibrosarcoma (MAF) basic leucine zipper protein (*MAFK*), which are Involved in this pathway (Huang, 2014). A *C/C* genotype at rs11080344 in *NOS2A*, a C/C genotype at rs2070401 in BACH1 and a *G/A* or *A/A* genotype at rs4720833 in *MAFK*, independently conferred ATDILI susceptibility (Huang, 2014).

2.16 Non-genetic factors that may be associated with Isoniazid induced hepatotoxicity

Age has been correlated to an increased risk of hepatotoxicity (Ramappa and Aithal, 2013); It was observed that anti-Tb drug induced hepatotoxicity was lower among younger age groups compared to elderly patients (Gaude et al., 2015). In a meta-analysis, a higher frequency of clinical hepatitis (6.9%) was observed in children receiving isoniazid and rifampicin in comparison to 2.7% in adults (Ramappa and Aithal, 2013).

Men and women might have differences in susceptibility to drug induced liver injury (Chalasani and Bjornsson, 2011). Women are more susceptible to liver injury associated with halothane, flucloxacillin, isoniazid, nitrofurantoin, chlorpromazine or erythromycin (Chalasani and Bjornsson, 2011). In addition pregnant women, have increased incidence of isoniazid hepatotoxicity especially In the third trimester and initial three months post-partum (Ramappa and Aithal, 2013).

High alcohol intake increases the risk of Anti-Tb drug induced liver injury; in a tuberculosis cohort 42.2% of the cases composed mostly of alcoholics developed ATDILI (Gaude et al., 2015). Sufficient consumption of nutrients, is crucial for the integrity of liver metabolism and detoxification of TB drugs (Ramappa and Aithal, 2013). A poor nutritional status may increase the frequency of anti TB drug induced hepatotoxicity; a weight loss of at least 2kg developing within 4 weeks during TB treatment is a notable risk factor for Anti-TB drug induced liver injury (Ramappa and Aithal, 2013).

Drug interactions can also increase isoniazid induced hepatotoxicity. In Moroccan patients, it was observed that the combined use of isoniazid and rifampicin was associated with an increased risk of Anti-Tb drug induced liver injury. Rifampicin induces isoniazid hydrolase, increasing hydrazine production (El Bouazzi et al., 2016).

Hepatitis B or C might also be risk factors for hepatotoxicity of anti-TB agents; in hepatitis C patients it was observed that there was a higher incidence of ATDILI (Kim et al., 2016). Similarly in a meta-analysis chronic hepatitis B was seen to increase the risk of hepatotoxicity ((Wang et al., 2016).

2.17 Identified Gaps in Literature

As seen from, section 2.16 and 2.15, it is evident that, other factors are likely to attribute to ATDILI, as such these gaps exist in the context of the Kenyan population. In the Kenyan population, there is need to identify the prevalence of HLADQA1*0102 and its constituent risk to confer ATDILI, similarly there is need to comprehensively evaluate the pro-inflammatory cytokine TNF-α and, to evaluate the TNF-α-308 G>A gene's role in ATDILI. In addition, the other factors CYP2E1, MNSOD, UGT, NOS2A, BACH1, MAFK and there corresponding ATDILI related genes, also need to be evaluated in the context of the Kenyan population, so as to give a more intricate evaluation for Anti TB drug induced liver injury. In terms of biomarkers for liver injury, there is need to develop diagnostic tools that can be used for early detection of liver injury, this is because ALT and AST are more specific for identification of hepatic injury that occurs after long term TB treatment.

CHAPTER THREE METHODS

3.1 Study design

The study conducted was a cross sectional study targeting adult HIV positive patients on IPT attending the CCC at KNH. The study entailed collection of patient demographic data and blood sample collection, which was later used for genotyping.

3.2 Study site

The study was conducted at the Comprehensive Care Centre (CCC) of KNH. The KNH is the largest hospital in East and Central Africa. It was established in 1901 as the Native Civil hospital. It has an Accident and Emergency department, 24 theatres (16 specialized), 22 outpatient clinics and 50 wards. The KNH CCC is an outpatient clinic within the hospital that offers specialized care and treatment to HIV infected patients. It has over 5000 patients enrolled on care who usually visit the clinic for routine medical follow up and prescription refills. Other services also include viral load testing, CD4 cell count monitoring, liver function tests and renal function tests.

3.3 Study Population

The study population was made up of patients above 18 years of age on IPT at the CCC seen between February and August 2019.

3.4 Inclusion and Exclusion Criteria

Participants included in the study were HIV patients on Isoniazid Preventive Therapy for at least 12 weeks, of either gender, who gave written informed consent and were above 18 years of age. Participants who were excluded were those who declined to give consent, had hepatic dysfunction (ALT > 200 ULN), had renal dysfunction (GFR <60ml/min/1.73m^2) at the time of, had acute viral hepatitis, were pregnant and aged below 18 years at initiation of isoniazid preventive therapy.

3.6 Sample size

The method described in Kelsey et al (1996), for estimation of sample size in a cross-sectional study was used. Consequently, the StatCalc utility of the data management and analysis software Epiinfo^{TM} version 7.2 was used to calculate the target sample size using the Kelsey method.

For this calculation the following inputs were used; Two-sided confidence level was set at the conventional 95%, minimum statistical power was set at the conventional 80%, expected ratio of unexposed (fast acetylators) to exposed (slow acetylators) was set as 0.6 (38% fast acetylators vs 62% slow acetylators) (Oluka,2012), estimated prevalence of elevated ALT in unexposed (fast acetylators) was set as 10%, estimated odds ratio was set at 4.5 based on the association between *NAT2* slow acetylators and anti-TB drug induced liver injury reported by (Mushiroda et al., 2016). The target sample size calculated based on the above assumptions and inputs was 110.

3.7 Sampling and Participant Recruitment

Sampling and participant recruitment was done at the CCC, convenient sampling was employed whereby a contracted health officer, recruited all potential and available participants eligible for the study, based on the inclusion and exclusion criteria. This involved recruiting patients on subsequent follow up visits and on prescription refills, following which patient data was collected using data collection forms (appendix F).

3.8 Data collection

A data abstraction form was used to obtain patient data, the demographic data collected included, gender, age, marital status, body weight, height, body mass index, education, ethnicity, alcohol use, and smoking. Clinical data collected included, ALT levels, first-line ART regimen and duration, second-line ART regimen and duration, adverse drug reactions, pre-existing diseases, concomitant medications, and CD4 cell counts (appendix F).

3.9 Blood sample collection and Processing

Blood samples were obtained from participants, who satisfied the inclusion criteria. Blood collection was done in the bleeding room of the CCC by an experienced phlebotomist. From each patient, 5ml of blood was obtained from the antecubital vein and was immediately transferred to an EDTA vacutainer. The blood was centrifuged; 3ml plasma (upper portion) was separated out and stored at -20°C and approximately 2ml of whole blood was stored at -20°C to be used for genotyping.

3.9.1 Genotyping

DNA extraction

This was done at the University of Washington laboratory, situated at the Clinical Chemistry Department University of Nairobi using the Qiaamp® DNA mini Kit as per manufacturer's instructions.

Equipment

The equipment used included sterile micro centrifuge tubes, pipettes, a heat block, spin columns and collection tubes (supplied with the kit), a vortexing machine, a centrifuge and a water bath.

DNA extraction procedure

The Qiaamp® DNA mini Kit was used for DNA extraction in accordance to manufacturer's instructions and it was composed of 20 µl Proteinase K, 200 µl PBS (Phosphate buffered saline). The buffers used were buffer AI (lysis buffer), AW1 and AW2 Buffer (wash Buffers) and AE Buffer (elution Buffer). DNA extraction was done from thawed whole blood using Qiaamp® DNA mini Kit described above as per the manufacturer's protocol.

200µL of thawed whole blood samples were pipetted into sterile micro centrifuge tubes, 20µl of proteinase K was then added to the whole blood samples. This was followed by adding 200µL of the lysis buffer (Buffer AL) to the samples to allow lysis of the whole blood samples to take place. This mixture was then vortexed for 15 seconds followed by incubation at 56ºC for 10 minutes. The mixture was centrifuged at 6000RCF for 1 minute, after which 200µL of ethanol was added to the lysates.

The mixture was centrifuged again at 6000 RCF for 1 minute and the resulting samples were transferred to spin columns with collection tubes. At 6000RCF, centrifugation was done and the collection tubes containing the filtrates were discarded.

For the purification step, 500 µL AW1 (wash buffer) was pipetted to the sterile Qiaamp® spin columns containing the lysates, the contents were then centrifuged at 6000RCF for 1 minute and resultant filtrates from the collection tubes were discarded. The spin columns were then transferred to sterile Qiaamp collection tubes and 500 µL AW2 (wash buffer) was added to the spin columns. The spin columns were consequently centrifuged at 20,000 RCF for 3 minutes. The resultant filtrate was discarded and spin columns were transferred into sterile collection tubes, that were spun at 20,000RCF for 1 minute from which the collection tubes containing the filtrate were discarded. Finally, 200 µL of AE buffer (elution buffer) was added to the spin columns which were transferred to 1.5-mL micro centrifuge tubes and spun at 6000RCF for 1 minute. The eluted purified genomic DNA were then transferred into 1.5 ml micro-centrifuge tubes and spin columns discarded. Following extraction, the DNA purity was verified, using a nanodrop spectrophotometer at the 260/280 ratio absorbance measurement. The DNA was then stored in a freezer at -20ºC for further processing.

3.9.2 DNA amplification

DNA amplification was done at University of Washington laboratory, situated at the Clinical Chemistry Department, University of Nairobi and was achieved on a conventional PCR thermal cycler (Thermofisher scientific).The reaction mix was composed of nuclease free water 27.7µl, x10 PCR buffer 5 µl, magnesium chloride 5µl, DNTP mix 5 µl, *NAT2* forward and reverse primer (Inqaba Biotec) 2.5 µl each, taq polymerase 0.3 µl and template DNA 2 µl with a final reaction volume of 50 µl .as shown in table 3.0 below.

Table 3.0:-Preparation of the reaction mix for PCR reaction.

COMPONENTS	96 well plate 50 µl, reaction
nuclease free water	27.7 µl,
x10 PCR buffer	5 µl,
magnesium chloride	5 µl,
DNTP mix	5 µl,
NAT2 forward primer GTTTTTCTTGCTTAGGGGATC	2.5 µl,
NAT2 reverse primer ATTAGTGAGTTGCGTGATACATA	2,5 µl,
taq polymerase	0.3 µl,
template DNA	2 µl,
Final volume	50 µl,

DNA amplification was achieved under the following conditions: An initial hold cycle for 2 minutes at 95°c, followed by 30 cycles of denaturation at 95°c for 30 seconds, annealing at 55°c for 30 seconds, extension at 72°c for 1 minute and a final hold cycle at 72°c for 10 minutes. After conventional PCR, PCR products were viewed on 2% agarose gel to confirm amplification. Figure 4.0 in the results section, shows the results of the gel electrophoresis following PCR amplification.

3.9.3 DNA sequencing

DNA sequencing was performed at the University of Nairobi Institute of Tropical and Infectious Diseases (UNITID) and was achieved on Veriti 96 well thermal cycler (Applied Biosystems) and 3730 DNA analyzer (Thermofisher scientific). For quality control, FASTQC was used to check the sequence quality. The steps included purification, cycle sequencing, capillary electrophoresis for DNA analysis and FASTQC Quality Control.

Purification

For this step 2.5 µl, of PCR product was mixed with 1 µl, of ExoSap (Exonuclease alkaline Phosphatase), to form a reaction volume of 3.5µl. This was then incubated to degrade excess primers and nucleotides at 37°C for 15 minutes followed by another incubation step to inactivate the ExoSap at 80°c for 15 minutes and finally a cooling step at 4°c for 5 minutes.

Cycle Sequencing

This step included, preparation of the reaction mix, PCR amplification and purification of the sequencing reaction using BigDye XTerminator. This step was achieved on the Veriti 96 well thermal cycler (Applied Biosystems). In the preparation step for the reaction mix, the reaction mix consisted of big dye terminator 1 µl, big dye terminator sequencing buffer 2 µl, forward primer 1 µl, molecular grade water 2.5 µl, and template DNA 3.5 µl with a final reaction volume of 10 µl, as shown in table 3.1.

Table 3.1: Preparation of the reaction mix for cycle sequencing

COMPONENTS	96 well plate 10 µl, reaction
Big dye terminator	1 µl,
Big dye terminator sequencing buffer	2 µl,
Forward primer GTTTTTCTTGCTTAGGGGATC	1 µl,
Molecular grade water	2.5 µl,
Template DNA	3.5 µl,
Final volume	10 µl,

The PCR amplification step for cycle sequencing was achieved under the following conditions, incubation at 96ºC for 1 minute followed by 25 cycles of denaturation at 96ºC for 10 seconds, annealing at 50ºC for 5 seconds, extension at 60ºC for 4 minutes and a final hold at 4ºC.

The reaction mix from the PCR cycle sequencing step underwent clean-up using the BigDye XTerminator. The reaction mix for the big dye termination consisted of 10 µl BigDye XTerminator bead solution and 45 µl SAM solution. In this step, 55 µl of bead mix (BigDye XTerminator and SAM solution) was dispensed in each well of the 96 well reaction plates. The plates were then sealed with transparent adhesive films followed by vortexing for 20 minutes at 1800RPM and centrifugation at 1000g for 2 minutes, finally the plates were stored at -20ºC prior to capillary electrophoresis.

Capillary electrophoresis

Capillary electrophoresis was used for the separation and identification of the fragments of fluorescently labeled DNA following cycle sequencing; this was achieved on the 3730 DNA analyzer (Thermo fisher scientific). The detected sequences were viewed on the Seqscape software and Snapgene viewer as shown in figure 4.0. Consequently, the sequences were checked and assessed for quality using FastQc as shown in figure 4.1, in the result section.

Homology similarity search

In order to identify alleles in this study population, A homology search was initially performed on a total of 80 sequences which were obtained following cycle sequencing. The online nucleotide Basic local alignment search tool (BLASTn) which is available at https://blast.ncbi.nlm.nih.gov/Blast.cgi and the bioinformatics software tool Unipro UGENE version 36 (Okonechnikov et al., 2012), were used. Each sequence was uploaded to the online BLASTn database and queried for homology against similar or related sequences in the nucleotide BLASTn database, so as to determine the relationship between the available *NAT2* sequences in the database and the obtained nucleotide sequences. This evaluation is shown in chapter 4.

Sequence alignment and SNP detection

Pairwise sequence alignment was done using the Unipro UGENE version 36 software (Okonechnikov et al., 2012). For sequence alignment, the exon of the *NAT2* reference sequence from human chromosome 8 GRCh38.p13 was obtained from the NCBI website found at https://www.ncbi.nlm.nih.gov/nuccore/CM000670.2?report. To identify SNPs (single nucleotide polymorphism), the exon regions of the identified alleles were aligned by pairwise alignment to the exon of the *NAT2* reference sequence, this allowed the subsequent identification of SNP positions.

3.9.4 Variables and Definitions

Observed genotypes were categorized into 2 categories, fast and slow acetylator genotypes, with the genotypes associated with the fast acetylation phenotype labeled as fast while genotypes associated with the slow acetylation phenotype labeled as slow. According to McDonagh et al, *NAT2* genotypes are classified into three phenotypes: slow acetylator (two slow alleles), intermediate acetylator (one slow and one rapid allele), and rapid acetylator (two rapid alleles, sometimes referred to as 'fast') *(McDonagh et al., 2014).*

ALT levels were transformed into a binary variable that consisted of 2 categories, ALT levels >40IU/L and ALT levels < 40IU/L Serum ALT levels >40IU/L were considered an indication of elevated ALT levels while levels < 40IU/L were considered as normal levels based on Kenyatta National hospital reference values (Olago-Rakuomi, 2013). The independent predictor variables were gender, alcohol status, smoking status, comorbidities and genotypic status.

3.9.5 Data management

Data was entered in a Microsoft Excel (2007) spreadsheet and the data transferred to SPSS version 25. The data was double checked by the investigator during data entry. This was also done for data generated following DNA sample processing. Any document linking the collected data to the patient files including the raw data was kept under lock and key and only accessible by the researcher.

3.9.6 Data Analysis

All continuous variables were tested for normal distribution using the Shapiro-wilk test. Standard deviation and mean were used to give a summary of variables that were normally distributed and those that were not normally distributed were expressed as the median and inter-quartile range. To investigate for associations, the chi-square test was used to assess the relationship between categorical variables so as to determine the factors associated with elevated ALT levels. In the event where sample sizes were small as outlined in the contingency table fisher's exact test was employed.

Statistical significance was accepted at $P \leq 0.05$. In addition the spearman correlation test was used to investigate correlations for non categorical variables which were not normally distributed as informed by the Shapiro-wilk test. Similarly Statistical significance was accepted at $P \leq 0.05$. The Hardy Weinberg equation for estimating proportions in genotypes was used to determine the prevalence of the genotypes and phenotypes in this population. The Hardy-Weinberg principle states that in a large population where there is no genetic drift, and in the absence of selection, migration and mutation, the allelic frequencies remain constant from generation to generation. If mating is random, the genotypic frequencies are related to the allelic frequencies by the square expansion of allelic frequencies (Neal et al., 2018). whereby, the frequency of genotypes in a population can be predicted from the frequency of alleles as a binomial distribution that is, if there are 2 alleles in a population with frequency P and Q where (P + Q = 1), the homozygotes will have frequencies P^2 and Q^2 and heterozygotes 2pq, in other words the expansion of $(P + Q)^2$ (Flint, 2017). To determine the independent predictors of hepatotoxicity, multivariate logistic regression analysis was carried out.

Multivariate regression analysis was carried out using SPSS statistics version 25 (IBM USA). For the analysis the outcome variable of interest was ALT levels which was used as a proxy indicator for hepatotoxicity, In this analysis, the variables, gender, age, bmi, comorbidities, smoking status, and alcohol were analyzed. Categorical variables were transformed into a binary format of either 1 or 0, in excel 2007. In the analysis, Binary and continuous data served as inputs for the SPSS tool, whereby a multivariate logistic regression analysis was then carried out. ALT levels above 40IU/L, were considered hepatotoxic and those below this threshold were considered normal as informed by the current Kenyatta National Hospital ALT levels (Olago-Rakuomi, 2013) .

3.9.7 Ethical considerations

Permission to do the study was given by Kenyatta National Hospital/University of Nairobi Research and Ethics committee (Ref: KNH-ERC/A/499). The letter granting ethical approval to conduct the study is appended at (Appendix A). In addition, as outlined in the Volunteer information and consent form (Appendix B), informed consent was obtained and patient data that was collected was kept private and confidential by the use of codes instead of patient identification information.

CHAPTER 4:- RESULTS

The results of this study are presented in three different parts. Genomic analysis, Clinical and socio-demographic characteristics of study participants, prevalence of *NAT2* variant alleles and genotypes, ALT levels of study participants, and factors that influenced ALT levels.

4.0 Genomic Analysis

Figure 4.0: *Chromatogram Peak distribution (Snap gene viewer version 5.2.2)*

Figure 4.1:*FASTQC Quality Control check.*
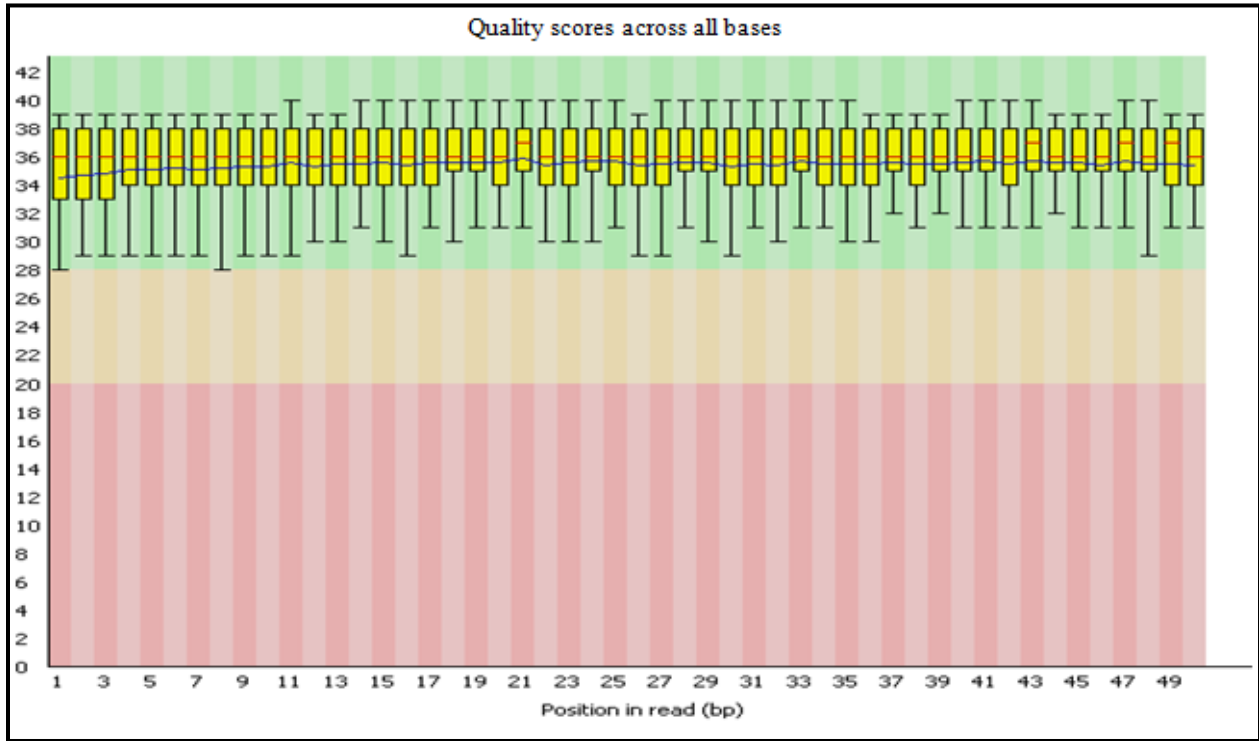
Figure 4.2 *Agarose gel electrophoresis of 873bp N acetyl transferase 2 gene in human samples (from left to right) lane 113 molecular ladder 1kb, lane 88 – 99 human samples*
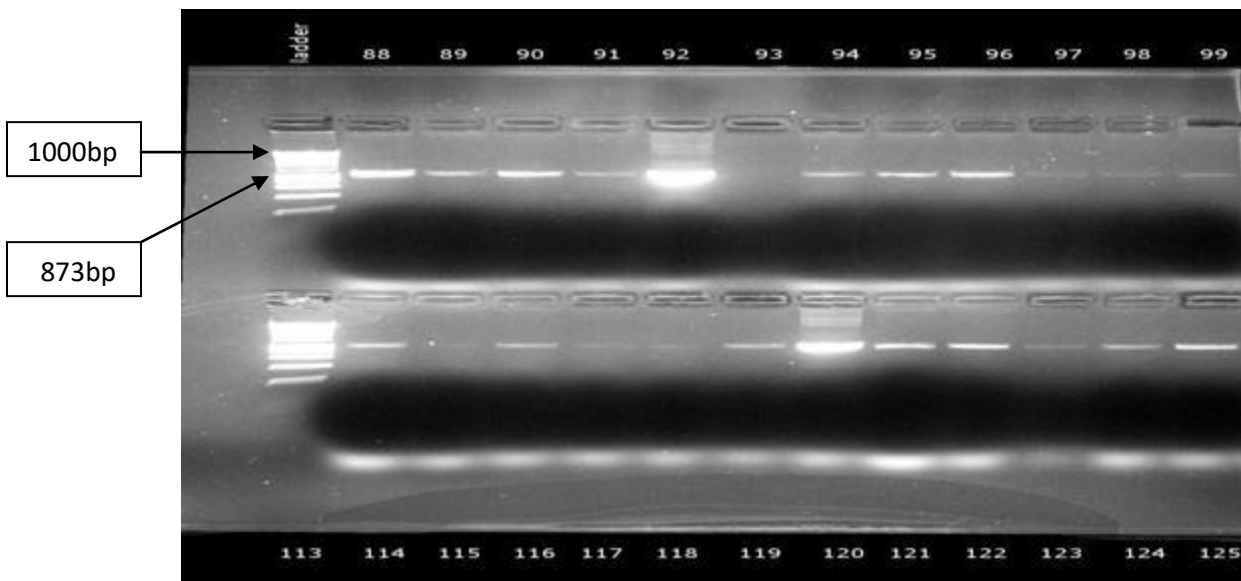
Sequence Analysis

Using the Basic Local Alignment tool, nucleotide sequences from the 873 bp amplicons were obtained and examined using (BLASTn). The results obtained using the tool, showed that 41 out of the 80 sequences, were closely related to NAT2 genes with a percentage identitiy > 95% as shown in figure 4.3

	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
✓	Homo sapiens isolate FBon09_1_NAT2*5A arylamine N-acetyltransferase 2 (NAT2) gene, complete cds	Homo sapiens	1055	1055	82%	0.0	100.00%	1396	KR231338.1

Figure 4.3:- *Depiction to display similarity between detected NAT2 genes and deposited reference genes in the BLAST database.*

In the subsequent analysis, Unipro UGENE version 36 (Okonechnikov et al., 2012), was employed for the detection of SNPS in the obtained sequences. In the preliminary investigation, the exon of the reference sequence from human chromosome 8, GRCh38.p13 was initially obtained from the NCBI website https://www.ncbi.nlm.nih.gov/nuccore/CM000670.2?report and was used to detect and identify the SNPs (single nucleotide polymorphism). A total of 41 sequences were used in the pairwise alignment, 39 sequences could not be comprehensively resolved in the BLAST database and hence could not be identified; as such, they were not included in this investigation. In the investigation, the exon regions of the obtained *NAT2* query sequences were aligned using pairwise alignment to the exon of the 873bp *NAT2* reference genome and subsequently the SNPs were identified using Unipro UGENE (Okonechnikov et al., 2012). Figure 4.4 shows the pairwise alignment for the detection of SNP's between 1 of the test sequences used in this analysis.
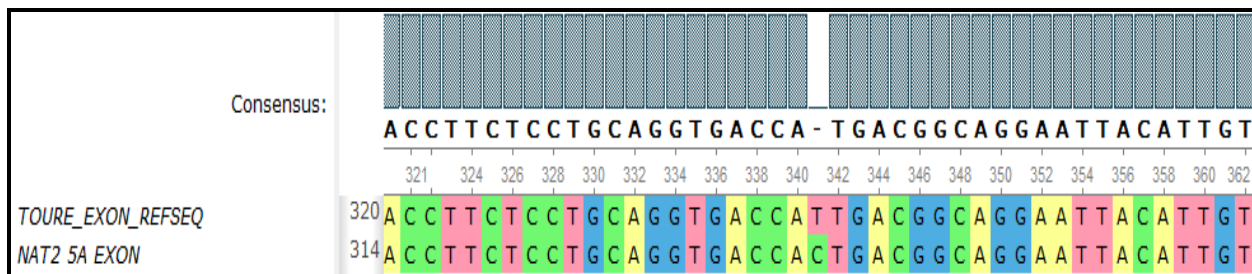
Figure 4.4:- *Pairwise alignment between test sequence exon and NAT2*5A exon.*

At position 341 there is a substitution of the thymine nucleotide in the reference sequence to cytosine nucleotide in the NAT2*5A exon.
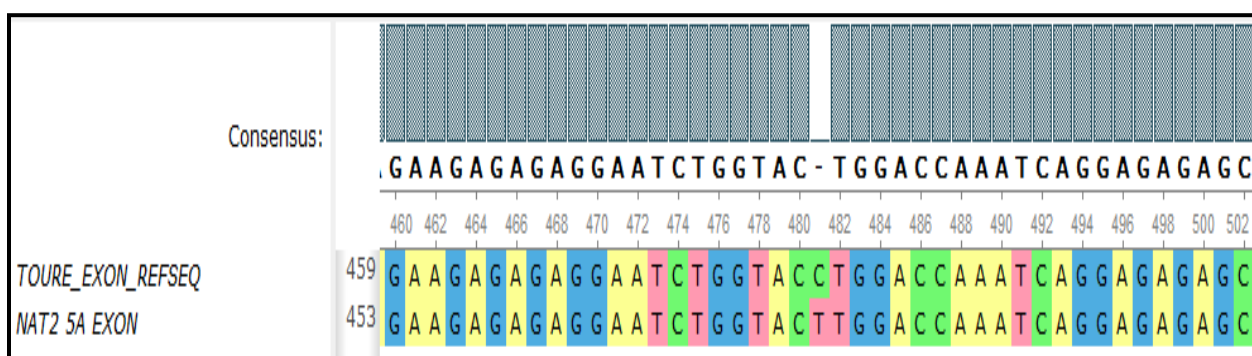
Figure 4.5:- *Pairwise alignment between test sequence exon and NAT2*5A exon.*

At position 481 there is a substitution of the cytosine nucleotide in the reference sequence to a thymine nucleotide in the NAT2*5A exon.
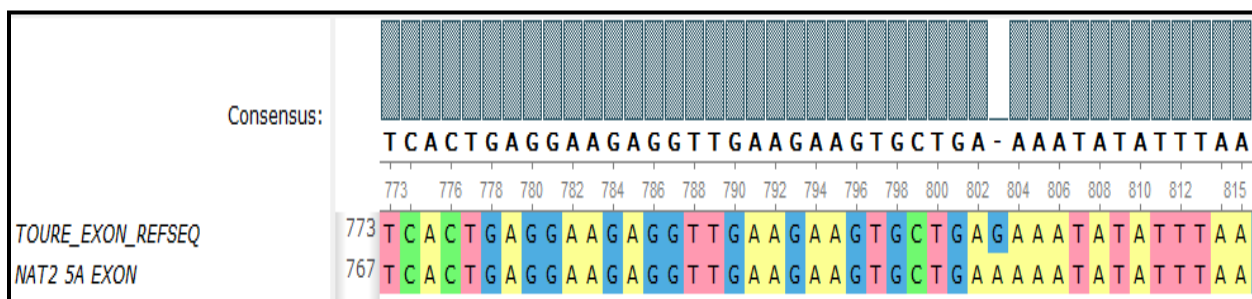
Figure 4.6:- *Pairwise alignment between test sequence exon and NAT2*5A exon.*

At position 803 there is a substitution of the guanine nucleotide in the reference sequence to an Adenine nucleotide in the NAT2*5A exon.

The SNPs identified for this test sequence which was inferred as *NAT2*5A* based on pairwise alignment to the reference exon were SNPs 341 C-T, 481 T-C and 803 G-A, which is consistent

with literature findings. This strategy was as a result employed to analyze all the 41 sequences and hence identify the type of *NAT2* allele present.

The identification of subsequent *NAT2* alleles also took into account the reported SNP data found at https://www.snpedia.com/index.php/NAT2 and the *NAT2* gene nomenclature committee found at http://nat.mbg.duth.gr/ to accurately identify the type of *NAT2* allele present based on the identified SNP positions obtained from pairwise alignment.

4.1 Socio - Demographic Characteristics of the study participants

These characteristics are summarized in table 4.1. A total of 110 participants took part in this study of which majority were female 77 (70%). The mean age for this population was 46.7 years [SD = 10.4] ranging from (19 - 72 yrs). The median body weight was 70 kg [inter-quartile range (IQR) 62.7-80 kg], with median Body Mass Index of 25.75 kg/m^2 [IQR 23.15 – 29.26 kg/m^2], the mean height was 163.8 cm, with a [SD = 8.18]. Majority of the participants were Bantus 82 (75%), while 28 (25%) were Nilotes.

Participants with (degree and diploma) college education were 24(22%) and 17(15%) respectively. Majority of the participants were married 52(48%) whereas 44(40%) reported alcohol use and only 3(3%) were smokers.

4.2 Clinical Characteristics of the study participants

The clinical characteristics are shown in table 4.2. These characteristics were collected after 12 weeks of IPT, The median ALT levels while on HAART was 22u/L, [IQR 21-31u/L], while the median CD4 cell count was 492 cells/mm^3 [IQR 324 – 680 cells/mm^3]. Participants with comorbidities were 23 (21%) while 87 (79%) had no other illness. Details of ART regimen are shown in table 4.2. The first line regimens that the participants used included 55 (50%) on Tenofovir disoproxil (TDF) + Lamivudine (3TC) + Efavirenz (EFV), 40 (36%) on tenofovir disoproxil (TDF) + Lamivudine (3TC) + Dolutegravir (DTG) and 1 (1%) participant on Zidovudine (AZT) + Lamivudine (3TC) + Nevirapine (NVP).

The second line regimens included 4 participants (3.6%) on Atazanavir/ritonavir (ATZ/r) + Zidovudine (AZT) + Lamivudine (3TC) and 5 (4.5%) on Tenofovir disoproxil (TDF) + Lamivudine (3TC) + Zidovudine (AZT).

Table 4.1 Socio-Demographic Characteristics of HIV patients on IPT at Kenyatta National Hospital CCC

Variable	Characteristic	n(%)	Mean(SD)	Median(IQR)
Age (years)			46.7(10.5)	
Sex	Male	33 (30)		
	Female	77 (70)		
Weight at diagnosis (kg)				70 (62-80)
Height (cm)		163.8		
BMI (kg/m^2)				25.7(23.1-29.2)
Ethno linguistic groups				
	Bantus	82 (75)		
	Nilotes	28 (25)		
Education level				
	Degree	24 (21)		
	Diploma	17 (15)		
	Secondary	42 (38)		
	Primary	21 (19)		
	None	7 (6)		
Marital status				
	Married	48 (44)		
	Single	23 (20)		
	Divorced	11 (10)		
	Widowed	15 (13)		
	Separated	8 (7)		
	Unknown	5 (4)		
Smoking				
	No	97(88)		
	Yes	3(3)		
	Unknown	10(9)		
Alcohol use				
	No	53(48)		
	Yes	47(42)		
	Unknown	10(9)		

In addition to isoniazid for IPT, other concurrent medication used were pyridoxine, nifedipine, hydrochlorothiazide, co-trimoxazole, fluconazole, metformin, insulin, lorsartan and carbamazepine. The clinical characteristics are presented in Table 4.2.

Table 4.2: clinical characteristics of HIV patients on IPT at the CCC of Kenyatta National Hospital

Variable	Characteristic	n(%)	Mean(SD)	Median(IQR)
Alcohol use				
	No	44(40)		
	Yes	58(53)		
	Unknown	8(7)		
ALT Levels at IPT				22IU/L(21-31)
	Normal ($\leq$40 u/l)	103 (94)		
	Elevated (>40 u/l)	7 (6)		
CD4 cell count				492cells/mm^3(324-680)
	$\leq$ 250 cells/mm^3	17 (15)		
	> 250 cells/mm^3	85 (77)		
	Unknown	8(7)		
Comorbidities				
	None	87(79)		
	Hypertension	14(13)		
	Diabetes	2(2)		
	Other conditions	7(6)		
Regimens during HAART				
(First line)	TDF + 3TC + EFV	55(50)		
	TDF + 3TC + DTG	40(36)		
	AZT + 3TC + NVP	1(1)		
(Second line)	ATZ/r + AZT + 3TC	4(4)		
	TDF + 3TC + AZT	5(5)		
	Unknown	5(5)		

4.3 Prevalence of *NAT2* alleles and genotypes

4.3.1Overall prevalence of *NAT2* alleles and genotypes

In this study population, 80 samples were sequenced. This was followed up by genotyping which included a BLAST search and pairwise alignment, of these 80 sequences 41 sequences displayed good sequence homology, with percentage identities close to or equal to 100%. 39 sequences could not be resolved, hence could not be identified using the online BLAST tool The frequency of NAT2 genotypes was thus determined in 41 individuals, and the alleles which were determined are *NAT2*4, NAT2*5A, NAT2*5B, NAT2*5D, NAT2*6B, NAT2*7B, NAT2*12A* and *NAT2*14G*. The genotypes which were determined are *NAT2*4*4, NAT2*5A*5A, NAT2*5B*5B, NAT2*5D*5D, NAT2*6B*6B, NAT2*6C*6C, NAT2*7B*12A* and *NAT2*14G*14G*. These alleles and genotypes were identified from DNA sample processing as outlined in section 4.0.

Table 4.3 shows the summary statistics for NAT2 allele frequencies. All the alleles determined in the study population apart from *NAT2*4* and *NAT2*7A, NAT2*12A* were slow acetylator alleles, and therefore the number of homozygous recessive genotypes in this population was 36. This was used to calculate the allelic and genotypic frequencies based on the Hardy Weinberg principle.

Table 4.3: *NAT2* Allele Frequencies in HIV patients on IPT at the CCC of Kenyatta National Hospital

NAT2 Allele Frequency Table		
NAT2 haplotypes	SNP	Frequency(%)
*NAT2*4*	None	1%
*NAT2*5A*	T341C, C481T	2%
*NAT2*5B*	T341C, C481T, A803G	9%
*NAT2*5D*	T341C	2%
*NAT2*6B*	G590A	6%
*NAT2*6C*	C282T,G590A,G803A	9%
*NAT2*7B*	C282T,G857A	4%
*NAT2*12A*	A803G	4%
*NAT2*14G*	G191A,C282T,A803G	4%

4.3.2 *NAT2* Genotypes and deduced Phenotypes

Predicted phenotypes were derived from the NAT2 genome nomenclature website found at http://nat.mbg.duth.gr based on the given diploid haplotype combination. If the combination consisted of two alleles with reported low activity, it was considered a slow acetylator allele; similarly, if a diploid combination consisted of two duplicate alleles with reported fast activity or two alleles with one reported fast and the other reported low activity, it was considered a fast acetylator allele. Table 4.4 displays the inferred phenotypes.

Table 4.4: *NAT2* Genotype Frequencies in HIV patients on IPT at the CCC of Kenyatta National Hospital

NAT2 Genotype Frequency Table			
Genotype	N	Frequency(%)	Deduced phenotype
*NAT2*4*4*	1	1%	Rapid
*NAT2*5A*5A*	2	2%	Slow
*NAT2*5B*5B*	10	9%	Slow
*NAT2*5D*5D*	3	2%	Slow
*NAT2*6B*6B*	7	6%	Slow
*NAT2*6C*6C*	10	9%	Slow
*NAT2*7B*12A*	4	4%	Rapid
*NAT2*14G*14G*	4	4%	Slow

Based on the Hardy-Weinberg principle, the calculated proportion of the slow acetylator alleles in this population was 56% and fast acetylator alleles was 44%, which corresponded to 62 and 48 study participants with the slow acetylator alleles and fast acetylator alleles respectively, in this study sample. The prevalence of homozygous dominant *NAT2* genotypes in this population was (21)19%, heterozygous genotypes were (55)50% while the homozygous recessive genotypes were (36)31%, these frequencies conformed to Hardy weinberg proportions.

4.3.3 *NAT2* genotypes and allele frequencies in the ethno-linguistic groups

The *NAT2* allele and genotype frequencies are presented in Table 4.6 and 4.7 respectively in this population, there were 2 Kenyan ethno-linguistic groups, Bantus and Nilotes. Bantu participants with the slow acetylator alleles were 24(22%), while Bantu participants with the fast acetylator alleles were 3(3%); Nilote participants with the fast acetylator alleles were 2(2%) while Nilote participants with the slow acetylator alleles were 12(11%). The data presented in table 4.5 shows the 41 followed-up sequences from the two ethno-linguistic groups that were genotyped and phenotypes inferred as previously described in section 4.3.2 above.

Table 4.5 *NAT2* Genotypes in Ethno-linguistic groups in HIV patients on IPT at the CCC of Kenyatta National Hospital

NAT2 GENOTYPE FREQUENCY IN ETHNO-LINGUISTIC GROUPS			
Genotypes **RA (Rapid Acetylators)**	**BANTUS**	**NILOTES**	**TOTALS**
*NAT2*4*4*	1	0	1
*NAT2*7B*12*	2	2	4
TOTALS	3	2	5
SA (Slow Acetylators)			
*NAT2*5A*5A*	2	0	2
*NAT2*5B*5B*	6	4	10
*NAT2*5D*5D*	2	1	3
*NAT2*6B*6B*	7	0	7
*NAT2*6C*6C*	6	4	10
*NAT2*14G*14G*	1	3	4
TOTALS	24	12	36
SUM TOTAL	27	14	41

In the subsequent analysis, on fisher's exact test, there was no significant difference in the distribution of *NAT2* genotypes between the ethno-linguistic groups (P = 1.00). Table 4.6 shows the overall *NAT2* allele frequency in the ethno-linguistic groups.

Table 4.6: Overall *NAT2* Allele Frequency in Ethno-Linguistic Groups in HIV patients on IPT at the CCC of Kenyatta National Hospital

NAT2 ALLELE FREQUENCY IN ETHNO-LINGUISTIC GROUPS			
Alleles	**Alleles in BANTU (P%)**	**Alleles in NILOTES (P%)**	**TOTAL**
*NAT2*4*	2(2%)	0	2
*NAT2*5A*	4(4%)	0	4
*NAT2*5B*	12(11%)	8(7%)	20
*NAT2*5D*	4(4%)	2(2%)	6
*NAT2*6B*	14(13%)	0	14
*NAT2*6C*	12(11%)	8(7%)	20
*NAT2*7B*	2(2%)	2(2%)	4
*NAT2*12A*	2(2%)	2(2%)	4
*NAT2*14G*	2(2%)	6(5%)	8
TOTAL	54	28	82

The deduced phenotypes based on the ethno-linguistic groups were also determined and are shown in table 4.7.

Table 4.7: *NAT2* **Deduced Phenotypes in Ethno-Linguistic Groups in HIV patients on IPT at the CCC of Kenyatta National Hospital**

NAT2 DEDUCED PHENOTYPE IN ETHNO-LINGUISTIC GROUPS				
Genotypes	**BANTUS**	**NILOTES**	**DEDUCED PHENOTYPE**	**FREQUENCY(%)**
*NAT2*4*4*	1	0	fast	1%
*NAT2*5A*5A*	2	0	slow	2%
*NAT2*5B*5B*	6	4	Slow	9%
*NAT2*5D*5D*	2	1	Slow	3%
*NAT2*6B*6B*	7	0	Slow	6%
*NAT2*6C*6C*	6	4	Slow	9%
*NAT2*7B*12A*	2	2	Fast	4%
*NAT2*14G*14G*	1	3	slow	4%

4.3.4 Distribution of *NAT2* genotypes and alleles by gender

Among the genotyped study participants, there were 18 males and 18 females with the slow acetylator alleles, and there were 2 males and 3 females with the fast acetylator alleles. There was no significant association between gender and *NAT2* genotypes (p = 1.00). Table 4.8 shows the genotypic frequencies based on gender.

Table 4.8: *NAT2* **Genotype Frequency based on Gender in HIV patients on IPT at the CCC of Kenyatta National Hospital**

NAT2 GENOTYPE FREQUENCY BASED ON GENDER			
Genotypes *RA (Rapid Acetylators)*	**MALE**	**FEMALE**	**TOTALS**
*NAT2*4*4*	0	1	1
*NAT2*7B*12*	2	2	4
TOTALS	2	3	5
SA (Slow Acetylators)			
*NAT2*5A*5A*	1	1	2
*NAT2*5B*5B*	4	6	10
*NAT2*5D*5D*	2	1	3
*NAT2*6B*6B*	3	4	7
*NAT2*6C*6C*	6	4	10
*NAT2*14G*14G*	2	2	4
TOTALS	18	18	36
SUM TOTAL	20	21	41

Based on Gender the observed Allele frequencies are also outlined as shown in table 4.9.

Table 4.9: *NAT2* **Allele Frequencies according to Gender in HIV patients on IPT at the CCC of Kenyatta National Hospital**

NAT2 ALLELE FREQUENCY AMONG GENDER			
Alleles	**Alleles in Males (P%)**	**Alleles in Females (P%)**	**TOTAL**
*NAT2*4*	0	2(2%)	2
*NAT2*5A*	2(2%)	2(2%)	4
*NAT2*5B*	8(7%)	12(11%)	20
*NAT2*5D*	4(4%)	2(2%)	6
*NAT2*6B*	6(5%)	8(7%)	14
*NAT2*6C*	12(11%)	8(7%)	20
*NAT2*7B*	2(2%)	2(2%)	4
*NAT2*12A*	2(2%)	2(2%)	4
*NAT2*14G*	4(4%)	4(4%)	8
TOTAL	40	42	82

The deduced phenotypes based on gender were also determined and are shown in table 4.10

Table 4.10: *NAT2* **Deduced Phenotypes among Gender in HIV patients on IPT at the CCC of Kenyatta National Hospital**

NAT2 DEDUCED PHENOTYPES AMONG GENDER				
Genotypes	**MALES**	**FEMALES**	**DEDUCED PHENOTYPE**	**FREQUENCY(%)**
*NAT2*4*4*	0	1	Fast	1(1%)
*NAT2*5A*5A*	1	1	Slow	2(2%)
*NAT2*5B*5B*	4	6	Slow	10(9%)
*NAT2*5D*5D*	2	1	Slow	3(3%)
*NAT2*6B*6B*	3	4	Slow	7(6%)
*NAT2*6C*6C*	6	4	Slow	10(9%)
*NAT2*7B*12A*	2	2	fast	4(4%)
*NAT2*14G*14G*	2	2	slow	4(4%)

4.4 Factors influencing ALT levels

4.4.1 Effect of *NAT2* phenotypes on ALT levels

Table 4.11: Effect of *NAT2* phenotypes on ALT levels in HIV patients on IPT at the CCC of Kenyatta National Hospital

	ALT LEVELS		
Genotypes	**Above 40IU/l**	**Below 40IU/l**	**TOTALS**
RA (Rapid Acetylators)			
*NAT2*4*4*	0	1	1
*NAT2*7B*12*	1	3	4
TOTALS	1	4	5
SA (Slow Acetylators)			
*NAT2*5A*5A*	1	1	2
*NAT2*5B*5B*	0	10	10
*NAT2*5D*5D*	0	3	3
*NAT2*6B*6B*	1	6	7
*NAT2*6C*6C*	0	10	10
*NAT2*14G*14G*	0	4	4
TOTALS	2	34	36
SUM TOTAL	3	38	41

On Fisher's exact test, it was observed that there was no significant association between ALT levels and the *NAT2* genotypes (P = 0.330).

Table 4.12: Factors affecting ALT levels in HIV patients on IPT at the CCC of Kenyatta National Hospital

	ALT LEVELS			
	above 40IU/L	below 40IU/L	Totals	
Alcohol intake				P value
Alcohol	4	43	47	0.577
No alcohol	5	48	53	
Totals	9	91	100	
Smoking status				
Smoking	0	3	3	0.751
Non-smoking	9	88	97	
Totals	9	91	100	
Comorbidities				
Comorbidities	4	19	23	0.127
No-comorbidities	6	81	87	
Totals	10	100	110	
Gender				
Female	6	71	77	0.346
Male	4	29	33	
Totals	10	100	110	

4.4.2 Effect of alcohol on ALT levels

On Fisher's exact test, there was no association between alcohol intake and ALT levels (P = 0.577). A summary of the statistics is shown in Table 4.12.

4.4.3 Effect of Smoking on ALT levels

The smoking status and ALT levels were investigated for association. The Chi-square test of independence found no association between the smoking status and the ALT levels (P = 0.751); similarly. The smoking status and ALT levels are presented in Table 4.12.

4.4.4 Effect of comorbidities on ALT levels

It was observed that there was no association between the comorbidities and the patient ALT levels (P = 0.127), a large majority of the population who had comorbidities had ALT levels below 40IU/L. The summary statistics are presented in Table 4.12.

4.4.5 Effect of gender on ALT levels

For this analysis, gender was compared to the two categories of ALT levels; ALT levels above 40IU/L and ALT levels below 40IU/L. On Fisher's exact test, it was observed that there was no significant association between gender and ALT levels (P = 0.346), similarly on the correlation test, The summary statistics are presented in Table 4.12

4.4.6 Effect of age on ALT levels

In this analysis, age was compared to ALT levels. On the spearman rank correlation test it was observed that there was no significant correlation between age and ALT levels (P = 0.577), The summary statistics are presented in Table 4.13.

Table 4.13:- Spearman correlation test for factors affecting ALT levels in HIV patients on IPT at the CCC of Kenyatta National Hospital.

Spearman correlation			
	Alt level	**Age**	**BMI**
Alt level	1	-0.055	0.149
(sig)	0	0.577	0.119
Age	-0.055	1	0.278
(sig)	0.577	0	0.003
BMI	0.149	0.278	1
(sig)	0.119	0.003	0

4.4.7 Effect of BMI on ALT levels

On the spearman rank correlation test it was observed that there was no significant correlation between BMI and ALT levels (P = 0.119), The summary statistics are presented in Table 4.13.

4.5 Logistic regression analysis

To investigate for the independent predictors of hepatotoxicity as determined by ALT levels, the variables gender, age, bmi, comorbidities, smoking status, and alcohol, were subjected to multivariate logistic regression analysis. On the test for multicollinearity, it was observed that there was no correlation between ALT levels and the independent predictor variables: - gender, age, bmi, comorbidities, smoking status, and alcohol ($P > 0.05$). In this analysis, the hosmer-lemeshow test was significant at ($P = 0.908$) implying that the logistic regression model was a good fit for modeling the data (Muchabaiwa, 2013). In the analysis, the *NAT2* genotype variable was not included in the final regression model due to incomparable samples as well as the lack of a significant association observed between the *NAT2* genotypes and ALT levels as seen in section 4.4 above.

On the logistic regression analysis, it was observed that the independent predictor variables; gender, age, bmi, comorbidities, smoking status, and alcohol, were not statistically associated with elevated ALT levels ($P > 0.05$). The summary of determinants of ALT levels is presented in Table 4.14.

Table 4.14: Determinants of ALT levels in HIV patients on IPT at the CCC of Kenyatta National Hospital

	Adjusted odds ratio	P value	95% C.I for Exp	
			Lower	Upper
Alcohol	1.121	0.881	0.250	5.037
Smoking	0	0.999	0	
Comorbidities	2.902	0.185	0.601	14.014
Gender	1.877	0.426	0.399	8.840
Age	0.589	0.575	0.092	3.761
BMI	3.115	0.116	0.754	12.861

CHAPTER 5 - DISCUSSIONS, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

This study set to investigate the relationship between Acetylator status and isoniazid induced hepatotoxicity (as indicated by elevated ALT levels) in patients on isoniazid preventive therapy at Kenyatta National Hospital between the period of February and August 2019. Among the 110 study participants, 80 samples were sequenced while 30 samples were not sequenced so as to mitigate the cost around sequencing. Following sequencing, genotyping was performed, which included a BLAST search and pairwise alignment to the reference *NAT2* exon to determine present alleles and genotypes. The observed genotype data were then used to infer phenotypes using the *NAT2* gene nomenclature committee classification and categorization obtained from the *NAT2* SNPedia websites, as outlined in chapter 4. 41 sequences displayed good sequence homology with percentage identities close to or equal to 100%. 39 sequences could not be resolved in the online BLAST tool and hence could not be identified. These sequences were used to supplement the genotypic frequency data described in Chapter 4.

Among the 41 genotyped sequences, there was a high frequency of the *NAT2*5B* (9%) and *NAT2*6C* (9%) alleles compared to *NAT2*7B* (4%) and *NAT2*14G* (4%) alleles in this population. This observation was comparable to similar studies on *NAT2* alleles, for instance a study done in Kenya reported a high frequency for the *NAT2*5* allele and *NAT2*6* allele in the population (Oluka et al., 2014). In South Africa a similar study on *NAT2* genotypes reported that the most frequent allele was *NAT2*5* (Mthiyane et al., 2020). A study done in Ethiopia also reported similar results with *NAT2*5* and *NAT2*6* having the highest frequencies compared to *NAT2*7* and *NAT2*14* (Aklillu et al., 2018).

In this study population, there were 2 ethno-linguistic groups, the Bantus and Nilotes. Among the 41 genotyped sequences, there were more Bantu 28(25%) participants with the slow *NAT2* alleles compared to the Nilotes 16(15%). In this study, there was a higher frequency of *NAT2*6*(15%) and *NAT2*5*(13%) alleles in both ethnic groups compared to *NAT2*4*(1%), *NAT2*7*(4%), *NAT2*12* (4%) and *NAT2*14* (4%) alleles. This was similar in comparison to a study in Kenya which reported a high prevalence of *NAT2*5* and *NAT2*6* alleles in Bantus (38.3%), Eastern Nilotes(41.6%) and Western Nilotes (32.6%). Similarly, the prevalence of *NAT2*6* alleles in the same study was Bantus (22.9%), Eastern Nilotes (26.6%) and Western Nilotes (22.8%). These were higher compared to the prevalence of *NAT2*7* and *NAT2*14* in the same population (Oluka et al., 2014).

The distribution of *NAT2* genotypes in the 41 genotyped sequences from the two populations was also investigated. It was observed that there was no association between the distribution of *NAT2* genotypes and the Ethno-linguistic groups. In the same Kenyan study, it was found that there were no significant differences in the distribution of *NAT2* genotypes between the three Kenyan populations (Oluka et al., 2014) this was similar by comparison to this study.

This study also investigated the distribution of *NAT2* genotypes by gender among the 41 genotyped sequences from the same population. In the subsequent analysis, it was observed that there were no significant differences in the distribution of *NAT2* genotypes and gender. This was similar to a study done in Pakistan on acetylation status which found the effect of gender on NAT 2 acetylation status to be non-significant (Akhter et al., 2019).

Similarly, there was no significant association between ALT levels and *NAT2* phenotypes (P = 0.330). Some studies however report Slow/intermediate *NAT2* acetylators as more likely to experience hepatotoxicity than rapid acetylators (Richardson et al., 2019). In contrast other studies report for a lack of a significant association between acetylator status and anti-tuberculoisis drug induced hepatotoxicity (Xiaozhen Lv et al., 2012). As a result, it is clear that more research on the effect of *NAT2* genotypes on anti-tuberculosis drug-induced hepatotoxicity is required to better explain associations or lack thereof.

This study showed that there was no significant association between alcohol intake and ALT levels. In comparison to other studies, alcohol intake was indicated as a significant factor for the elevation of ALT levels (Wang et al., 2002). Another study also corroborated this, more frequent and higher amount of alcohol consumption was found to adversely affect the liver physiology (Gogoi et al., 2017). In this study, the population of participants on alcohol consumption was moderately larger than the participants on no-alcohol consumption. To investigate the effect of alcohol consumption on ALT levels more comprehensively there is need for a larger sample size.

On the effect of smoking on ALT levels, this study found no significant association between smoking and ALT levels, this is likely because the majority of participants in this population were non-smokers. Some studies have however reported an association between smoking and elevated ALT levels (C.-S. Wang et al., 2002). In a study in Libya for example, cigarette smoking raised alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), plasma total cholesterol and triglycerides in a smoker group when compared with a non-smoker group (Alsalhen & Abdalsalam, 2014).

In this study, there was no association between comorbidities and ALT levels, this is likely because majority of the study population 87(79%) had no comorbidities and of those who had,

only 4(4%) had elevated ALT levels when compared to the baseline; in contrast to other studies, comorbidities have been reported to affect the pharmacokinetics of isoniazid which ultimately affects plasma isoniazid concentration and as a result can be linked to hepatotoxicity. In one such study, Diabetes Melittus status appeared to influence Pharmacokinetics and pharmacodynamic relationships for isoniazid and rifampicin (Alfarisi et al., 2018) as such it is also important, that extensive studies be done on the various comorbidities associated with isoniazid and other anti-TB related medication such as pyrazinamide, streptomycin and other anti-tubercular medications.

In this study, age, BMI, smoking status, alcohol status, comorbidities and gender were not significant as independent predictors of elevated ALT levels as an indication of isoniazid induced hepatotoxicity, this observation was similar from the results obtained in the chi-square test for independence as outlined in chapter 4. This is in contrast to other studies where pre-existing liver diseases, taking hepatotoxic drugs and having lower serum albumin were found to be significant factors associated with the development of anti-tuberculosis drug-induced hepatitis (Gezahegn et al., 2020). Similarly, extra-pulmonary TB, having a comorbid condition, alcohol consumption and being geriatric have also been found to have an increased risk of developing drug induced hepatotoxicity (Molla et al., 2021). As such, other factors that are likely to predict isoniazid-induced hepatotoxicity in a large population sample should also be collected as part of the demographic data.

5.2 Conclusion

The study found no significant association between *NAT2* phenotypes and ALT levels (P = 0.33). However it found that there was a high frequency of *NAT2*5* and *NAT2*6* alleles compared to *NAT2*7* and *NAT2*14* alleles among the 41 genotyped sequences in this study population.

The study also found that there was a higher frequency of *NAT2*5* and *NAT2*6* genotypes in both ethnic groups compared to *NAT2*7* and *14 genotypes among the 41 genotyped sequences in this study population. There was no association between the distribution of *NAT2* genotypes and the Ethno-linguistic groups (P = 1.00), among the 41 genotyped sequences in this study population.

It also found that there was no significant association between gender and the distribution of *NAT2* genotypes among the 41 genotyped sequences in this study population (P = 1.00). Similarly, in this study, there was no association between ALT levels and the factors age, BMI, smoking status, alcohol status, gender and comorbidities.

5.3 Study Limitations

The small sample size used in the study is a likely contributor to why the independent predictor variables were found to not be significant for the outcome variable. In addition, due to the high cost of DNA sequencing, only eighty participants were sequenced and even so there were 41 sequences that could be comprehensively genotyped. In addition, other polymorphisms of drug metabolizing enzymes were not considered in this study, and these may affect isoniazid hepatotoxicity. Plasma Isoniazid concentration was also not considered in this study, this may also affect hepatotoxicity.

Despite the fact that there were more Bantus than Nilotes and females than males in this study population, it is likely that bantus and females were overrepresented; however, it is also likely that these two demographics are a true reflection of the Kenyan population, as evidenced by the 2019 Kenyan population (Kenya National Bureau of Statistics, 2019) and housing census and data obtained from the statista website ("Kenya - total population by gender 2020," 2021), which show females to be the majority gender in Kenya. Similarly, according to data obtained from the world population review website, bantus make up the majority of the population in Kenya, accounting for 60% of the population ("Kenya population," 2021).

In addition, there was no routine liver function tests such as ALT or AST tests that were done as part of Isoniazid preventive therapy at Kenyatta National Hospital. As a result, the ALT levels used in this study were determined at the start of the study, after patients had completed 12 weeks of IPT after which, ALT tests were carried out. Information was obtained from data collection forms filled by study participants. Some sections were unfilled.or missing.

5.4 Recommendations

5.4.1 Recommendations for further research

For a better powered study, a large sample size is recommended. In addition, more studies on the influence of anti-TB medication such as isoniazid, rifampicin, pyrazinamide and streptomycin on patient health should be done. This should be conducted through periodic drug monitoring studies for assessing drug plasma concentration and genetic polymorphism studies with relation to drug metabolizing enzymes. In addition to these studies, the factors associated with the adverse effects of anti-TB medication taken over an extended period of time should also be investigated.

5.4.2 Recommendations for policy practice

In terms of patient care, periodic routine testing for adverse effects of drugs among patients on anti-TB medication as mentioned above is also recommended, in particular, it is important to

assess the period/duration in which liver function tests are to be considered effective for a definitive diagnosis for liver hepatotoxicity, this is because patients at the start of therapy may show low or high levels of liver enzymes depending on current regimens, duration of previous treatments, drug pharmacokinetic profile and other factors unique to the individual patient. In addition, caregivers, stakeholders and patients should also undertake to give or seek information that relates to the adverse effects of short and long term anti-TB related medication such as isoniazid, rifampicin etc.

REFERENCES

Adithan, C., Subathra, A., 2016. NAT2 gene polymorphism: Covert drug interaction causing phenytoin toxicity. Indian J. Med. Res. 143, 542–544. https://doi.org/10.4103/0971-5916.187101

Akhter, N., Iqbal, T., Jamil, A., Akram, M., Tahir, I.M., Munir, N., 2019. Determination of Arylamine N-Acetyltransferase 2 Acetylation Genotype by PCR and Phenotyping Using Dapsone Through High-Pressure Liquid Chromatography Assay: A Gender Wise Study. Dose-Response 17, 155932581985553. https://doi.org/10.1177/1559325819855537

Aklillu, E., Carrillo, J. A., Makonnen, E., Bertilsson, L., & Djordjevic, N. (2018). N-Acetyltransferase-2 (NAT2) phenotype is influenced by genotype-environment interaction in Ethiopians. *European Journal of Clinical Pharmacology*, *74*(7), 903–911. https://doi.org/10.1007/s00228-018-2448-y

Alfarisi, O., Mave, V., Gaikwad, S., Sahasrabudhe, T., Ramachandran, G., Kumar, H., Gupte, N., Kulkarni, V., Deshmukh, S., Atre, S., Raskar, S., Lokhande, R., Barthwal, M., Kakrani, A., Chon, S., Gupta, A., Golub, J. E., & Dooley, K. E. (2018). Effect of Diabetes Mellitus on the Pharmacokinetics and Pharmacodynamics of Tuberculosis Treatment. *Antimicrobial Agents and Chemotherapy*, *62*(11), e01383-18, /aac/62/11/e01383-18.atom. https://doi.org/10.1128/AAC.01383-18

Alsalhen, K. S., & Abdalsalam, R. D. (2014). Effect of cigarette smoking on liver functions: a comparative study conducted among smokers and non-smokers male in El-beida City, Libya. *International Current Pharmaceutical Journal*, *3*(7), 291–295. https://doi.org/10.3329/icpj.v3i7.19077

Asif, M., 2013. International Journal of Pharmaceutical Chemistry a review on potent antitubercular agent isoniazid and its analogues Mohammad Asif. Int. J. Pharm. Chem. 2, 110–120. https://doi.org/10.7439/ijpc.v2i4.755

Ayele, H.T., Van Mourik, M.S.M., Debray, T.P.A., Bonten, M.J.M., 2015. Isoniazid prophylactic therapy for the prevention of tuberculosis in HIV infected adults: A systematic review and meta-analysis of randomized trials. PLoS ONE 10, 1–16. https://doi.org/10.1371/journal.pone.0142290

Azuma, J., Ohno, M., Kubota, R., Yokota, S., Nagai, T., Tsuyuguchi, K., Okuda, Y., Takashima, T., Kamimura, S., Fujio, Y., Kawase, I., 2013. NAT2 genotype guided regimen reduces isoniazid-induced liver injury and early treatment failure in the 6-month four-drug standard treatment of tuberculosis: A randomized controlled trial for pharmacogenetics-based therapy. Eur. J. Clin. Pharmacol. 69, 1091–1101. https://doi.org/10.1007/s00228-012-1429-9

Bardou, F., Raynaud, C., Ramos, C., Lanéelle, M.A., Lanéelle, G., 1998. Mechanism of isoniazid uptake in Mycobacterium tuberculosis. Microbiology 144, 2539–2544. https://doi.org/10.1099/00221287-144-9-2539

Boelsterli, U.A., Lee, K.K., 2014. Mechanisms of isoniazid-induced idiosyncratic liver injury: Emerging role of mitochondrial stress. J. Gastroenterol. Hepatol. Aust. 29, 678–687. https://doi.org/10.1111/jgh.12516

Brewer, C. T., Yang, L., Edwards, A., Lu, Y., Low, J., Wu, J., Lee, R. E., & Chen, T. (2019). The Isoniazid Metabolites Hydrazine and Pyridoxal Isonicotinoyl Hydrazone Modulate Heme Biosynthesis. *Toxicological Sciences*, *168*(1), 209–224. https://doi.org/10.1093/toxsci/kfy294

Briggs, M.A., Emerson, C., Modi, S., Taylor, N. kenji, Date, 2015. Use of Isoniazid Preventive Therapy for Tuberculosis Prophylaxis Among People Living With HIV / AIDS : A Review of the Literature. JAIDS J. Acquir. Immune Defic. Syndr. 68, 297–305. https://doi.org/10.1097/QAI.0000000000000497

Brito, T.C., Possuelo, L.G., Valim, A.R.M., Todendi, P.F., Ribeiro, A.W., Gregianini, T.S., Jarczewski, C.A., Hutz, M.H., Rossetti, M.L.R., Zaha, A., 2014. Polymorphisms in CYP2E1, GSTM1 and GSTT1 and anti-tuberculosis drug-induced hepatotoxicity. An. Acad. Bras. Cienc. 86, 855–865. https://doi.org/10.1590/0001-3765201420130350

Cai, Y., Yi, J.Y., Zhou, C.H., Shen, X.Z., 2012. Pharmacogenetic Study of Drug-Metabolising Enzyme Polymorphisms on the Risk of Anti-Tuberculosis Drug-Induced Liver Injury: A Meta-Analysis. PLoS ONE 7, 1–8. https://doi.org/10.1371/journal.pone.0047769

centers for disease control., 2013. Core Curriculum on Tuberculosis : What the Clinician Should Know. Cent. Dis. Control Prev. Natl. Cent. HIVAIDS Viral Hepat. STD TB Prev. Div. Tuberc. Elimin. 1–320. https://doi.org/10.1128/IAI.00057-06

Chalasani, N., Bjornsson, E., 2011. Risk Factors for Idiosyncratic Drug-Induced Liver Injury. Gastroenterology 138, 2246–2259. https://doi.org/10.1053/j.gastro.2010.04.001.Risk

Chan, S.L., Chua, A.P.G., Aminkeng, F., Chee, C.B.E., Jin, S., Loh, M., Gan, S.H., Wang, Y.T., Brunham, L.R., 2017. Association and clinical utility of NAT2 in the prediction of isoniazid-induced liver injury in Singaporean patients. PLoS ONE 12, 1–11. https://doi.org/10.1371/journal.pone.0186200

Chiriac, A, Brzezinski, P., Chiriac, AE, Foia, L., Mihaila, D., Solovan, C., 2015. Isoniazid – induced chronic cutaneous Lupus erythematosus. http://dx.doi.org/10.3126/njdvl.v13i1.14306

Cojutti, P., Duranti, S., Isola, M., Baraldo, M., Viale, P., Bassetti, M., Pea, F., 2016. Might isoniazid plasma exposure be a valuable predictor of drug-related hepatotoxicity risk among adult patients with TB? J. Antimicrob. Chemother. 71, 1323–1329. https://doi.org/10.1093/jac/dkv490

Ebeshi, B.U., Bolaji, O.O., Masimirembwa, C.M., 2011. Arylamine N-acetyltransferase 2 (NAT2) single nucleotide polymorphisms ' frequencies in Nigerian populations. Afr. J. Pharm. Pharmacol. Res. 1, 1–6.

El Bouazzi, O., Hammi, S., Bourkadi, J.E., Tebaa, A., Tanani, D.S., Soulaymani-Bencheikh, R., Badrane, N., Bengueddour, R., 2016. First line anti-tuberculosis induced hepatotoxicity: Incidence and risk factors. Pan Afr. Med. J. 25, 1–11. https://doi.org/10.11604/pamj.2016.25.167.10060

Fan, W., 2014. The role of potential antioxidant in medicinal drug- induced oxidative stress. Missouri university of Science and Technology. https://scholarsmine.mst.edu/cgi/viewcontent.cgi?article=3328&context=doctoral_dissertations

Flint, J., 2017. Deep Reads: How I learnt to love population genetics. PLoS Genet. 13, 1–6. https://doi.org/10.1371/journal.pgen.1007140

Gaude, G., Chaudhury, A., Hattiholi, J., 2015. Drug-induced hepatitis and the risk factors for liver injury in pulmonary tuberculosis patients. J. Fam. Med. Prim. Care 4, 238. https://doi.org/10.4103/2249-4863.154661

Gezahegn, L. K., Argaw, E., Assefa, B., Geberesilassie, A., & Hagazi, M. (2020). Magnitude, outcome, and associated factors of anti-tuberculosis drug-induced hepatitis among tuberculosis patients in a tertiary hospital in North Ethiopia: A cross-sectional study. *PLOS ONE*, *15*(11), e0241346. https://doi.org/10.1371/journal.pone.0241346

Gichuki, J., & Mategula, D. (2021). Characterisation of tuberculosis mortality in informal settlements in Nairobi, Kenya: Analysis of data between 2002 and 2016. *BMC Infectious Diseases*, *21*(1), 718. https://doi.org/10.1186/s12879-021-06464-2

Gogoi, J. B., Bhasin, M., Behera, J. K., Gairola, K., & Baruah, H. (2017). A study of effect of alcohol on liver function tests (LFT) in Garhwal hills, India. *International Journal of Research in Medical Sciences*, *6*(1), 94. https://doi.org/10.18203/2320-6012.ijrms20175536

Guaoua, S., Ratbi, I., Laarabi, F.Z., Elalaoui, S.C., Jaouad, I.C., Barkat, A., Sefiani, A., 2014. Distribution of allelic and genotypic frequencies of NAT2 and CYP2E1variants in Moroccan population. BMC Genet. 15, 156. https://doi.org/10.1186/s12863-014-0156-x

Guidelines for Management of Tuberculosis and Leprosy in kenya. (2013). *Guidelines for Management of Tuberculosis and Leprosy in Kenya*. http://guidelines.health.go.ke:8000/media/TB_Treatment_GUIDELINES_2013.pdf

Hein, D.W., 2006. N-acetyltransferase 2 genetic polymorphism: Effects of carcinogen and haplotype on urinary bladder cancer risk. Oncogene 25, 1649–1658. https://doi.org/10.1038/sj.onc.1209374

Hsieh, Y. L., Jahn, A., Menzies, N. A., Yaesoubi, R., Salomon, J. A., Girma, B., Gunde, L., Eaton, J. W., Auld, A., Odo, M., Kiyiika, C. N., Kalua, T., Chiwandira, B., Mpunga, J. U., Mbendra, K., Corbett, L., Hosseinipour, M. C., Cohen, T., & Kunkel, A. (2021). *An evaluation of 6-month versus continuous isoniazid preventive therapy for M. tuberculosis in adults living with HIV/AIDS in Malawi*. 17.

Huang, Y.S., 2014. Recent progress in genetic variation and risk of antituberculosis drug-induced liver injury. J Chin Med Assoc 77, 169–173. https://doi.org/10.1016/j.jcma.2014.01.010

Isa, S.E., Ebonyi, A.O., Shehu, N.Y., Idoko, P., Anejo-Okopi, J.A., Simji, G., Odesanya, R.U., Abah, I.O., Jimoh, H.O., 2016. Antituberculosis drugs and hepatotoxicity among hospitalized patients in Jos, Nigeria. Int. J. Mycobacteriology 5, 21–26. https://doi.org/10.1016/j.ijmyco.2015.10.001

Jaramillo-Valverde, L., Levano, K. S., Tarazona, D. D., Capristano, S., Zegarra-Chapoñan, R., Sanchez, C., Yufra-Picardo, V. M., Tarazona-Santos, E., Ugarte-Gil, C., & Guio, H. (2022). NAT2 and CYP2E1 polymorphisms and antituberculosis drug-induced hepatotoxicity in Peruvian patients. *Molecular Genetics & Genomic Medicine*, *10*(8). https://doi.org/10.1002/mgg3.1987

Jena, L., Waghmare, P., Kashikar, S., Kumar, S., Harinath, B.C., 2014. Computational approach to understanding the mechanism of action of isoniazid, an anti-TB drug. Int. J. Mycobacteriology 3, 276–282. https://doi.org/10.1016/j.ijmyco.2014.08.003

Kabbara, W.K., Sarkis, A.T., Saroufim, P.G., 2016. Acute and Fatal Isoniazid-Induced Hepatotoxicity: A Case Report and Review of the Literature. Case Rep. Infect. Dis. 2016, 1–6. https://doi.org/10.1155/2016/3617408

Kagaya, H., Miura, M., Niioka, T., Saito, M., Numakura, K., Habuchi, T., Satoh, S., 2012. Influence of NAT2 Polymorphisms on Sulfamethoxazole Pharmacokinetics in Renal Transplant Recipients. Antimicrob. Agents Chemother. 56, 825–829. https://doi.org/10.1128/AAC.05037-11

Khan, S., Mandal, R. K., Elasbali, A. M., Dar, S. A., Jawed, A., Wahid, M., Mahto, H., Lohani, M., Mishra, B. N., Akhter, N., Rabaan, A. A., & Haque, S. (2019). Pharmacogenetic association between NAT2 gene polymorphisms and isoniazid induced hepatotoxicity: Trial sequence meta-analysis as evidence. *Bioscience Reports*, *39*(1), BSR20180845. https://doi.org/10.1042/BSR20180845

Kenya - total population by gender 2020 [WWW Document], 2021. . Statista. URL https://www.statista.com/statistics/967855/total-population-of-kenya-by-gender/ (accessed 11.23.21).

Kenya National Bureau of Statistics (Ed.), 2019. 2019 Kenya population and housing census, 8th ed. Kenya National Bureau of Statistics, Nairobi. http://housingfinanceafrica.org/app/uploads/VOLUME-II-KPHC-2019.pdf

Kenya population [WWW Document], 2021. . worldpopulationreview.com. URL https://worldpopulationreview.com/countries/kenya-population (accessed 11.23.21).

Kim, Lee, S.S., Lee, C.M., Kim, Hong Jun, Ha, C.Y., Kim, Hyun Jin, Kim, T.H., Jung, W.T., Lee, O.J., Hong, J.W., You, H.S., Cho, H.C., 2016. Hepatitis C and not Hepatitis B virus is a risk factor for anti-tuberculosis drug induced liver injury. BMC Infect. Dis. 16, 50. https://doi.org/10.1186/s12879-016-1344-2

Kim, Sang-Heon, Kim, Sang-Hoon, Yoon, H.J., Shin, D.H., Park, S.S., Kim, Y.-S., Park, J.-S., Jee, Y.-K., 2012. TNF-α genetic polymorphism −308G/A and antituberculosis drug-induced hepatitis. Liver Int. 32, 809–814. https://doi.org/10.1111/j.1478-3231.2011.02697.x

Laborde, J., Deraeve, C., & Bernardes-Génisson, V. (2017). Update of Antitubercular Prodrugs from a Molecular Perspective: Mechanisms of Action, Bioactivation Pathways, and Associated Resistance. *ChemMedChem*, *12*(20), 1657–1676. https://doi.org/10.1002/cmdc.201700424

Lai, E. C.-C., Liang, H.-Y., Huang, Y.-C., Huang, W.-I., Chao, P.-H., Chen, W.-W., & Weng, M.-Y. (2021). Association between 9-month isoniazid prophylaxis of latent tuberculosis and severe hepatitis in patients treated with TNF inhibitors. *Scientific Reports*, *11*, 18013. https://doi.org/10.1038/s41598-021-97444-8

Lakkakula, S., Mohan Pathapati, R., Chaubey, G., Munirajan, A.K., Lakkakula, B.V.K.S., Maram, R., 2014. NAT2 genetic variations among South Indian populations. Hum. Genome Var. 1, 14014. https://doi.org/10.1038/hgv.2014.14

Lala, V., Zubair, M., & Minter, D. A. (2022). *Liver Function Tests—StatPearls—NCBI Bookshelf.pdf*. StatPearls Publishing LLC. https://pubmed.ncbi.nlm.nih.gov/29494096/

Little, K. M., Khundi, M., Barnes, G. L., Ngwira, L. G., Nkhoma, A., Makombe, S., Corbett, E. L., Chaisson, R. E., & Dowdy, D. W. (2018). Predictors of isoniazid preventive therapy completion among adults newly diagnosed with HIV in rural Malawi. *The International Journal of Tuberculosis and Lung Disease*, *22*(4), 371–377. https://doi.org/10.5588/ijtld.16.0836

Lv, Xiaozhen, Tang, S., Xia, Y., Zhang, Y., Wu, S., Yang, Z., Li, X., Tu, D., Chen, Y., Deng, P., Ma, Y., Chen, D., Chen, R., Zhan, S., 2012. NAT2 genetic polymorphisms and anti-tuberculosis drug-induced hepatotoxicity in Chinese community population. Ann. Hepatol. 11, 700–707. https://doi.org/10.1016/S1665-2681(19)31446-2

Marriner, G.A., Nayyar, A., Uh, E., Wong, S.Y., Mukherjee, T., Via, L.E., Carroll, M., Edwards, R.L., Gruber, T.D., Choi, I., Lee, J., Arora, K., England, K.D., Helena I.M. Boshoff, and C.E.B.I., 2011. The Medicinal Chemistry of Tuberculosis Chemotherapy. Third World Dis. https://doi.org/10.1007/7355

Martindale, W., & Sweetman, S. (2020). *Martindale: The complete drug reference* (40th ed). Pharmaceutical press.

Matei, L. (2015). *Synthesis of new Isoniazid Derivatives with potential implications in the Individualized Therapy of Tuberculosis* (p. 23). https://www.chimie.unibuc.ro/images/teze_de_doctorat/2015...Matei/Matei-en.pdf

Matejcic, M. (2013). *Identification Of Genetic Polymorphisms Associated With Oesophageal Squamous Cell Carcinoma Risk In South Africa*. University of Cape Town. https://open.uct.ac.za/bitstream/item/2914/thesis_hsf_2013_matejcic_m.pdf?sequence...

McDonagh, E.M., Boukouvala, S., Aklillu, E., Hein, D.W., B, R., Altman, Klein, and T.E., 2014. Very Important Pharmacogene information for N-acetyltransferase 2.

Pharmacogenet Genomics 24, 409–25.
https://doi.org/10.1097/FPC.0000000000000062.PharmGKB

metushi, I., Sanders, C., Lee, W.M., Uetrecht, J., 2014. Detection of Anti-Isoniazid and Anti-CYP Antibodies in Patients with Isoniazid-Induced Liver Failure. Hepatology 21, 129–139. https://doi.org/10.5588/ijtld.16.0716.Isoniazid

Metushi, I., Uetrecht, J., Phillips, E., 2016. Mechanism of isoniazid-induced hepatotoxicity: Then and now. Br. J. Clin. Pharmacol. 81, 1030–1036. https://doi.org/10.1111/bcp.12885

Molla, Y., Wubetu, M., & Dessie, B. (2021). Anti-Tuberculosis Drug Induced Hepatotoxicity and Associated Factors among Tuberculosis Patients at Selected Hospitals, Ethiopia. *Hepatic Medicine: Evidence and Research*, *Volume 13*, 1–8. https://doi.org/10.2147/HMER.S290542

Mthiyane, T., Millard, J., Adamson, J., Balakrishna, Y., Connolly, C., Owen, A., Rustomjee, R., Dheda, K., McIlleron, H., & Pym, A. S. (2020). *N* -Acetyltransferase 2 Genotypes among Zulu-Speaking South Africans and Isoniazid and *N* -Acetyl-Isoniazid Pharmacokinetics during Antituberculosis Treatment. *Antimicrobial Agents and Chemotherapy*, *64*(4), e02376-19, /aac/64/4/AAC.02376-19.atom. https://doi.org/10.1128/AAC.02376-19

Muchabaiwa, H. (2013). *Logistic Regression To Determine Significant Factors Associated With Share Price Change*. University of Suuth Africa. https://core.ac.uk/download/pdf/43173688.pdf

Mushiroda, T., Yanai, H., Yoshiyama, T., Sasaki, Y., Okumura, M., Ogata, H., Tokunaga, K., 2016. Development of a prediction system for anti-tuberculosis drug-induced liver injury in Japanese patients. Hum. Genome Var. 3, 16014. https://doi.org/10.1038/hgv.2016.14

Mwangi, P. M. (2016). *Implementation Of Isoniazid Preventive Therapy Among Hiv Infected Children In Three Health Facilities In Nairobi County*. Nairobi. http://erepository.uonbi.ac.ke:8080/xmlui/handle/11295/99719

National AIDS & STI Control Program. (2018). *ICAP_CQUIN_Kenya-ARV-Guidelines-2018-Final_20thAug2018.pdf*. http://cquin.icap.columbia.edu/wp-content/uploads/2017/04/ICAP_CQUIN_Kenya-ARV-Guidelines-2018-Final_20thAug2018.pdf

National Tuberculosis Leprosy and Lung Disease Program. (2016). *Kenya TB Prevalence Survey*. http://www.nltp.co.ke/tb-prevalence-survey-2015-2016/

Neal, D., Sataloff, R.T., Johns, M.M., Kost, K.M., 2018. Introduction to Population Biology, 2nd ed. Cambridge University press, Saskatoon. https://doi.org/doi:10.1017/9781139107976

Ng, C.-S., Hasnat, A., Al Maruf, A., Ahmed, M.U., Pirmohamed, M., Day, C.P., Aithal, G.P., Daly, A.K., 2014. N-acetyltransferase 2 (NAT2) genotype as a risk factor for development of drug-induced liver injury relating to antituberculosis drug treatment in a

mixed-ethnicity patient group. Eur. J. Clin. Pharmacol. 70, 1079–1086. https://doi.org/10.1007/s00228-014-1703-0

Ngongondo, M., Miyahara, S., Hughes, M.D., Sun, X., Bisson, G.P., Gupta, A., Kumwenda, J., Lavenberg, J.A., Torres, T.S., Nyirenda, M., Kidonge, K.K., Hosseinpour, M.C., Team, =AIDS Clinical Trials Group A5274 (REMEMBER) Study, 2018. Hepatotoxicity during Isoniazid Preventive Therapy and Antiretroviral Therapy in People Living with HIV with Severe Immunosuppression: a Secondary Analysis of a Multi-country Open-label Randomized Controlled Clinical Trial. J. Acquir. Immune Defic. Syndr. 1999. https://doi.org/10.1097/qai.0000000000001641

Olago-Rakuomi, A. A. (2013). *Prevalence of Abnormal Liver Function Tests in Rheumatoid Arthritis* [University of Nairobi,kenya].http://erepository.uonbi.ac.ke/bitstream//bitstre am/handle/11295/101717/Olago-Rakuomi,Agatha%20A_Prevalence%20of%20AAbn ormal%20Liver%20Function%20in%20Rheumatoid%20Arthritis.pdf?sequence=1

Okonechnikov, K., Golosova, O., Fursov, M., the UGENE team, 2012. Unipro UGENE: a unified bioinformatics toolkit. Bioinformatics 28, 1166–1167. https://doi.org/10.1093/bioinformatics/bts091

Olmos, G., Lladó, J., 2014. Tumor necrosis factor alpha: A link between neuroinflammation and excitotoxicity. Mediators Inflamm. 2014, 12. https://doi.org/10.1155/2014/861231

Oluka, M. N. (2012). *Pharmacogenetics of Drug Metabolizing Enzymes and Clinical Implications in Selected Kenyan Populations* (p. 293) [University of Nairobi, Kenya]. http://erepository.uonbi.ac.ke/handle/11295/8988

Oluka, M.N., Matimba, A., Okalebo, F.A., Osanjo, G.O., Guantai, A.N., Masimirembwa, C.M., 2014. Characterization of inter-ethnic genetic and GSTs in the Bantu and Nilotic populations of Kenya and implications for the chemotherapy of infectious diseases. Afr. J. Pharmacol. Ther. 3, 38–46.

Perwitasari, D.A., Atthobari, J., Wilffert, B., 2015. Pharmacogenetics of isoniazid-induced hepatotoxicity. Drug Metab. Rev. 47, 222–228. https://doi.org/10.3109/03602532.2014.984070

Ramachandran, G., Swaminathan, S., 2012. Role of pharmacogenomics in the treatment of tuberculosis: A review. Pharmacogenomics Pers. Med. 5, 89–98. https://doi.org/10.2147/PGPM.S15454

Ramappa, V., Aithal, G.P., 2013. Hepatotoxicity Related to Anti-tuberculosis Drugs: Mechanisms and Management. J. Clin. Exp. Hepatol. 3, 37–49. https://doi.org/10.1016/j.jceh.2012.12.001

Richardson, M., Kirkham, J., Dwan, K., Sloan, D., Davies, G., Jorgensen, A., 2017. Influence of genetic variants on toxicity to anti-tubercular agents: A systematic review and meta-analysis (protocol). Syst. Rev. 6, 1–10. https://doi.org/10.1186/s13643-017-0533-4

Russom, M., Berhane, A., Debesai, M., Andom, H., Mehari, D. T., Zeremariam, Z., Gebrehiwet, S., Tesfamichael, N., Said, S., & Ahmed, H. (2019). *Challenges of Hepatotoxicity Associated with Isoniazid Preventive Therapy among People Living with HIV in Eritrea*. 228.

Ross, J. M., Badje, A., Rangaka, M. X., Walker, A. S., Shapiro, A. E., Thomas, K. K., Anglaret, X., Eholie, S., Gabillard, D., Boulle, A., Maartens, G., Wilkinson, R. J., Ford, N., Golub, J. E., Williams, B. G., & Barnabas, R. V. (2021). Isoniazid preventive therapy plus antiretroviral therapy for the prevention of tuberculosis: A systematic review and meta-analysis of individual participant data. *The Lancet HIV*, *8*(1), e8–e15. https://doi.org/10.1016/S2352-3018(20)30299-X

.

Santos, N.P.C., Callegari-Jacques, S.M., Dos Santos, A.K.C.R., Silva, C.A., Vallinoto, A.C.R., Fernandes, D.C.R.O., De Carvalho, D.C., Santos, S.E.B., Hutz, M.H., 2013. N-acetyl transferase 2 and cytochrome P450 2E1 genes and isoniazid-induced hepatotoxicity in Brazilian patients. Int. J. Tuberc. Lung Dis. 17, 499–504. https://doi.org/10.5588/ijtld.12.0645

Saxena, N., Srivastava, N., Shukla, P., & Tripathi, G. K. (2019). The Drug Discovery Development for Treatment of Tuberculosis. *Journal of Drug Delivery*, 18.

Seng, K., Hee, K., Soon, G., Chew, N., Khoo, S.H., Lee, S., 2015. Population Pharmacokinetic Analysis of Isoniazid, Acetylisoniazid, and Isonicotinic Acid in Healthy Volunteers. Antimicrob. Agents Chemother. 59, 6791–6799. https://doi.org/10.1128/AAC.01244-15.Address

Sharma, S.K., Balamurugan, A., Saha, P.K., Pandey, R.M., Mehra, N.K., 2002. Evaluation of clinical and immunogenetic risk factors for the development of hepatotoxicity during antituberculosis treatment. Am. J. Respir. Crit. Care Med. 166, 916–919. https://doi.org/10.1164/rccm.2108091

Sharma, S.K., Jha, B.K., Sharma, A., Sreenivas, V., Upadhyay, V., Jaisinghani, C., Singla, R., Mishra, H.K., Soneja, M., 2016. Genetic polymorphisms of N-acetyltransferase 2 & susceptibility to antituberculosis drug-induced hepatotoxicity. Indian J Med Res 144, 924–928. https://doi.org/10.4103/ijmr.IJMR

Shi, J., Xie, M., Wang, J., Xu, Y., Liu, X., 2015. Susceptibility of N-acetyltransferase 2 slow acetylators to antituberculosis drug-induced liver injury: a meta-analysis. Pharmacogenomics 16, 2083–2097. https://doi.org/10.2217/pgs.15.144

Shu, C.C., Lee, C.H., Lee, M.C., Wang, J.Y., Yu, C.J., Lee, L.N., 2013. Hepatotoxicity due to first-line anti-tuberculosis drugs: A five-year experience in a Taiwan medical centre. Int. J. Tuberc. Lung Dis. 17, 934–939. https://doi.org/10.5588/ijtld.12.0782

Sim, E., Abuhammad, A., Ryan, A., 2014. Arylamine N-acetyltransferases: From drug metabolism and pharmacogenetics to drug discovery. Br. J. Pharmacol. 171, 2705–2725. https://doi.org/10.1111/bph.12598

Singh, A., Prasad, R., Balasubramanian, V., Gupta, N., Gupta, P., 2015. Prevalence of adverse drug reaction with first-line drugs among patients treated for pulmonary tuberculosis. Clin. Epidemiol. Glob. Health 3, S80–S90. https://doi.org/10.1016/j.cegh.2015.10.005

Spinasse, L.B., Santos, A.R., Suffys, P.N., Muxfeldt, E.S., Salles, G.F., 2014. Different phenotypes of the *NAT2* gene influences hydralazine antihypertensive response in

patients with resistant hypertension. Pharmacogenomics 15, 169–178. https://doi.org/10.2217/pgs.13.202

Steele, M.A., Burk, R.F., DesPrez, R.M., 1991. Toxic hepatitis with isoniazid and rifampin: A meta-analysis. Chest 99, 465–471. https://doi.org/10.1378/chest.99.2.465

Teixeira, L.D.F., Quinhones Pires Lopes, M., Suffys, P.N., Santos, A.R., 2013. Tuberculosis Pharmacogenetics : State of The Art, in: Tuberculosis - Current Issues in Diagnosis and Management. INTECH Open Access Publisher, 2013, pp. 105–126. https://doi.org/10.5772/54984

Teklay, G., Teklu, T., Legesse, B., Tedla, K., Klinkenberg, E., 2016. Barriers in the implementation of isoniazid preventive therapy for people living with HIV in Northern Ethiopia: A mixed quantitative and qualitative study. BMC Public Health 16, 1–9. https://doi.org/10.1186/s12889-016-3525-8

UNAIDS, 2017. HIV and AIDS in Kenya | AVERT [WWW Document]. Glob. Inf. Educ. HIV AIDS. URL https://www.avert.org/professionals/hiv-around-world/sub-saharan-africa/kenya (accessed 3.15.18).

Unissa, A.N., Subbian, S., Hanna, L.E., Selvakumar, N., 2016. Overview on mechanisms of isoniazid action and resistance in Mycobacterium tuberculosis. Infect. Genet. Evol. 45, 474–492. https://doi.org/10.1016/j.meegid.2016.09.004

University of Cape Town. (2018). *TST no longer needed prior to INH prophylaxis (IPT): National DOH Circular | Medicines Information Centre*. http://www.mic.uct.ac.za/MICNews/Sep18/TST

Vögeli, B., Rosenthal, R. G., Stoffel, G. M. M., Wagner, T., Kiefer, P., Cortina, N. S., Shima, S., & Erb, T. J. (2018). InhA, the enoyl-thioester reductase from Mycobacterium tuberculosis forms a covalent adduct during catalysis. *The Journal of Biological Chemistry*, *293*(44), 17200–17207. https://doi.org/10.1074/jbc.RA118.005405

wang, pengcheng, Pradhan, K., Zhong, X. bo, Ma, X., 2016. Isoniazid metabolism and hepatotoxicity. Acta Pharm. Sin. B 6, 384–392. https://doi.org/10.1016/j.apsb.2016.07.014

Wang, C.-S., Wang, S.-T., Chang, T.-T., Yao, W.-J., & Chou, P. (2002). Smoking and Alanine Aminotransferase Levels in Hepatitis C Virus Infection: Implications for Prevention of Hepatitis C Virus Progression. *Archives of Internal Medicine*, *162*(7), 811. https://doi.org/10.1001/archinte.162.7.811

Wang, Huang, Y.-S., Lin, M.-H., Huang, B., Perng, C.-L., Lin, H.-C., 2016. Chronic hepatitis B infection and risk of antituberculosis drug-induced liver injury: Systematic review and meta-analysis. J. Chin. Med. Assoc. 79, 368–374. https://doi.org/10.1016/j.jcma.2015.12.006

Wang, P., Pradhan, K., Zhong, X. bo, Ma, X., 2016. Isoniazid metabolism and hepatotoxicity. Acta Pharm. Sin. B 6, 384–392. https://doi.org/10.1016/j.apsb.2016.07.014

Wattanapokayakit, S., Mushiroda, T., Yanai, H., Wichukchinda, N., Chuchottawon, C., Nedsuwan, S., Rojanawiwat, A., Denjanta, S., Kantima, T., Wongyai, J., Suwankesawong, W., Rungapiromnan, W., Kidkeukarun, R., Bamrungram, W., Chaiwong, A., Suvichapanich, S., Mahasirimongkol, S., Tokunaga, K., 2016. NAT2 slow acetylator associated with anti-tuberculosis drug- induced liver injury in Thai patients 8.

Werely, C. J. (2012). *Pharmacogenetics of Arylamine N-acetyltransferase genes in South African populations*. Stellenbosch : Stellenbosch University. http://scholar.sun.ac.za/handle/10019.1/71832

WHO. (2022, October 27). *Tuberculosis (TB)*. https://www.who.int/news-room/fact-sheets/detail/tuberculosis

WHO. (2015a). *HIV / AIDS TB / HIV facts 2015 Challenges and key issues*. http://www.who.int/hiv/topics/tb/tbhiv_facts_2015/en/

World Health Organization. (2021). *WHO global lists of high burden countries for tuberculosis (TB), TB/HIV and multidrug/rifampicin-resistant TB (MDR/RR-TB), 2021–2025: Background document*. World Health Organization. https://apps.who.int/iris/handle/10665/341980

WHO. (2017a). *Global TuberculosisReport*,http://www.who.int/tb/publications/global_report/en/

World Health Organization. (2022). *Global tuberculosis report 2022*. World Health Organization. https://apps.who.int/iris/handle/10665/363752

WHO. (2022, October 27). *Tuberculosis (TB)*. https://www.who.int/news-room/fact-sheets/detail/tuberculosis

WHO. (2018). WHO | Tuberculosis. In *WHO*. World Health Organization. http://www.who.int/mediacentre/factsheets/fs104/en/

World Health Organization, 2015. WHO | Recommendation on 36 months isoniazid preventive therapy to adults and adolescents living with HIV in resource-constrained and high TB and HIV-prevalence settings: 2015 update.

Wu, H., Dombrovsky, L., Tempel, W., Martin, F., Loppnau, P., Goodfellow, G.H., Grant, D.M., Plotnikov, A.N., 2007. Structural Basis of Substrate-binding Specificity of Human Arylamine N-Acetyltransferases. J. Biol. Chem. 282, 30189–30197. https://doi.org/10.1074/jbc.M704138200

Xiang, Y., Ma, L., Wu, W., Liu, W., Li, Y., Zhu, X., Wang, Q., Ma, J., Cao, M., Wang, Q., Yao, X., Yang, L., Wubuli, A., Merle, C., Milligan, P., Mao, Y., Gu, J., Xin, X., 2014. The incidence of liver injury in uyghur patients treated for TB in Xinjiang Uyghur Autonomous Region, China, and its association with hepatic enzyme polymorphisms

NAT2, CYP2E1, GSTM1 and GSTT1. PLoS ONE 9, 1–8.
https://doi.org/10.1371/journal.pone.0085905

Yirdaw, K.D., Jerene, D., Gashu, Z., Edginton, M.E., Kumar, A.M., Letamo, Y., Feleke, B., Teklu, A.M., Zewdu, S., Weiss, B., Ruff, A., 2014. Beneficial effect of isoniazid preventive therapy and antiretroviral therapy on the incidence of tuberculosis in people living with HIV in Ethiopia. PloS One 9, 1–8. https://doi.org/10.1371/journal.pone.0104557

Yuliwulandari, R., Prayuni, K., Susilowati, R. W., M Sofro, A. S., Tokunaga, K., & Shin, J.-G. (2019). NAT2 slow acetylator is associated with anti-tuberculosis drug-induced liver injury severity in indonesian population. *Pharmacogenomics*, *20*(18), 1303–1311. https://doi.org/10.2217/pgs-2019-0131

Zabinski, R.F., Blanchard, J.S., 1997. The Requirement for Manganese and Oxygen in the Isoniazid-Dependent Inactivation of Mycobacterium tuberculosis Enoyl Reductase. J. Am. Chem. Soc. 2331–2332. https://doi.org/10.1021/ja9639731

Zhou, X., Ma, Z., Dong, D., Wu, B., 2013. Arylamine N-acetyltransferases : a. Bitish J. Pharmacol. 169, 748–760. https://doi.org/10.1111/bph.12182

APPENDICES

Appendix A- KNH/UoN Ethical Approval

UNIVERSITY OF NAIROBI
COLLEGE OF HEALTH SCIENCES
P O BOX 19676 Code 00202
Telegrams: varsity
Tel: (254-020) 2726300 Ext 44355

KNH-UON ERC
Email: uonknh_erc@uonbi.ac.ke
Website: http://www.erc.uonbi.ac.ke
Facebook: https://www.facebook.com/uonknh.erc
Twitter: @UONKNH_ERC https://twitter.com/UONKNH_ERC

KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi

August 30 2018

Ref: KNH-ERC/A/325

Akumu Reveszt Maugo
Reg. No.U52/88146/2016
Dept. of Pharmacology and Pharmacognosy
School of Pharmacy
College of Health Sciences
University of Nairobi

Dear Akumu

RESEARCH PROPOSAL – COMPARATIVE PREVALENCE OF POLYMORPHISMS OF N-ACETYL TRANSFERASE 2 IN PATIENTS ON ISONIAZID PREVENTIVE THERAPY WITH AND WITHOUT ALANINE AMINOTRANSFERASE ELEVATION AT KENYATTA NATIONAL HOSPITAL
(P449/06/2018)

This is to inform you that the KNH- UoN Ethics & Research Committee (KNH- UoN ERC) has reviewed and **approved** your above research proposal. The approval period is 30th August 2018 – 29th August 2019.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH-UoN ERC before implementation.
c) Death and life threatening problems and serious adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH-UoN ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH- UoN ERC within 72 hours.
e) Clearance for export of biological specimens must be obtained from KNH- UoN ERC for each batch of shipment.
f) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
g) Submission of an *executive summary* report within 90 days upon completion of the study. This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/ or plagiarism.

Protect to discover

66

For more details consult the KNH- UoN ERC website http://www.erc.uonbi.ac.ke

Yours sincerely,

PROF. M. L. CHINDIA
SECRETARY, KNH-UoN ERC

c.c.   The Principal, College of Health Sciences, UoN
        The Director, CS, KNH
        The Chairperson, KNH-UON ERC
        The Assistant Director, Health Information, KNH
        The Dean, School of Pharmacy, UON
        The Chair, Dept. of Pharmacology and Pharmacognosy, UON
        Supervisors: Dr. Eric M. Guantai, Dr. Margaret N. Oluka, Dr. James Kimotho

Protect to discover

Appendix B: Kenyatta national Hospital Research Authorization letter

INTERNAL MEMO

Kenyatta National Hospital

OFFICE OF THE HEAD OF DEPARTMENT – LABORATORY MEDICINE

"Email: laboratoryknh@gmail.com" - Exten: 44121

Ref: KNH/DLM/60/VOL.1/   Date: 17th December, 2018

Reverszt Akumu Maugo
Department of pharmacy
Nairobi
Mobile: 0712866492

Dear Reveszt,
RE: PERMISSION TO CARRY OUT RESEARCH AT KNH

Reference is made to your study Consent application request dated dated11th December 2018for a study entitled **"COMPARATIVE PREVALENCE OF POLYMORPHISMS OF N-ACETYL TRANSFERASE 2 IN PATIENTS ON ISONIAZID PREVENTIVE THERAPY WITH AND WITHOUT ALANINE AMINOTRANSFERASE ELEVATION AT KENYATTA NATIONAL HOSPITAL".**The request also includesrequest for permission to transport samples for further analysis in the Department of Pharmacology (College of Health Sciences) and KEMRI Laboratories.

The Department of Laboratory Medicine supports research in the hospital provided the researchercomplies with the regulations for conducting research. Among theDepartmental regulations for conducting research it is provided that, if the Researcher is Not a Clinical Staff or Medical Laboratory Professional affiliated to Kenyatta National Hospital, they must identify an authorized person or collaborator though whom the results will be dispatched and released for the purpose of their study.You have indicated that you are not a registered health professional but you have included Dr. David Wata, a Pharmacist in the Kenyatta National Hospitalas one of your collaborator. Dr. Wata therefore be the responsible person as a collaborator, for the release of data and any other issues related to the dissemination of yourstudy findings.

The Research study is required to have been registered at the Research and Programs Department of the Hospital, a study number obtained and commitment given to share the results. If the tests in your study are going to be run on the patients as part of their management, the cost in the tests will be limited to those items and the process that are covered in the request and the payment receipt. Please note that any assistantwho may be required to handle any samples on your behalf will have to demonstrate evidence of authority and compliance by the relevant regulatory authority. This authority therefore gives permission for the collection of samples in Comprehensive Care Unit andtheir transportationto the Department of Pharmacy (UON) and the KEMRI Laboratories.
Kindly liaise with the in-charge ofComprehensive Care Laboratory Section for furtherassistance and facilitation.

Yours sincerely,

Dr. Mary K. Mungania
For: HOD – LABORATORY MEDICINE
CC:
    -HOD- Research and Programs
    -Dr. David Wata- Pharmacy Dept-KNH
    -I/C Comprehensive Care Laboratory Section

1

Appendix C: Study Registration Certificate

KNH/R&P/FORM/01

KENYATTA NATIONAL HOSPITAL
P.O. Box 20723-00202 Nairobi

Tel.: 2726300/2726450/2726565
Research & Programs: Ext. 44705
Fax: 2725272
Email: knhresearch@gmail.com

Study Registration Certificate

1. Name of the Principal Investigator/Researcher
   Akumu Revesat Maugo

2. Email address: revesatakumu@gmail.com   Tel No. 0712866492

3. Contact person (if different from PI)..

4. Email address: ....   Tel No. ....

5. Study Title
   Comparative Prevalence of Polymorphisms on N-Acetyl Transferase 2 in patients on Isoniazid preventive therapy with & without Alanine Aminotransferase elevation at Kenyatta N. Hospital

6. Department where the study will be conducted Comprehensive Care Centre
   (Please attach copy of Abstract)

7. Endorsed by Research Coordinator of the KNH Department where the study will be conducted.
   Name: Dr. C. Chiwana   Signature ....   Date 4/12/18

8. Endorsed by KNH Head of Department where study will be conducted.
   Name: Dr P. N Munuvi   Signature ....   Date 3/12/18

9. KNH UoN Ethics Research Committee approved study number P449/06/2018
   (Please attach copy of ERC approval)

10. I Akumu Revesat Maugo commit to submit a report of my study
    findings to the Department where the study will be conducted and to the Department of Research
    and Programs.

    Signature.................   Date 3/12/18

11. Study Registration number (Dept/Number/Year)_____
    (To be completed by Research and Programs Department)

12. Research and Program Stamp _____

All studies conducted at Kenyatta National Hospital **must** be registered with the Department of
Research and Programs and investigators **must commit** to share results with the hospital.

Version 2: August. 2014

Appendix D: Volunteer information and consent form

Consenting process

This document is a consent form; it has information about the study and shall be discussed with you by the investigators. Please study it carefully and feel free to seek any clarification especially concerning terminologies or procedures that may not be clear to you. Once you understand and agree to take part, you are requested to sign your name on this form. You should understand the following general principles which apply to all participants in a medical research.

I. Your agreement to participate in this study is voluntary

II. You may withdraw from the study anytime without necessarily giving a reason for your withdrawal

III. Refusal to participate in the research will not affect the services that you are entitled to receive in the clinic

TITLE: GENETIC POLYMORPHISMS OF N-ACETYL TRANSFERASE 2 AND THE RISK OF HEPATOTOXICITY IN PATIENTS ON ISONIAZID PREVENTIVE THERAPY AT KENYATTA NATIONAL HOSPITAL

Introduction to the study

Isoniazid is a drug used to treat tuberculosis. It is a key component of HIV therapy, and is used to manage tuberculosis through isoniazid preventive therapy, since it acts as a first line Anti-TB drug. However patients may experience hepatotoxicity while on therapy. This is critical since patients might not only experience treatment failure but can also die due to permanent liver failure.

Hepatotoxicity may be due to genetic variations in the enzyme N-acetyl transferase 2 (*NAT2*), which is an enzyme involved in the metabolism of isoniazid. Genetic variations in the *NAT2* enzyme may lead to isoniazid induced hepatotoxicity. I am investigating the comparative prevalence of N-acetyl transferase 2 in patients on isoniazid preventive therapy with and without Alanine transaminases (ALT) elevation. Samples may be taken abroad for secondary analysis. Permission is requested from you to enroll in this medical research study.

Purpose of the study

The primary objective is to compare the prevalence of SNPs of *NAT2* in patients on isoniazid preventive therapy with and without ALT elevation.

Procedures to be followed

With your permission we will go through your medical records to obtain information on laboratory investigations which have been conducted since you were initiated on isoniazid preventive therapy. We will also check if you have suffered any bad reactions to isoniazid. You will be asked a few questions about your ethnicity, if you are using any other drugs (prescription/over the counter) or herbal products, level of education, marital status, whether you drink or smoke, how regularly you take medicine and whether you experienced bad reactions to drugs that you are taking. We will also collect a blood sample from you.

Selection criteria

You will be selected to take part in this study if you meet the following criteria

   I.   You have been on isoniazid preventive therapy for at least 4 weeks
  II.   You are aged above 18 years
 III.   You have given written informed consent to take part in the study

Risks or and discomfort

There are no risks involved in this study to you

Rights and safety

To safeguard your rights and safety as a participant taking part in this study, the Kenyatta National Hospital/University of Nairobi Research and Ethics Committee will review the study protocol and the informed consent process before commencing the research.

Benefits

The study may be of benefit to you and other HIV patients on isoniazid preventive therapy in that it will be used to enhance treatment outcomes in patients with *NAT2* polymorphisms that may experience hepatotoxicity. It may also inform policy makers on the need to review guidelines on pharmacogenomics testing.

Confidentiality

All the information that we collect for the purpose of this research will be kept confidential. Your name will not be used during data handling or in any resulting publications. Codes will be

used instead. Your medical records will be kept under lock and key and information will be accessible only to authorized persons only.

Contacts

If you have any questions concerning this study, you may ask them now or contact Reveszt Akumu on mobile number 0712866492

Principal investigator

Akumu Reveszt Maugo
Department of pharmacology and Pharmacognosy
School of Pharmacy,
University of Nairobi
P.O Box, 35079- Nairobi. Tel: 0712-866492

Supervisors

Dr. Eric M. Guantai, PhD

Department of Pharmacology and Pharmacognosy,
School of Pharmacy, University of Nairobi

Dr. Margaret N. Oluka, **PhD**

Department of Pharmacology and Pharmacognosy
School of Pharmacy, University of Nairobi

Dr. James Kimotho, PhD

Innovation & Technology Transfer Division

Kenya Medical Research Institute

Consent Declaration form

TITLE: GENETIC POLYMORPHISMS OF N-ACETYL TRANSFERASE 2 AND THE RISK OF HEPATOTOXICITY IN PATIENTS ON ISONIAZID PREVENTIVE THERAPY AT KENYATTA NATIONAL HOSPITAL

I _____ (name of participant), being 18 years of age and more and having full capacity to consent, hereby do consent to voluntarily participate in the study. The nature of the study has been explained to me by the principal investigator and have been given the opportunity to ask questions concerning the study which have been answered to my satisfaction, The benefits and risks have been clearly explained to me and I'm aware that I am free to withdraw from this study at any point and this will not jeopardize the care that I receive at the hospital.

I therefore give consent to be interviewed and answer the questionnaire and that information from my file can also be used having understood the purpose of the study

Signature…………………………………………

Date………………………………………………

Researcher's declaration statement

I………………………………………………….being the study researcher have adequately explained to the above named participant on the nature and purpose of the study and has agreed to voluntary participate in the study

Signature…………………………………………..

Date………………………………………………..

Contacts…………………………………………...

Appendix E Fomu ya maelezo ya kukubali

Hati hii ni fomu ya ridhaa; ina habari kuhusu utafiti na itajadiliwa na wewe na wachunguzi. Tafadhali jifunze kwa uangalifu na ujisikie huru kutafuta ufafanuzi wowote hasa kuhusu maneno na taratibu ambazo haziwezi kuwa wazi kwako. Mara tu unapoelewa na kukubali kushiriki, unatakiwa kusaini jina lako kwenye fomu hii. Unapaswa kuelewa kanuni zifuatazo kwa wahusika wote katika utafiti wa matibabu.

   I. Mkataba wako wa kushiriki kwa utafiti huu ni kwa hiari
  II. Unaweza kuondoka kutoka kwenye utafiti wakati wowote bila lazima ya kutoa sababu ya uondoaji wako
 III. Kukataa kushiriki katika utafiti hautaathiri huduma ambazo una haki ya kupokea katika kliniki

Mada ya utafiti: Ulinganisho wa uenezi wa polymorphism ya N-acetyl transferase kati ya wagonjwa kwenye matibabu ya kuzuia kifua kikuu (TB) kupitia isoniazid wenye upungufu wa alanine aminotransferase (ALT) na wagonjwa kwenye matibabu hayo wasiokuwa na upunguzi wa (ALT) katika hospitali ya kitaifa ya Kenyatta

Utangulizi wa utafiti

Isoniazid ni dawa inayotumika kutibu kifua kikuu. Ni sehemu muhimu katika tiba ya virusi vya ukimwi (VVU), Na hutumiwa kusimamia kifua kikuu kwa njia ya tiba inayoitwa kuzuia kifua kikuu kwa kutumia dawa ya isoniazid au isoniazid preventive therapy kwa kimombo. Ingawa Isoniazidhutumika kama dawa ya kwanza ya kupambana na kifua kikuu, wagonjwa wanaweza kuugua kwa kushindwa kwa ini wakati wa tiba. Hii ni muhimu kwa sababu wagonjwa hawawezi tu kupata upungufu wa tiba lakini pia wanaweza kufa kutokana na kushindwa kwa ini.

kushindwa kwa ini inaweza kuwa kutokana na tofauti za maumbile katika enzyme N-acetyl transferase (*NAT2*) ambayo ni enzyme inayohusika katika kimetaboliki ya isoniazid. Tofauti za kimaumbile katika enzyme ya *NAT2* inaweza kusababisha hepatotoxicity kutokana na matumizi ya isoniazid. Nafanya utafiti kuhusu ulinganishowa uenezi wa polymorphism ya N-acetyl transferase (*NAT2*) katika wagonjwa waliowekwa kwenye matibabu ya isoniazid, ambao wana uwepo au kutokuwepo wa mwongezeko, wa kiwango cha Alanine aminotranferase (ALT). Sampuli zina weza kuchukuliwa nje ya nchi kwa ajili ya uchambuzi wa secondari. Ruhusa inaombwa kwako kwa kujiandikisha katika utafiti huu wa matibabu.

Lengo la utafiti

Lengo kuu ni kutafuta ulinganisho wa uenezi wa polymorphism ya N-acetyl transferase kati ya wagonjwa katika matibabu ya isoniazid ambao wana mwongezeko wa Kiwango cha Alanine aminotransferase na wagonjwa ambao hawana mwongezeko.

Taratibu za kufuatiwa

Kwa ruhusa yako tutapitia kumbukumbu zako za matibabu ili kupata taarifa juu ya uchunguzi wa maabara uliofanywa tangu ulipoanzishwa matibabu ya kuzuia Kifua kikuu kupitia dawa ya isoniazid (IPT). Tutaangalia pia ikiwa umeathiriwa na isoniazid. Utaulizwa maswali machache kuhusu ukabila wako, ikiwa unatumia madawa mengine yoyote au bidhaa za mitishamba, kiwango cha elimu, hali ya ndoa, ikiwa unakunywa au unavuta sigara, ni mara ngapi unachukua dawa na ikiwa umeathirika na madawa unayochukua. Pia tutakusanya sampuli ya damu kutoka kwako

Vigezo vya Uchaguzi

Utachaguliwa kushiriki katika utafiti huu ikiwa unakidhi vigezo vifuatavyo

  I. Umekuwa kwenye tiba ya kuzuia kifua kikuu kwa kutumia dawa ya isoniazid kwa angalau wiki 4

 II. Una miaka angalau 18 na zaidi

III. Umetoa idhini iliyoandikwa yenye ujuzi kushiriki katika utafiti

Hatari

Hakuna hatari zinazohusika katika utafiti huu kwako

Haki na usalama

ili kulinda haki zako na usalama kama mshiriki anayejihusisha katika utafiti huu, Kamati ya Chuo Kikuu cha Utafiti na Maadili ya Nairobi itashughulikia itifaki ya utafiti na mchakato wa ruhusa kabla ya kuanza utafiti.

Faida

Utafiti huu unaweza kuwa kwa faida kwako na wagonjwa wengine wa VVU kwenye tiba ya kuzuia kifua kikuu kwa kutumia isoniazid (IPT) kwa kuwa itatumika kuongeza matokeo ya matibabu kwa wagonjwa wenye polymorphisms ya *NAT2* ambao wanaweza kupata hepatotoxicity. Inaweza pia kuwajulisha watunga sera juu ya haja ya kurekebisha miongozo juu ya upimaji wa pharmacogenomics.

Hakikisho la Usiri

Taarifa zote tunayokusanya kwa ajili ya utafiti huu zitahifadhiwa kama siri. Jina lako halitatumiwa wakati wa utunzaji wa data au katika machapisho yoyote yaliyotokana.Nambari za siri zitatumiwa badala yake. Kumbukumbu zako za matibabu zitahifadhiwa chini ya kufungwa na ufunguo na habari zitapatikana kwa watu wenye mamlaka tu.

Mawasiliano

Ikiwa una maswali yoyote kuhusu utafiti huu, unaweza kuyauliza sasa au wasiliana na Reveszt Akumu kwenye simu ya mkononi 0712866492

Mtafiti mkuu

Akumu Reveszt Maugo

Idara ya pharmakolojia na Pharmakognosia

Shule ya Pharmacy,

Chuo Kikuu cha Nairobi

SLP, 35079- Nairobi. Simu: 0712-866492

Wasimamizi

Dr. Eric M, Guantai, PhD	**Dr. James Kimotho, PhD**
Idara ya pharmacology na Pharmacognosy	Idara ya Ubunifu na Teknolojia
Shule ya Pharmacy, Chuo Kikuu cha Nairobi	Taasisi ya utafiti wa tiba ya Kenya

Dr. Margaret N Oluka, PhD

Idara ya pharmacology na Pharmacognosy

Shule ya Pharmacy, Chuo Kikuu cha Nairobi

Fomu ya Azimio la kibali

Mada ya utafiti:-ULINGANISHO WA UENEZI WA POLYMORPHISM YA N-ACETYL TRANSFERASE2 KATI YA WAGONJWA KWENYE MATIBABU YA KUZUIA KIFUA KIKUU (TB) KUPITIA ISONIAZID WENYE UPUNGUFU WA (ALT) NA WAGONJWA KWENYE MATIBABU HAYO WASIOKUWA NA UPUNGUZI WA ALANINE AMINOTRANSFERASE (ALT) KATIKA HOSPITALI YA KITAIFA YA KENYATTA.

Mimi _____ (jina la mshiriki), nikiwa na umri wa miaka 18 au zaidi na kuwa na akili timamu ya kushiriki kwenye utafiti huu ninakubali kushiriki kwa utafiti huu. Hali ya utafiti na yatakayofanyikanimeelezwa kwa ufasaha na mtafiti mkuu, nimepewa fursa ya kuuliza maswali kuhusu utafiti na nimepata ufafanuzi wa kuridhika. Faida na hatari zimeelezewa wazi na ninajua kwamba Mimi niko huru kujiondoa kwenye utafiti huu wakati wowote na hii haiwezi kuharibu huduma ninayopokea katika hospitali.

Kwa hiyo ninaruhusu kuhojiwa na kujibu maswali na kuchukuliwa kwa taarifa za matibabn yangu katika faili yangu kwa madhumuni ya utafiti huu

Sahihi………………………………………

Tarehe………………………………………

Azimio la mtafiti

Mimi……………………………………………….nikiwa mtafiti wa utafiti huu nimeelezea kwa kutosha kwa mshiriki juu ya asili na madhumuni ya utafiti na amekubali kushiriki kwa hiari katika utafiti

Sahihi………………………………………..   Simu…………………………………

Tarehe……………………………………….

Appendix F: data abstraction form

Data Collector _____ **Date of Collection** _____

Patient study number _____

A) Patient Demographics

Age (years) _____   Weight _____

Body Mass Index (BMI)   _____

Height   _____

Gender   ☐ Male   ☐ Female

Marital status   ☐ Married   ☐ Single   ☐ Divorced

Highest education level   ☐ Degree   ☐ Diploma   ☐ High School   Primary ☐

Ethnicity   Mother _____   Father _____

Smoker   ☐ Yes   ☐ No

Alcohol use   ☐ Never   ☐ Occasionally   ☐ Regularly

B) Medical history

Date diagnosed with HIV _____

Date of HAART initiation _____

Date of IPT initiation _____

Dose of IPT _____

Concurrent medical conditions at the time of data collection

1. _____

2. _____

3. _____

4. _____

5. _____

Time last meal was taken _____ (to be collected during sample collection)

Allergies   **Yes** ☐   **No** ☐

If **Yes** give details_____

_____

C) Laboratory Parameters at Baseline

	Date	Value	Normal Value
ALT			
CDF cell count			

Laboratory Parameters during treatment

Date						
Parameter	**1st**	**2nd**	**3rd**	**4th**	**5th**	**6th**
ALT						
CDF cell count						

D) Medication History

(Only list the medication that patient has been taking at least 2 weeks before and after sample collection)

Drug name	Dose	Frequency
	Antiretrovirals	
1.		
2.		
3.		
4.		
	Others	
5.		
6.		
7.		
8.		
9.		
10.		
11.		

F) Adverse Drug Reactions
List of adverse drug reactions experienced while in treatment

1._____   5._____

2._____   6._____

3._____

4._____

_____28-11-2022
DR MARGARET OLUKA- Supervisor

Thesis - GENETIC POLYMORPHISMS OF N-ACETYL TRANSFERASE 2 AND THE RISK OF HEPATOTOXICITY IN PATIENTS ON ISONIAZID PREVENTIVE THERAPY AT KENYATTA NATIONAL HOSPITAL

by Reveszt Akumu

Thesis - GENETIC POLYMORPHISMS OF N-ACETYL TRANSFERASE 2 AND THE RISK OF HEPATOTOXICITY IN PATIENTS ON ISONIAZID PREVENTIVE THERAPY AT KENYATTA NATIONAL HOSPITAL

5   journals.uonbi.ac.ke
     Internet Source   1 %

6   cquin.icap.columbia.edu
     Internet Source   1 %

7   www.nature.com
     Internet Source   1 %

8   Zhou, Xiaotong, Zhiguo Ma, Dong Dong, and
     Baojian Wu. "Arylamine N-acetyltransferases: <1 %
     a structural perspective : Understanding of
     NATs with their 3D structures", British Journal
     of Pharmacology, 2013.
     Publication

9   bpspubs.onlinelibrary.wiley.com
     Internet Source   <1 %

10   jscholarship.library.jhu.edu
     Internet Source   <1 %

11   "Posters", Hepatology, 2020
     Publication   <1 %

12   Jonathan Flint. "Deep Reads: How I learnt to
     love population genetics", PLOS Genetics,   <1 %
     2017
     Publication

13   Doll, M.A.. "Determination of
     HumanNAT2Acetylator Genotype by   <1 %
     Restriction Fragment-Length Polymorphism

and Allele-Specific Amplification", Analytical Biochemistry, 199511
Publication

14   Dyah Aryani Perwitasari, Jarir Atthobari, Bob Wilffert. "Pharmacogenetics of isoniazid-induced hepatotoxicity", Drug Metabolism Reviews, 2014   <1%
Publication

15   Submitted to October University for Modern Sciences and Arts (MSA)   <1%
Student Paper

16   daneshyari.com   <1%
Internet Source

17   www.coursehero.com   <1%
Internet Source

18   Pengcheng Wang, Komal Pradhan, Xiao-bo Zhong, Xiaochao Ma. "Isoniazid metabolism and hepatotoxicity", Acta Pharmaceutica Sinica B, 2016   <1%
Publication

19   www.e-trd.org   <1%
Internet Source

20   antiinfectivedrugs.com   <1%
Internet Source

21   www.pharmgkb.org   <1%
Internet Source

22 L. G. Possuelo. "Association of slow N-acetyltransferase 2 profile and anti-TB drug-induced hepatotoxicity in patients from Southern Brazil", European Journal of Clinical Pharmacology, 07/2008
Publication

<1%

23 faces.ucsf.edu
Internet Source

<1%

24 Metushi, Imir G, Corron Sanders, William M. Lee, and Jack Uetrecht. "Detection of anti-isoniazid and anti-cyp antibodies in patients with isoniazid-induced liver failure", Hepatology, 2013.
Publication

<1%

25 Naga Chalasani, Einar Björnsson. "Risk Factors for Idiosyncratic Drug-Induced Liver Injury", Gastroenterology, 2010
Publication

<1%

26 Submitted to Olivet Nazarene University
Student Paper

<1%

27 www.lcgdbzz.org
Internet Source

<1%

28 dspace.knust.edu.gh
Internet Source

<1%

29 Hossein Khalili, Shamileh Fouladdel, Mohammad Sistanizad, Mahboobeh

<1%

Hajiabdolbaghi, Ebrahim Azizi. "Association of N-Acetyltransferase-2 Genotypes and Anti-Tuberculosis Induced Liver Injury: First Case-Controlled Study from Iran", Current Drug Safety, 2011
Publication

30   Submitted to University of Glamorgan   <1%
      Student Paper

31   repositorio.ufpe.br   <1%
      Internet Source

32   Submitted to University of Ulster   <1%
      Student Paper

33   hdl.handle.net   <1%
      Internet Source

34   phcfm.org   <1%
      Internet Source

35   uir.unisa.ac.za   <1%
      Internet Source

36   repository-tnmgrmu.ac.in   <1%
      Internet Source

37   mts.intechopen.com   <1%
      Internet Source

38   nagasaki-u.repo.nii.ac.jp   <1%
      Internet Source

39   www.bioflux.com.ro