CORRELATION OF ACETYLATOR STATUS, PHENYLTHIOCARBAMIDE (PTC) TASTE SENSITIVITY AND BLOOD GROUPS OF TUBERCULOSIS (TB) PATIENTS ACCESSING MEDICARE IN ALUSHI MEDICAL CENTRE, AKWANGA.

BY

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NSU/MSC/BMB/0012/17/18

A DESERTATION SUBMITTED TO THE SCHOOL OF POS-GRADUATE STUDIES, NASARAWA STATE UNIVERSITY, KEFFI, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER'S OF SCIENCE (M.SC) DEGREE IN BIOCHEMISTRY.

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DECLARATION

I hereby declare that this project has been written by me and it is a report of my research work. It has not been presented in any previous application for master's degree. All quotations are indicated and sources of information specifically acknowledged by means of references.

AMBI, LAMI JAMES

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CERTIFICATION

The dissertation "Correlation of Acetylator Status, Phenylthiocarbamide (PTC) taste Sensitivity and Blood groups of Tuberculosis patients accessing Medicare in Alushi Medical Center, Akwanga" meets the regulations governing the award of M.Sc. Biochemistry of the School of Postgraduate Studies, Nasarawa State University, Keffi, and is approved for its contribution to knowledge.

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DEDICATION

I dedicate this research work to Almighty God and to my beloved father, late Mr. James Ambi.

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ABSTRACT

Isoniazid also known as isonicotinylhydrazide (INH), is an antibiotic used for the treatment of tuberculosis, it is highly effective against Mycobacterium tuberculosis. The drug is metabolized by acetylation, which varies among individuals. Therefore for effective treatment outcomes that is free from hepatotoxicity, an individual's acetylator status must be determined.The correlation of blood groups, acetylator status and phenylthiocarbamide taste sensitivity in Tuberculosis (TB) patients accessing Medicare in Evangelical Reformed Church of Christ (ERCC), Alushi Medical Centre, Akwanga was determined. A total of 150 TB patients: 102 (68%) males and 48 (48%) females. The blood group test was conducted using the tile method. Out of the 150 samples 16 (10.6%) were group A+, 5 (3.3%) group A-, 39(26.0%) group B+, 5 (3.3%) group B-, 66 (44.0%) group O+, 8 (5.0%) group O-, 10 (6.7%) group AB+ and 1 (0.7%) were group AB-. The acetylator phenotype was achieved by measuring the percentage of acetylisoniazid in the urine after a unique oral dose of 300mg of acetylators. TB patients were treated for two months with standard regimen combining rifampicin, isoniazid, pyrazinamide and ethambutol. Tasters and non-tasters of PTC were determined among the participants using PTC taste strips. Exactly 127 (84.7%) and 23 (15.3%) were tasters and non-tasters respectively. Using pearson correlation, the results obtained showed a significantly ($p<0.01$) strong positive correlation (correlation coefficient of 0.99) between the blood group and sensitivity (positive) to PTC of TB patients. However, the correlation coefficient between blood group and inability (negative) to taste PTC was 0.527, indicating a weaker and non-significant ($p<0.01$) positive correlation was also obtained (correlation coefficient of 0.852) between the blood group and fast acetylators. Similarly, significant ($p<0.05$) positive correlation was obtained between blood group and the slow acetylators, with a correlation coefficient of 0.767. The results showed a significant ($p<0.01$) positive correlation (correlation coefficient of 0.852); a near-perfect correlation between the blood group of TB patients attending Alushi Hospital and the fast acetylators. Also, significant ($p<0.05$) positive correlation was obtained between blood group and the slow acetylators, with a correlation coefficient 0.767.There was a significant correlation between the blood group and the acetylator phenotype of TB patients. However, there was no correlation between blood group and inability to taste PTC.

LIST OF ABBREVIATIONS

ATP: Adenosine Triphosphate

PTU: phenylthiourea

ATT: Anti-tuberculosis treatment

INA: isonicotinic acid

NADH: nicotinamide adenine dinucleotide (NAD) + hydrogen (H).

HIV: Human Immunodeficiency Virus

AIDs: Acquired Immune Deficiency Syndrome

DOT: Direct Observed Treatment

SNPs: Single Nucleotide Polymorphism

NAT: *N-acetyltansferase*

M.tb: *Mycobacterium* Tuberculosis

ALT: *Alanine Aminotransferase*

AcHz: acetylhydrazine

Hz: hydrazine

SA: slow acetylator

FA: fast acetylator

INH: isoniazid

AclINH: acetylisoniazid

TB: tuberculosis

%aclINH: percentage isoniazid

nm: nanometer

OD: optical density

cm^{-3}: cubic centimetre

PTC: phenylthiocarbamide

$moldm^{-3}$: Molar concentration

ERCC: Evangelical Reformed Church of Christ

RBCs: red blood cells

Rh: rhesus factor

DiAcHz: diacetylhydrazine

P450: cytochrome P450

RIF: Rifampicin

PZA: Pyrazinamide

E: Ethambutol

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Genetic variation among humans is important in classifying and comparing them. Human genetic variations refer to the genetic differences that exist in the human population. There are no genetically identical human beings; even monozygotic twins have infrequent genetic differences due to mutations that occur during development and gene copy- number variations (Bruder *et al.*, 2008). These variants maybe of any given gene in the human population called polymorphism. In 2015, the typical difference between human genomes of two individual was estimated at 20 million base pairs (Auton *et al.,* 2015). As at 2017, there are a total of 324 million known variants from sequenced human genomes (NCBI, 2017).

Genetic variation among humans occurs on many scales, ranging from gross alteration in human karyotype to single nucleotide changes (Kidd *et al.*, 2008). A single nucleotide polymorphism (SNP) is a difference in a single neucleotide between members of one specie that occurs from the substitution of a single base pair. Single nucleotide polymorphisms (SNPs) are the most common type of sequence variation (Collins *et al.,* 1998). Others are single base exchanges, deletions and insertions (Thomas *et al., 2011)*. SNPs occur on average of about every 100 to 300 bases and these accounts for the major source of heterogeneity (Ke *et al., 2008*). Functional SNP affects some factors such as gene splicing or mRNA and these results in phenotypic differences among humans (Collins *et al.,* 1998).

N-acetyltransferases (NAT1 and NAT2) are key enzymes in conjugation of certain drugs and other xenobiotics with an arylamine structure (Riddle, *et al.,* 1971). The NATs are

polymorphic in population and are involved in the initial biotransformation metabolism of aromatic amines and hydralazines which catalyze the transfer of the acetyl group from acetyl CoA to the nitrogen of the substrate (Riddle *et al.,* 1971). The acetylation polymorphism was discovered more than 60 years ago as a result of the differences observed in tuberculosis patients to isoniazid toxicity (Hughes *et al.*, 1954). The NAT1 enzyme has limited substrate specificity which is p-aminobenzoic (Windmill *et* al., 2000) while NAT2 enzyme metabolizes a wide variety of therapeutic drugs such as dapsone, sulphadoxine, isoniazid, procainamide, hydralazine as well as exogenous chemicals that are present in diet (Sabbagh *et al.*, 2011). The NAT1 and NAT2 enzymes are encoded by two different genes known as NAT1 and NAT2 respectively both of which are located in the short arm of submetacentric human chromosome 8 (Boukouvala *et al.*, 2005).

Genetic variations in the NAT2 gene have been known to result in individual acetylation polymorphisms, thus several mutant genotypes of NAT2 have been recognized. There are three acetylator phenotypes that exist; they are the slow, intermediate and fast acetylator phenotypes (Higginson, 1976). The slow acetylator phenotype usually experiences toxicity from drugs such as isoniazid, procainamide, hydralazine and sulfonamides, while the fast acetylator phenotype may not respond to isoniazid and hydralazine in the management of tuberculosis and hypertension respectively (Ellad, 1976).

Phenylthiocarbamide (PTC) tasting ability is generally referred to as a simple genetic trait governed by a pair of alleles, dominant T for tasting and recessive t for non-tasting. Persons with genotype TT and Tt are tasters, while those with tt are non- taster. Tasters are persons who have the ability to taste the bitterness of PTC, while non-tasters cannot taste it. The ability to taste PTC has been correlated with the ability to taste bitter substances (Tepper, 1998). Also,

the ability or inability to taste PTC has been associated with some disorders or diseases. For example, non-tasters are said to be more susceptible to congenital arthritic cretinism (Fraser, 1961), nodular goiter (Facchini *et al.*, 1990), epilepsy (Pal, 2004), and dental caries (Rupesh *et al.*, 2006). While tuberculosis, diabetes, mongolism, gastric ulcers have been reported to be associated with the ability to taste PTC (Manlapas *et al.*, 1965).

According to World Health Organization (WHO) 2017 reports, tuberculosis (TB) is the ninth leading cause of death worldwide ranking above HIV/AIDS. In 2017, there were an estimated 1.3 million TB deaths among HIV-negative people (down from 1.7 million in the year 2000) while 10.4 million people are infected with the disease. One third of the global population is latently infected with *mycobacterium* tuberculosis (M.tb), which represents a huge pool of hosts at risk of M.tb reactivation (Zumla *et al.*, 2013).

Tuberculosis is one of the early diseases that caught hold of the human race and still ranks in the top ten infectious diseases known to kill humans (WHO, 2016). It affects about one-third of the world's population and so it can be considered as a global emergency that needs to be addressed urgently, since it is responsible for about two million deaths among those infected annually (WHO,2017). Current therapy for TB consists of multiple expensive antibiotics such as Isoniazid, Rifampicin, Pyrazinamide, and Ethambutol. This treatment is lengthy, usually up to six months for drug-susceptible, and nine months or more for drug-resistant variants of TB. Although current TB treatment eradicates M.tb from the host's body, however, there are reported cases of hepatotoxicity and other adverse side effects linked to individual's ability to acetylate the drugs, thereby causing a large number of patients to withdraw early from therapy.

Multiple strategies have been defined and implemented with the objective to stop TB related deaths (GPS-TB, 2013), and among them the greatest emphasis has been put on patient

management by using efficient medications. It appeared obvious that while TB is a serious scourge, deaths are reduced if treatments are completed with a long 6 months course of a combination of four to six antibiotics (WHO, 2010). Many factors however, can influence the completeness of treatment of which patient's adherence, side effects and adequacy of drug dosing play a key role (Kaishusha *et al.*, 2009), (NIH, 2013). Numerous studies have been reported in the literature demonstrating how an individual's genetic profile may be used for adjusting drug dosages and choosing a medication with minimal side effects to promote the patient's compliance (Kinzig-Schippers *et al.,* 2005). The polymorphism profile of isoniazid acetylation varies with race and ethnicity and its knowledge has been found useful in tailoring individual dosing to minimize side effects and maximize clinical outcomes (Junichi *et al.,* 2013), (Patin *et al.,* 2006).

It therefore beholds that new or alternative approach at determining individual acetylator status is necessary to streamline individual treatment regime to the person's ability to acetylate the drugs. Whereas much work has been reported on acetylator status all over the world (Mukanyangezi *et al.,* 2015), there is no much information on the correlation of acetylator status, PTC taste sensitivity, and blood groups in TB patients. This work is therefore aimed at establishing the relationship between the individual's acetylator status, PTC taste sensitivity and blood groups in TB patients accessing Medicare in a local resource setting in Nasarawa State.

1.2 Statement of the Problem

Despite the advances in the healthcare and treatment strategies for tuberculosis, the situation is getting complicated every year with more people suffering from the disease. Although current

TB treatment eradicates M.tb from the host body it also causes severe hepatotoxicity and other adverse side effects, resulting to a large number of patients withdrawing early from the therapy. This could be checked by assessing the acetylator status of individuals before administering the treatment, so as to ascertain how slow or fast the drugs can be metabolized in order to curtail the possible side effects. The procedure for determining individual acetylator status is time consuming and could be marred by other factors. This work therefore intends to provide an alternative that is dependable, fast, consistent and specific to individuals.

1.3 Aim of Study

The aim of this research is to determine the correlation of blood groups, acetylator status and phenylthiocarbamide taste sensitivity in TB patients accessing medicare in Alushi Medical Centre, Akwanga.

1.4 Objectives of Study

The objectives of the study is to determine the following in the TB patients accessing Medicare in Alushi Medical Centre, Akwanga, Nasarawa State,

1. The blood group of the sampled population
2. The acetylator status of the sampled population
3. The PTC threshold of the sampled population
4. The correlation of the blood group, acetylator phenotype and PTC taste sensitivity in the sampled population.

1.5 Significance of the Study

I. Findings from this study will help to generate a common or an alternative test that can be used to determine individual's acetylator status. This will help fast track diagnosis on individuals that are undergoing tuberculosis treatment.

II. It will provide useful information on the option of using PTC taste sensitivity or blood groups by clinicians in predicting individual acetylator status in prescription.

1.6 Scope of the Study

This research focuses on the correlation of blood groups, acetylator status and phenylthiocarbamide taste sensitivity in tuberculosis (TB) patients that are accessing medical treatment in Evangelical Reformed Church of Christ (ERCC) Hospital, Akwanga, Nasarawa State.

CHAPTER TWO

LITERATURE REVIEW

2.1 Meaning of Tuberculosis

Tuberculosis (TB) is a multisystemic infectious disease caused by *Mycobacterium tuberculosis* (or TB, TB germs), a rod-shaped bacterium. TB (TB may stand for the disease or the bacteria that cause the disease) is the most common cause of infectious disease-related mortality worldwide (about 10 million people worldwide were sick with TB in 2017, and about 1.3 million people died from TB worldwide in 2017 according to the World Health Organization (WHO). The most common site (about 85%) for TB to develop is in the pulmonary tract although it may infect other parts of the body. TB has been infecting humans for many centuries; evidence of TB infections has been found in cadavers that date back to about 8000 BC. The Greeks termed it as a wasting away disease (phthisis). For many European countries, TB caused death in about 25% of adults and was the leading cause of death in the U.S. until the early 1900s. Robert Koch discovered TB's cause to be *Mycobacterium tuberculosis* (M.tb) in 1882. With the increased knowledge of TB, public health initiatives, treatment methods like isolation of patients (quarantine), and the development of drugs to treat TB, has led to the reduction

in the incidence of the disease, especially in developed countries (www.medicinenet.com)

2.2 Types of Tuberculosis

There are many types of tuberculosis, but the main two types are termed either active or latent tuberculosis infection. Active TB is when the disease is actively producing symptoms and can be transmitted to other people while latent TB disease occurs when one is infected

with *Mycobacterium tuberculosis* bacteria, but the bacteria are not producing symptoms (usually due to the body's immune system suppressing the bacterial growth and spread) and have no TB bacteria in the sputum. People with latent TB usually cannot transfer *Mycobacterium tuberculosis* bacteria to others unless the immune system fails; the failure causes reactivation (bacterial growth is no longer suppressed) that results in active TB so the person becomes contagious (www.medicinenet.com).

2.3 Treatment of Tuberculosis

The current internationally accepted therapy is the Directly Observed Treatment, Short-Course (DOTS) for drug-susceptible TB which consists of multiple expensive antibiotics for a long period of time. (Tousif *et al.*, 2015). Adherence and compliance are critical for optimal efficacy of these drug regimens. The regimen chosen for treatment of TB is largely based on two indicators. These indicators are firstly, whether the patient has previously been treated for tuberculosis and secondly, the drug-susceptibility status of infecting bacteria. The WHO recommended first and second-line drugs for treatment of TB DOTS strategy for treatment of drug susceptible TB involves the administration of Isoniazid (INH), Rifampicin(RIF), Pyrazinamide (PZA), and Ethambutol (E) during the first two months in the active phase of the disease, followed by an additional four months of treatment with INH and Rifampicin. However, much longer and more extensive treatment in the form of combinatorial therapy is required for curing drug-resistant forms of TB (WHO, 2008).

2.4 Isoniazid

Isoniazid also known as isonicotinylhydrazide (INH), is an antibiotic used for the treatment of tuberculosis, it was first made in 1952 (Walker, 2012). Isonicotinylhydrazide (INH) is not only highly effective against *Mycobacterium*

tuberculosis, but is also widely affordable, inexpensive, and well tolerated (Hemanth *et al.*, 2012). INH is a low-molecular weight and water-soluble compound that can be rapidly absorbed from the gastrointestinal tract (Weber *et al.*, 1971). Pharmacokinetic properties of INH are affected by various patient-specific factors, like genetic status, age, comorbidities, and the co-administered food or drugs (Mach *et al.*, 2016), (Saktiawati *et al.*,2016) . The peak plasma concentration is achieved around 1–3 hours after administration of the drug (Ellard *et al.*, 1976). Meals containing high fats can decrease absorption of INH as revealed by the reduction of C_{max} by 51% and the increasing of T_{max} to 2 times (Peloquin *et al.*, 1999). Hence it is recommended to consume INH on an empty stomach. After absorption, INH diffuses into all tissues and body fluids rapidly, including cerebrospinal fluid, saliva, pleural and peritoneal exudates, bronchi and pulmonary alveoli (Bhandari *et al.*, 2013). INH also can be excreted into breast milk (Singh *et al.*, 2008).

For active tuberculosis it is often used together with rifampicin, pyrazinamide, and either streptomycin or ethambutol (WHO Model Formulary, 2008) while for latent tuberculosis it is often used alone (ASHSP, 2016). It is usually taken by mouth but may be used by injection into muscle. Isoniazid (INH) is an essential drug in the treatment of tuberculosis (TB).

2.5 Metabolism of Isoniazid

The primary step in its metabolism is acetylation to acetyl INH. The metabolizing enzyme is a hepatic N-acetyl transferase, which displays genetic polymorphism.

The rate of acetylation of INH is known to influence the response to treatment of TB patients. It was observed that the failure of once-weekly regimens was predominantly due to inadequate exposure and coverage that is, hours for which a bacteriostatic concentration of INH 0.2 µg/ml are maintained. (Sarma *et al.*, 1975). Weiner *et al.*, 1986 observed an association between INH acetylator status and treatment outcome in TB patients and further found that low INH concentrations in rapid acetylators was associated with failure/relapse. It is therefore essential that adequate INH concentrations are maintained in blood for good treatment outcome.

The major pathways of INH metabolism include: Acetylation to form AcINH through N-acetyltransferase (NAT) 2, Hydrolysis to produce isonicotinic acid (INA) and hydrazine (Hz) through amidase. AcINH can also be hydrolyzed to form INA and AcHz. In addition, Hz can be acetylated to AcHz and diacetylhydrazine (DiAcHz) (Reziosi, 2007). Hydrazine (Hz) and AcHz are further oxidized to reactive metabolites and involved in INH hepatotoxicity (Delaney & Timbrell, 1995), which was proposed to be mediated by microsomal P450s, especially CYP2E (Sarich *et al.*, 1999).

Besides these major metabolic pathways, INH can also conjugate with several endogenous metabolites (Khan *et al.*, 2016), including ketone acids, vitamin B6 (pyridoxal and pyridoxal 5-phosphate), and NAD^+. In addition, INH was found to disturb the homeostasis of endogenous metabolites, such as vitamin B6, bile acids, cholesterol, and triglycerides (Cilliers *et al.*, 2010). The major metabolic pathways of INH are enzymatic-dependent reactions, including acetylation and hydrolysis of INH by NAT and acyl amidase, respectively (Boelsterli and Lee, 2014). Catalase-peroxidase (KatG) of *Mycobacterium tuberculosis* (Mtb) and human

neutrophil myeloperoxidase can catalyze the formation of INH-NAD^+ adducts (Rozwarski *et al.,* 1998). Nevertheless, conjugation of INH with ketone acids and vitamin B6 are non-enzymatic reactions.

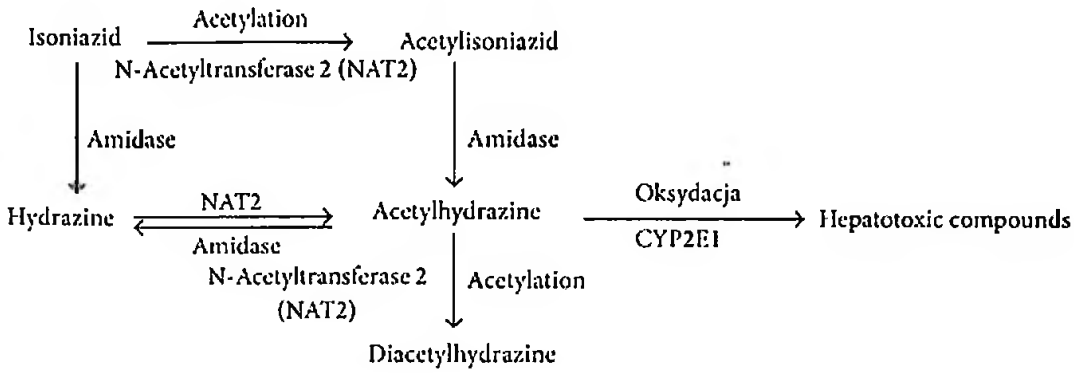

Figure 2.1:Biotransformation of Isoniazid

2.6 Mechanism of the Action of Isoniazid

Isoniazid is a prodrug that acts by inhibiting the formation of the mycobacterial cell wall. Isoniazid must be activated by a bacterial catalase-peroxidase enzyme in *Mycobacterium tuberculosis* called KatG (Suarez *et al.,* 2009). KatG catalyzes the formation of the isonicotinic acyl radical, which spontaneously couples with NADH to form the nicotinoyl-NAD adduct. This complex binds tightly to the enoyl-acyl carrier protein reductase InhA, thereby blocking the natural enoyl-AcpM substrate and the action of fatty acid synthase. This process inhibits the synthesis of mycolic acids, which are the required components of the mycobacterial cell wall. A range of radicals are produced by KatG activation of isoniazid, including nitric oxide, (Timmins *et al.*, 2004) which has also been shown to be important in the action of other antimycobacterial prodrug called pretomanid (Singh *et al* B., 2008).

2.7 Isoniazid Acetylator Phenotype

Several studies have shown that human subjects show a wide degree of variation in their capacity to acetylate INH to acetyl INH in spite of receiving similar prescribed INH doses (Ellard *et al.*, 1976). The human population is divided into three different phenotypic groups according to acetylation rate, that is, slow, intermediate, and fast acetylators (Anna *et al.*, 2013). Molecular techniques that are now available permit identification of three genotypes: rapid, intermediate and slow. Slow acetylators are known to be at a risk for most drug-induced toxicities, while rapid acetylators are likely to experience decreased therapeutic efficacy. It has been suggested that *NAT2* genotyping before therapy could be useful to predict adverse reactions and make dose adjustments, if necessary (Azuma *et al.*, 2013).

Isoniazid is metabolized internally to acetylisoniazid by NAT, which is then hydrolyzed to acetylhydrazine. Acetylhydrazine can be transformed into diacetylhydrazine by a process of acetylation or oxidized by cytochrome P-4502E1 (CYP2E1) to form hepatotoxic compounds. Low *N-acetyltransferase* (NAT) activity increases the risk of hepatic damage because the majority of acetylhydrazine is oxidized (Czech *et al,* 2005). Depending on its serum concentration, isoniazid can be both an inducer and an inhibitor of CYP2E1. High concentrations of INH repress the CYP2E1 activity, whereas low INH concentrations induce the enzyme (Czech *et al.,* 2005).

The hepatotoxic potential of INH is greatly influenced by the enzymatic isoform of NAT. Low NAT activity increases the risk of hepatocellular damage due to oxidation of acetylhydrazine by CYP2E1. N-Acetyltransferase 2 (NAT2) exhibits genetic polymorphisms. Different acetylation phenotypes within a population are the result of mutations in the NAT2 gene. These mutations influence the activity (leading to either high or low activity) of the NAT enzyme

(slow and fast acetylators, respectively) (Sim *et al.*, 2000). The NAT2 gene which is located on chromosome 8p22, is autosomal dominant and intronless, with a single open reading frame of 870 base pair. NAT2 enzyme detoxifies and inactivates drugs and xenobiotics in the liver. Polymorphisms of NAT2 confer slow, intermediate, or fast acetylator phenotypes with broad interethnic variations. There are currently known 53 NAT2 alleles, and each allelic variant reflects a combination of one, two, three, or four nucleotide substitutions. Within the coding region, there are seven missense mutations (G191A, T341C, A434C, G590A, A803G, A845C, and G857A) and four silent mutations (T111C, C282T, C481T, and C759A) (Hein *et al*, 2000). The wild type NAT2*4 allele is associated with the fast acetylator phenotype and does not have any nucleotide substitutions.

2.8 Roles of *N-acetyltransferases* (NATs) in Isoniazid Metabolism

NATs (EC 2.3.1.5, *N*-acetyltransferase, arylamine *N*-acetyltransferases) are a class of enzymes that catalyze the acetylation of arylamines from acetyl-CoA. It is widely found in different species, both in eukaryotes and prokaryotes (Payton *et al.*, 2001). NATs are responsible for acetylation of hydrazine drugs and carcinogenic aromatic amines, as well as endogenous molecules, such as serotonin (Sim *et al.*, 2014). NAT1 and NAT2 are the major NATs that are involved in the biotransformation of xenobiotics. The *NAT* genes are located in close vicinity in the genome and share high sequence identity (Blum *et al.*, 1990), but their expression profiles have distinct tissue distribution patterns and the enzymes have different substrate preferences (Hickman *et al.*, 1995). NAT1 is widely expressed in all tissues, including endocrine tissues, blood cells, neural tissue, liver, and the gastrointestinal tract, while NAT2 expression is limited to the liver and gastrointestinal tract (Windmill *et al.*, 2000). *p*-

Aminobenzoate and *p*-aminosalicylate, are prefer substrates of NAT1, whereas NAT2 preferentially metabolizes sulfamethazine, procainamide.

NAT2 is the dominant enzyme that catalyzes the acetylation of INH, Hz, and AcHz (Peretti *et al.*, 1987). NAT2 is involved in three steps of INH biotransformation, including deactivation (formation of AcINH), bioactivation (formation of AcHz), and detoxification (formation of DiAcHz). NAT2 is highly polymorphic and has been thought to be involved in INH hepatotoxicity (Walker *et al.*, 2009). Rapid acetylators have been proposed to have a higher risk of INH-induced liver injury than slow acetylators, which is based on the proposition of an increased rate of AcHz formation in rapid acetylators (Ohno *et al.*, 2000). Yamamoto *et al.*, 1986 In a study with 143 patients who received INH-containing regimens for anti-TB therapies, 18 patients with abnormal elevated levels and 18 patients with normal levels of serum aminotransferase were investigated. It was observed that 14 patients with abnormal serum aminotransferase were rapid acetylators, while only 7 were rapid acetylators in patients with a normal serum aminotransferase level. These results suggest that rapid acetylators have a higher risk of liver injury with INH therapies.

However, the later clinical studies found that the presence of slow acetylator alleles has a higher risk of INH hepatotoxicity Gupta *et al.*, 2013). In a study of 224 patients that received anti-TB treatment, slow acetylators had a much higher risk of liver toxicity than rapid acetylators (26.4 % *vs.* 11.1 %, P=0.013) (Walker *et al.*, 2009). Another study reported the risks of different acetylator status in INH hepatotoxicity in Brazilian patients. The risk of developing hepatitis was 22% for slow acetylators, while only 9.8 % for intermediate acetylators and 2.9 % for rapid acetylators (Teixeira *et al.*, 2011). The plasma levels of INH and AcHz are higher in slow acetylators than those in rapid acetylators, which contradict

previous findings (Mitchell *et al.,* 1975). Even though the acetylation rate of INH is slow in slow acetylators, the acetylation of AcHz is also slow, thus leading to a higher accumulation of AcHz in slow acetylators (Bing *et al.,* 20011). The clearance rate of INH is also slower in slow acetylators than in rapid acetylators (Seng *et al.,* 2015), which also contributes to the accumulation of INH in slow acetylators. A higher level of free INH might be the cause of the high incidence of liver injury directly as INH can bind to liver proteins and cause immune-mediated liver injury (Metushi *et al.,* 2012). Elevation of INH can also lead to an increase in Hz formation, which is supported by an increased rate of hydrolysis process of INH in slow acetylators than in rapid ones (Lauterburg *et al.,* 1981). Furthermore, decreasing the dose of INH in slow acetylators can reduce the incidence of INH hepatotoxicity (Azuma *et al.,* 2013). In a multicenter, paralleled, randomized, and controlled clinical trial with Japanese patients, treatment with a lower dose of INH (2.5 mg/kg) used for anti-TB therapy in slow acetylators significantly decreased the incidence of INH-induced liver injury than the standard treatment (5 mg/kg for all patients). In addition, NAT2 status also plays an important role in hepatotoxicity caused by INH and rifampicin combination therapies (Ohno *et al.,* 2000). In a study with 77 Japanese patients with INH + rifampicin treatment, the risk of liver injury was much higher in slow acetylators than in intermediate and rapid acetylators. Even though these clinical reports showed that slow acetylators have a higher risk of INH-induced liver injury with INH treatment in different populations (Chamorro *et al.,* 2013), (Gupta *et al.,*2013). Several other clinical observations showed modest or no significant difference of incidence of INH hepatotoxicity between different acetylators status (Cai *et al.,* 2012). Furthermore, the incidence of INH hepatotoxicity did not show significant correlation with *NAT2* status in different ethnic populations (Huang, 2014). The frequency of slow acetylators in the Asian

populations (10%–20%) is much lower than that in Caucasians and Africans (more than 50%) (Walker *et al.*, 2009), but the incidence of INH hepatotoxicity does not show such dramatic ethnic differences (Huang, 2014).

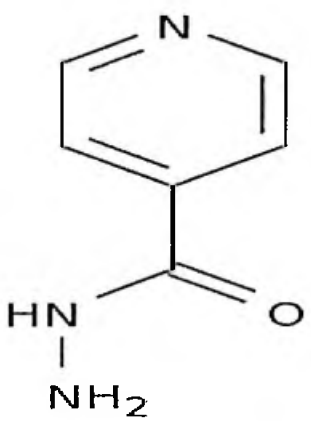

Figure 2.1: Structure of isoniazid

It is recommended that women with active tuberculosis who are pregnant or breastfeeding take isoniazid. Preventive therapy should be delayed until after giving birth. Nursing mothers excrete a relatively low and non-toxic concentration of INH in breast milk, and their babies are at low risk for side effects. Both pregnant women and infants being breastfed by mothers taking INH are expected to take vitamin B6 in its pyridoxine form in order to minimize the risk of peripheral nerve damage (Bothamley, 2001). Vitamin B6 is used to prevent isoniazid-induced B6 deficiency and neuropathy in people with a risk factor, such as pregnancy, lactation, HIV infection, alcoholism, diabetes, kidney failure, or malnutrition (Steichen *et al.*, 2006) Brand names of INH include Hydra, Hyzyd, Isovit, Laniazid, Nydrazid, Rimifon, and *Stanozide (*FDA, 2016).

2.9 Challenges of the Treatment of Tuberculosis Using Isoniazid

Despite the beneficial effects of INH, severe adverse effects especially peripheral neuropathy and hepatotoxicity are associated with INH therapies (Boelsterli *et al.*, 2014). Hepatotoxicity is the most serious complication arising from the first line of TB treatment. Isoniazid, rifampicin and pyrazinamide are potentially hepatotoxic drugs (Saukkonen *et al.*, 2006). About 10%–20% of patients consuming INH have a transient elevation of serum alanine aminotransferase (ALT) level. Most of the patients can adapt to it and their serum ALT levels return to normal without discontinuation, while some patients can develop severe liver injury and even liver failure (CDC, 2010). Clinically, INH-associated treatments usually cause a hepatocellular-type of liver injury, as characterized by a marked elevation of ALT but minimal increases in alkaline phosphatase (ALP) levels.

INH metabolism is thought to be associated with INH-induced liver injury (Tafazoli *et al.*, 2008; Cheng *et al.*, 2014,). Significantly, about 0.5% patients show hepatotoxicity to INH mono-therapy. The percentage was higher for combination therapies. It is not INH levels in the serum that is toxic but rather its metabolites, especially hydrazine. The enzyme N-acetyltransferase 2 (NAT-2) in humans acetylates INH to form acetylisoniazid, which further undergoes hydrolysis to generate acetylhydrazine. Polymorphic variants of NAT-2 redirect some INH towards an alternative oxidative pathway via P450 to generate hydrazine (Sinha and Mason, 2014). Acetylhydrazine and hydrazine participate in complex reactions to produce free radicals that generate an environment conducive to oxidative stress. INH metabolites bind covalently with cellular macromolecules, which clinically manifests primarily as hepatocellular steatosis and necrosis. Long-term intake of INH can cause peripheral neuritis and CNS effects, as well as sideroblastic anemia (Tousif *et al.*, 2015). Peripheral neuritis is linked to pyridoxine

(vitamin B6) depletion, and is the reason why pyridoxine supplementation is prescribed along with standard INH dosing for safety reasons (Dalle *et al.*, 2012).

Studies of INH hepatotoxicity in rats showed that AcINH and AcHz can cause hepatic necrosis; however, treatment with INH directly even at high dose and long term did not cause toxicity (Metushi *et al.*,2012). These results suggested INH metabolites are responsible for INH hepatotoxicity. Covalent binding of acetyl group to liver proteins were observed after treating rats with ^{14}C-acetyl-labeled AcINH but not with aromatic ring ^{14}C labeled AcINH, indicating that AcHz is responsible for INH hepatotoxicity in rats (Nelson *et al.*, 1976). Studies carried out in mice showed different results. When Hz or AcHz was administrated at a dose of 300 mg/kg to mice, Hz produced hepatic necrosis, macrovesicular degeneration, and steatosis, whereas AcHz did not (Richards *et al.*, 2004). This suggests that Hz is responsible for INH-induced liver injury in mice. In a rabbit model of INH-induced liver injury, the plasma level of Hz is correlated with the extent of INH-induced necrosis and steatosis, but plasma levels of INH and AcHz are not (Sarich *et al.*, 1996). In addition, Hz inhibits mitochondrial complex II and affects the function of electron transport chain and ATP production in mouse primary hepatocytes. Co-treatment with Hz and a complex I inhibitor can cause hepatocyte death (Lee *et al.*, 2013). Studies also found INH itself can bind to liver proteins and cause immune-mediated hepatotoxicity (Metushi and Uetrecht, 2014).

The adverse effects that are associated with INH therapy emerge from exposure to INH (Metushi *et al.*, 2016) and in particular to its toxic metabolites, Hz and AcHz, in the liver and brain (Bando *et al.*, 2011). Notably, both toxic metabolites are a substrate for NAT2 (Koizumi

et al., 1998), therefore, the enzyme is involved in both metabolite formation and subsequent detoxification. This results in an inevitable trade-off between treatment efficacy and drug-induced toxicity in INH-based chemotherapy. Although slow acetylators have an increased risk of adverse reactions due to a higher exposure to toxic metabolites (Ng *et al.,* 2014), fast acetylators, in turn, have to face reduced treatment efficacies as a result of the lower plasma half-life of the active drug (Pasipanodya *et al.,* 2012), however, patients are still given the same INH doses, regardless of their acetylator or health status.

Phenylthiocarbamide Taste Perception in Human Population

The human sense of taste consists of five different modalities, bitter, sweet, sour, salt, and umami (the taste elicited by glutamate), that are critical for nutrition and survival. Of these, bitter perception has a particularly important role, as it protects individuals from ingesting naturally toxic substances which typically taste bitter (Mueller *et al.,* 2005). Variation in taste sensitivity to the bitter compound phenylthiocarbamide (PTC) is one of the best known. Phenylthiocarbamide (PTC), also known as phenylthiourea (PTU), is an organosulfur thiourea containing a phenyl ring. It has the unusual property that it either tastes very bitter or is virtually tasteless, depending on the genetic makeup of the taster. Phenylthiocarbamide (PTC) tasting ability is generally referred to as a simple genetic trait governed by a pair of alleles, dominant T for tasting and recessive t for non-tasting. Persons with genotype TT and Tt are tasters, while those with tt are non- taster. Tasters are persons who have the ability to taste the bitterness of PTC, while non-tasters cannot taste it. The ability to taste PTC has been correlated with the ability to taste bitter substances (Tepper, 1998).

The receptor gene family consists of a single coding exon 1002 base pair long, encoding a 333 amino acid, 7-transmembrane domain Gprotein-coupled receptor (Kim *et al.,* 2003), that responds to bitter stimuli (Duffy *et al.,* 2004; Bufe *et al.,* 2005).

Two major forms of this bitter receptor gene were identified in most of the world's populations, designated as the 'major taster' form and the 'major non-taster' form. These two forms differ in 3 amino acid positions, numbers 49, 262, and 296 (Kim *et al.,* 2004). The major taster form contains a proline at position 49, an alanine at position 262, and a valine at position 296, while the non-taster form contains an alanine, a valine, and an isoleucine at these 3 positions, respectively. These two forms, called alleles, account for the bimodal distribution of taste thresholds and the classic recessive inheritance pattern is observed. Reports have observed that younger subjects are more sensitive than older subjects to the bitterness of 6-n-propylthiouracil (PROP) or PTC, with some suggesting that age modifies the genotype-phenotype relationship (Kim *et al.,* 2004). Allele frequencies for the bitter taste gene, TAS2R38 vary by racial/ethnic group and therefore when two racial groups are compared, they differ in phenotype as they differ in genotype (Wooding *et al.,* 2004). Factors like mutation, natural selection, inbreeding, genetic drift and miscegenation are known to play an important role in producing gene frequency differences in different humans.

Phenylthiocarbamide (PTC) tasting is believed to be generally determined by a single gene, TAS2R38 with two alleles; one for tasting which is dominant (TT or Tt) and the other which is recessive (tt) for nontasting. However, some studies have shown that there are other genes or environmental factors that influence PTC tasting which suggest that it does not follow the one gene, two allele myth of Mendelian genetics (Shivaprasad *et al.*, 2012).

Also, the ability or inability to taste PTC has been associated with some disorders or diseases. For example, non-tasters are said to be more susceptible to epilepsy (Pal, 2004), nodular goiter (Facchini *et al*, 1990), congenital arthritic cretinism (Fraser, 1961), and dental caries (Rupesh *et al.*, 2006). While tuberculosis, diabetes, mongolism, gastric ulcers have been reported to be associated with the ability to taste PTC (Manlapas *et al.*, 1965).

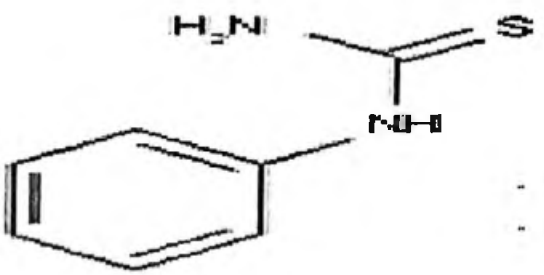

Figure 2.2: Structure of phenylthiocarbamide

ABO blood group system is the most studied trait in human genetics followed by the ability to taste phenylthiocarbamide (PTC) (Chibuisi *et al.*, 2010). These two traits have been extensively used in describing genetic variations among human populations around the world (Egesie *et al.*, 2008). The divergence in taste responses to PTC was first described by Fox (1932). Since then many investigators had described PTC sensitivity as a bimodal autosomal trait inherited in a simple Mendelian recessive pattern, with tasters being dominant (T) and non-tasters recessive (t) (Bakare *et al.*, 2009). Aside its importance in genetic and anthropological studies, PTC taste sensitivity has been shown to be important in food selection, which may affect individual metabolism and physiology (Davis, 1978). It was previously used in paternity testing before the advent of DNA markers (Cardullo *et al.*, 1951).

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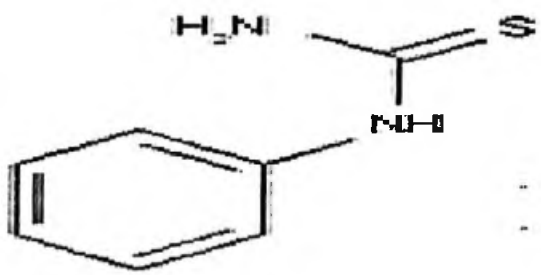

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2.2.2. The ABO Blood Group System

The ABO blood group are the first red cell antigens while the Rhesus blood group are the most immunogenic red cell antigens discovered. They express their character in transfusion and pregnancy as important histocompatibilty genes. (Dacie and Lewis,1994). ABO and Rhesus blood groups are of vital clinical and immunological importance due to their frequent occurrence and capacity for stimulation of antibodies, which may lead to intravascular hemolysis in cases of incompatible blood transfusion or pregnancy in majority of Rhesus 'D' (RhD)-negative individuals. The Rhesus antigen is present on red cell structural membrane (Carton and Agre, 1993), but not on white cells, platelets, tissue, or body fluids.

The ABO and Rhesus factor are the two most significant blood group systems in transfusion medicine (Dhruva *et al.,* 2015). These blood groups are being tested for all healthy blood donors as well as all patients prior to blood transfusion to ensure that the patients are given the right blood for transfusion (Bhagwat *et al.,* 2015). Pre-donation blood gouping of the donor is the preliminary result which is repeated again during pre-transfusion testing from the donor unit before issuing the blood for transfusion to the patient. ABO blood groups are one of the most studied blood systems among human populations due to its clinical, genetic and anthropological importance (Jeremiah, 2006). The ABO blood group is expressed by three alleles on chromosome 9 controlling four phenotypes; A, B, AB and O. (Rehman *et al.*, 2005). The grouping of ABO into blood groups is based on the antigenic properties on the surface membrane of the red blood cells (RBCs) (Garratty, 2005). Blood grouping among human populations has shown relevance in genetic studies, blood transfusion and forensic pathology (Chibuisi *et al.*, 2010). The classification of blood groups into type A, B, AB and O in ABO system, Rh positive and Rh-negative in Rh system is based on the presence or absence of

inherited antigenic substances on the surface of the red blood cells. The antigens may be proteins, carbohydrates, glycoproteins, and glycolipids depending on the blood group system (Rehma *et al.,* 2005). ABO and Rhesus (Rh) blood group antigens are hereditary characters which are useful in population genetic studies, researching population migration patterns, as well as resolving certain medicolegal issues, particularly of disputed paternity and more importantly in compatibility test in blood transfusion practice.

There are wide variations in racial distribution of ABO antigens. In Caucasian, prevalence of 46, 42, 9 and 3% (Wikipedia, 2012) were reported compared to 57, 20.5, 21.6 and 1.4% for O, A, B, and AB in Blacks respectively. (Chima *et al,* 2012).

The cellular expression of A and B antigens are determined by the H gene, which is inherited independently. This gene codes for an enzyme that converts a carbohydrate precursor into H substance. The A and B genes code for specific enzymes (glycosyl transferases), that converts H substance into A and B antigens by the terminal addition of N-acetyl-galactosamine and D-galactose, respectively. The O gene produces an inactive transferase, so that H substance persists unchanged in group O individuals.

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Study Area

This study was carried out on tuberculosis infected individuals assessing treatment in Evangelical Reformed Church of Christ (ERCC) Alushi Medical Centre, Akwanga Local Government Council of Nasarawa State, Nigeria. Evangelical Reformed Church of Christ (ERCC) Medical Centre is owned by Evangelical Reformed Church of Christ of Nigeria. The Medical Centre is situated along Akwanga-Keffi Road Nasarawa State. It is on latitude 8.9067 °N and longitude 8.4075 °E. The Medical centre accommodates both in and out patients infected with TB.

Figure 3.1 Map of Akwanga Local Government, Nasarawa State

3.1.4 Exclusion Criteria

Patients were not eligible to participate in the study if they are:

i. Diabetic

ii. Out-patients

3.1.5 Consent / Ethical Approval

Each individual's consent to participate in the research without payment was obtained. Similarly, ethical approval by the Board of Ethics and Research Committee of the Alushi Medical Centre, Akwanga was obtained.

3.2 METHODS

3.2.1 Sample Collection

Exactly one tablet (300 mg) of isoniazid was administered to patients under the direct strict supervision of medical staff. At approximately 8 hours, urine samples were collected from subjects, each into a labeled sterilized specimen container. The analysis was carried out within 2-4 hours of sample collection.

3.2.2 Condition of Urine Samples

Urine samples collected from subjects into specimen containers were used without any further processes.

3.3 Analytical Methods

3.3.1 Test for Sugar

Each urine specimen was first subjected to sugar test as described briefly; $5cm^3$ of Benedict's solution was put into a test tube followed by the addition of 8 drops of the urine sample. The mixture was heated under a spirit lamp for about five minutes. Samples that changes to red yellow colour change indicated the presence of sugar and were discarded because the urine of diabetics will give false reactions with isoniazid (Mason & Russel, 1971).

3.3.2 Phenotyping Method

The phenotype determination was carried out using a simple spectrophotometric method, which is a modification of Eidus &Hodgkin (1973) and Mukanyangezi *et al.*(2015). After a test dose of isoniazid, free isoniazid (INH) and its acetyl derivative (AcINH) were estimated in urine. The classification into slow acetylator (SA) and fast acetylator (FA) was based on the ratio of the metabolite acetylisoniazid to the total hydrazines excreted (% AcINH).

3.3.3 Analysis of Metabolized and Unmetabolized drugs in Urine Samples

To two 1-ml aliquots of urine (aliquot A and B), 0.5ml of 0.5M hydrochloric acid was added and the mixture was left for 15 minutes at room temperature. Acetylisoniazid (metabolized drug) was estimated direct from aliquot A while the total hydrazide content (that is both the metabolized and unmetabolized drug) was estimated in aliquot B as follows:

One drop of acetic anhydride (reagent grade) was added to aliquot B and then it was shaken for one minute.This was followed by the addition of one drop of 7 $moldm^{-3}$ sodium hydroxide

(to convert the free isoniazid artificially to acetylisoniazid).Two drops of distilled water was added to aliquot A in order to ensure equal volumes of the two solutions (aliquot A and B).

Both aliquots (A and B) were then neutralized with 0.5 cm^3 of 0.5 $moldm^{-3}$ sodium hydroxide. This was followed by the successive addition of the following:

1 cm^3 of 0.5 $moldm^{-3}$ potassium phosphate buffer pH 6.0 (freshly prepared).1 cm^3 of freshly prepared aqueous solution of potassium cyanide.4 cm^3 of freshly prepared solution of chloramine T^C, the mixture was shaken greatly using a stirrer for 2 minutes 5 cm^3 of reagent-grade acetone was added with thorough mixing.The optical density (OD) of the colour that was formed in the two aliquots were measured at a wavelength of 530 nm.

Since aliquot A contains only acetylisoniazid and aliquot B contains both acetylisoniazid and the free isoniazid (artificially converted to acetylisoniazid) the percentage of acetylation was calculated as follows:

$$\frac{\text{Optical density of A}}{\text{Optical density of B}} \times \frac{100}{1} \quad \text{---------- (2)}$$

An individual was classified as either slow or rapid acetylator depending on the percentage acetylation. Patients yielding a proportion lower than 70 % are considered as slow acetylators, while rapid acetylators produce values over 75 % with this method.

3.3.4. Phenylthiocarbamide (PTC) Tasting

Phenylthiocarbamide (PTC) taste strips (0.0143 mg of PTC/strip) were used. Each participant was given a PTC taste strip and were asked to put it on their tongue and allowed to be soaked

in their saliva before describing their perception to each taste strip (Igbeneghu *et al.*, 2016). Taste description of each participant was recorded.

3.3.5. Blood Grouping

The ABO blood group antigens test was performed using the tile method. The ABO blood grouping is based on agglutination of red blood cells by antibody (Waters, 1994). It was performed using commercially prepared monoclonal anti-A and anti-B according to the manufacturer's instructions. The following procedure was used:

The white tile was labeled A, B, AB, and D. A drop of anti-A, anti-B, anti-AB and anti-D sera were placed respectively on the label. A drop of test red cell suspension was added to each drop of typing antiserum.The mixtures of the cells and reagent were properly done using an applicator stick..Each mixture was Spread evenly on the slide over an area.The slide was tilted back and forth to observe agglutination. Tests that show no agglutination within two minutes were considered negative

3.4 Statistical Analysis

The data obtained were analyzed using descriptive analysis which showed the percentages of the blood group test, acetylator status and phenylthiocarbamide while Pearson correlation analysis was used to correlate blood group and acetylator status, as well as blood group and PTC taste sensitivity. These were carried out with the aid of IBM statistical product and service solution (SPSS) version 23.0. $P<0.01$ was considered significant for blood group and PTC taste sensitivity as well as fast acetylators while $P<0.05$ was considered significant for blood

group and slow acetylators. Where it is required, data were given as mean ±standard deviation. One-way ANOVA was used to compare the mean ages.

CHAPTER FOUR

RESULTS PRESENTATION AND ANALYSIS

RESULTS

4.1: Gender of Tuberculosis Patients

The gender of TB patients accessing Medicare in Alushi Medical Centre is presented in figure 4.1. The result clearly showed that 120 of the patients were males which correspond to 68% while 48 of them were female corresponding to 32% of the population.

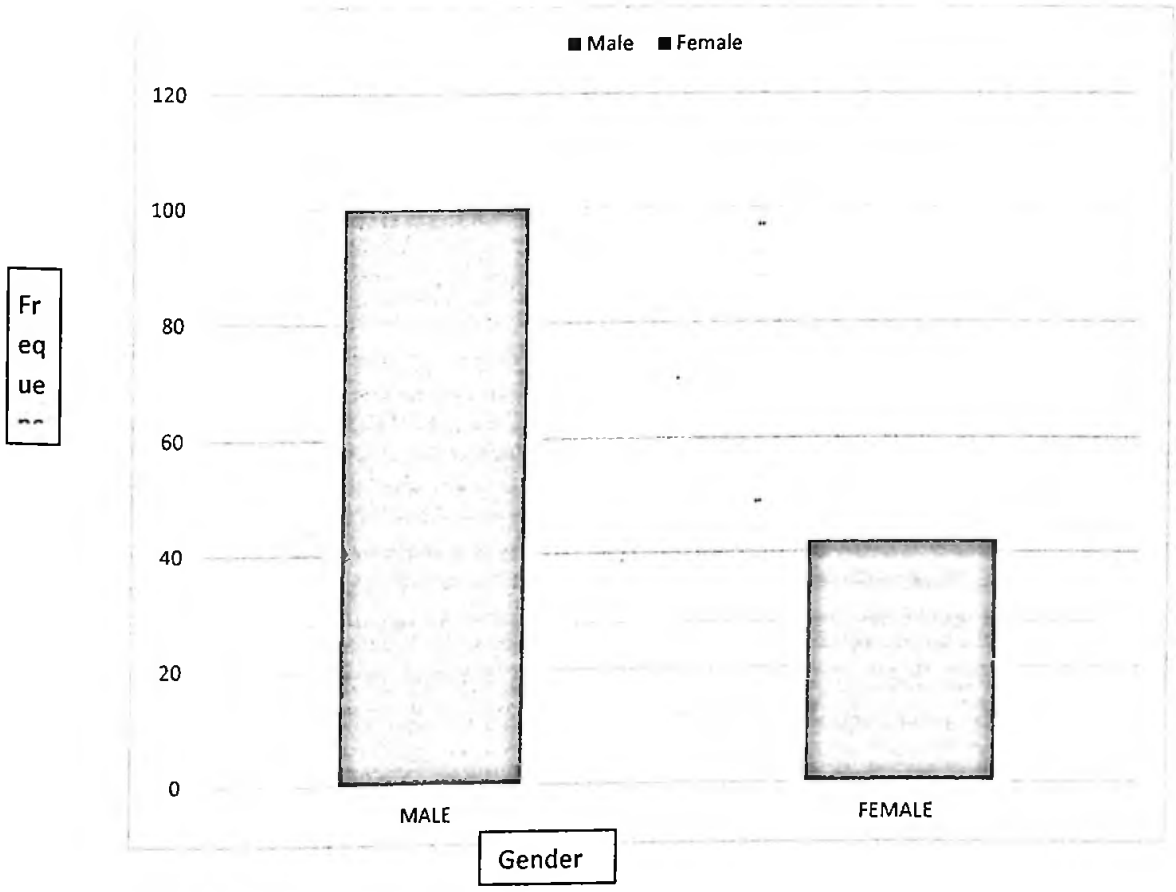

Figure 4.1: Gender of TB patients accessing Medicare in Alushi Medical Centre

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4.3: Blood Groups of Tuberculosis Patients

The blood groups of tuberculosis patients accessing Medicare in Alushi Medical Centre is presented in figure 4.3. The result clearly showed that 16 (10.7 %), 5 (3.3 %), 39 (26 %), 5 (3.3%), 66 (44 %), 8 (5.3 %), 10 (6.7 %) and 1 (0.7 %) of the population belong to the blood groups A^+, A^-, B^+, B^-, O^+, O^-, AB^+ and AB^- respectively.

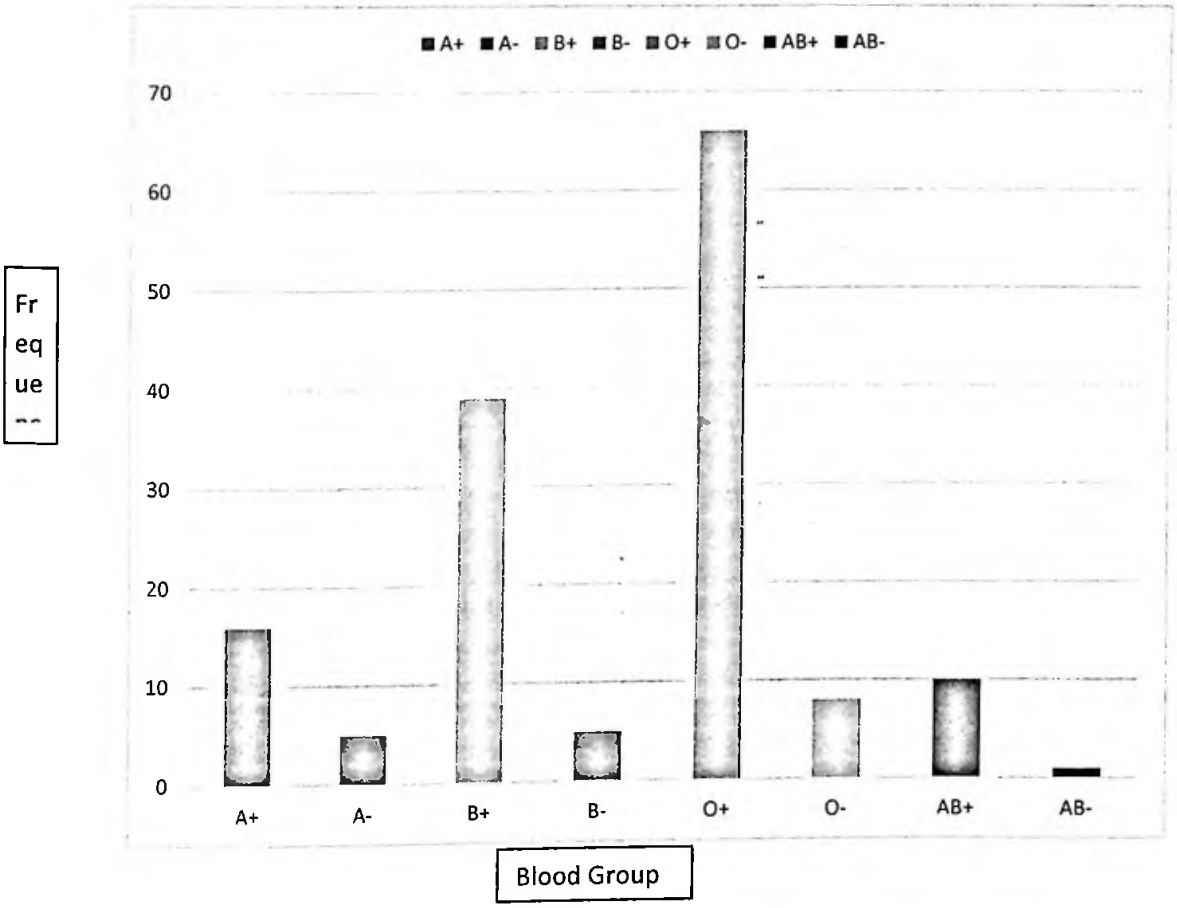

Figure 4.3: Blood Groups of Tuberculosis patients accessing Medicare in Alushi Medical Centre

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4.4: Acetylator Status of Tuberculosis Patients

The acetylator status of patients accessing Medicare in Alushi Medical Centre is presented in figure 4.4. The result clearly showed that 70 of the patients were fast acetylators which correspond to 47% while 80 of them were slow acetylators corresponding to 53%of the population.

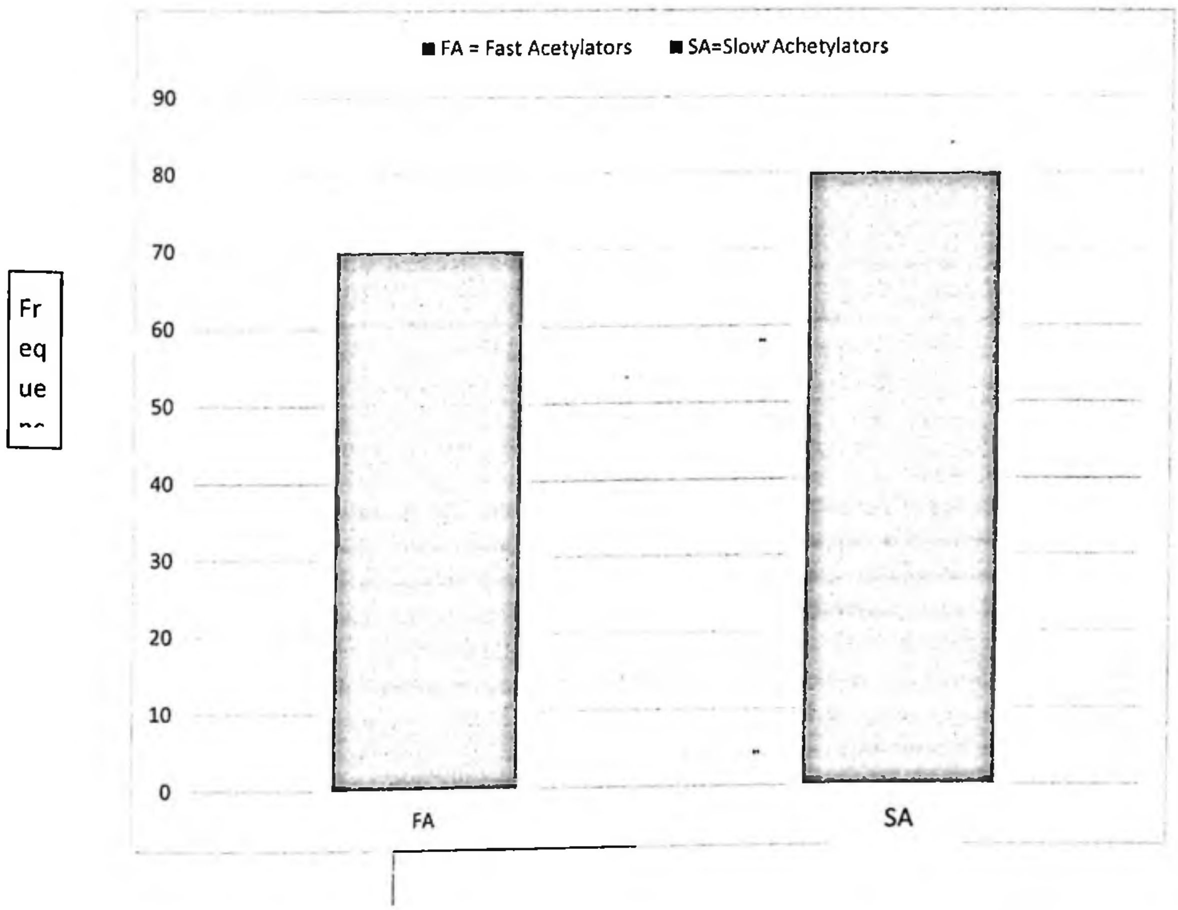

Figure 4.4: Acetylator Status of Tuberculosis Patients accessing Medicare in Alushi Medical Centre

4.5: Phenylthiocarbamide Taste Sensitivity of Patients

The Phenylthiocarbamide Taste Sensitivity of Patients of patients accessing Medicare in Alushi Medical Centre is presented in figure 4.5. The result clearly showed that 80 of the patients were tasters which correspond to 53.3% while 70 of them were non tasters corresponding to 46.7% of the population.

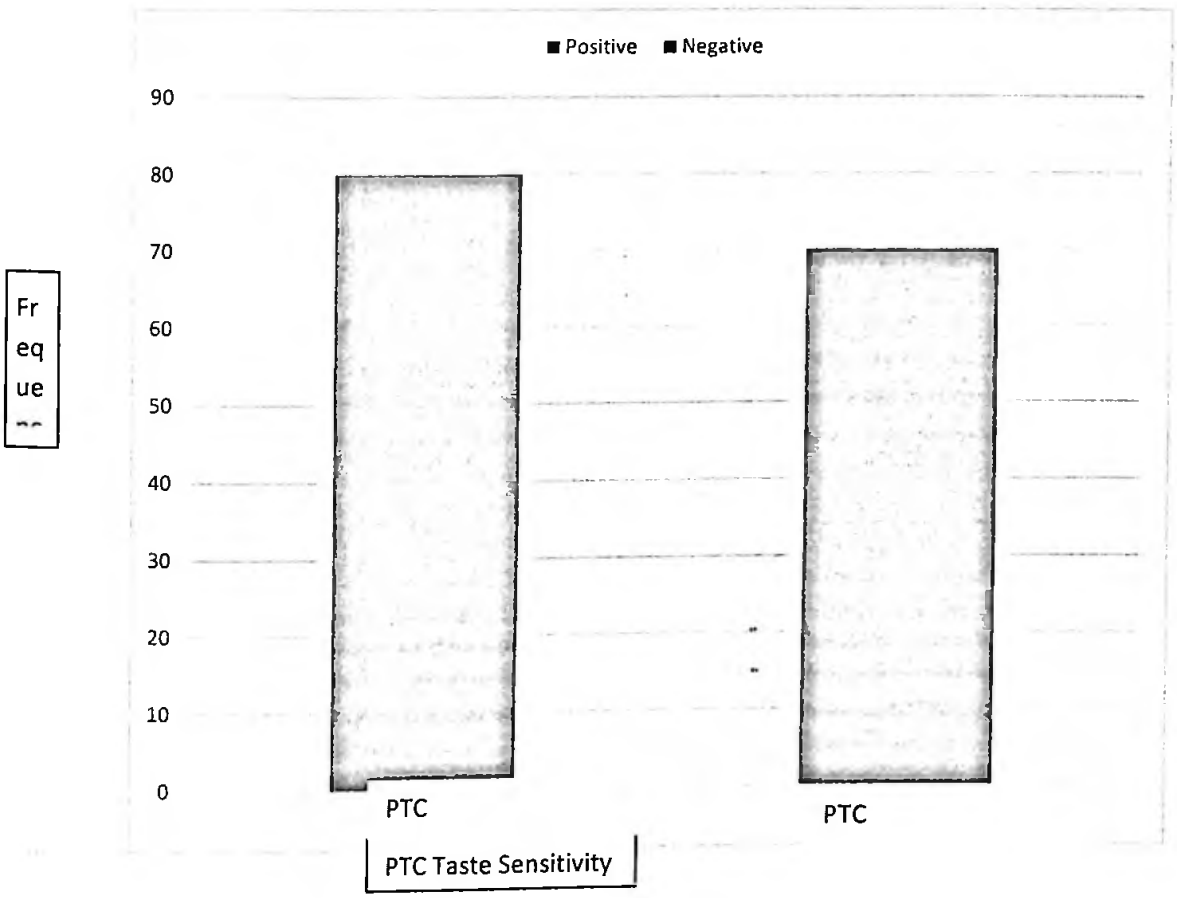

Figure 4.5: Phenylthiocarbamide Taste Sensitivity of TB Patients accessing Medicare in Alushi Medical Centre

4.6: Blood Group and the Acetylator Status of Sampled Population

Table 4.1 represents the results of various blood groups tests and their acetylator status which were presented as percentage acetylation. Values below 70% were considered as slow acetylators while above 70% were considered as fast acetylators. The table revealed a total of 16 A^+ blood group with 11 (7.30%) having fast acetylator values and 5 (3.30%) having slow acetylator values. Blood group A^- had a total of 5 samples representing 4 (2.70%) high acetylators and 1 (0.70%) slow acetylators. B^+ blood group showed 3 (2.00%) high acetylators and 36 (24.00%) slow acetylators while B^- blood group had 1 (0.70%) fast acetylator with 4 (2.70%) slow acetylators. A total of 66 O^+ samples were tested for acetylator status with 43 (28.70%) fast acetylators with 23 (15.30%) slow acetylators while O^- had 2 (1.30%) fast acetylators with 6 (4.0%) slow acetylators. A total of 10 AB^+ samples were tested and the results showed 6 (4.0%) fast acetylators with 4 (2.70%) slow acetylators while 1 (0.70%) AB^- blood group showed slow acetylation with no fast acetylator sample for the blood group.

Table 4.1: Blood Group and the Acetylator Status of Sampled Population.

Blood group	No. Of samples	No. Of fast acetylators (%)	No. of slow acetylators (%)
A^+	16(10.70%)	11.00 (7.3%)	5.00 (3.30%)
A^-	5(3.30%)	4.00 (2.70%)	1.00 (0.70%)
B^+	39((26.00%)	3.00 (2.00%)	36.00 (24.00%)
B^-	5(3.30%)	1.00 (0.70%)	4.00(2.70%)
O^+	66(44%)	43.00 (28.70%)	23.00(15.30%)
O^-	8((5.30%)	2.00 (1.30%)	6.00 (4.00%)
AB^+	10(6.70%)	6.00 (4.00%)	4.00 (2.70%)
AB^-	1(0.70%)	0.00 (0.00%)	1.00 (0.70%)

4.7: Correlation Coefficients of Blood Group and the Acetylator Status of sampled Population

The correlation coefficients of Blood group and the acetylator status of tuberculosis patients attending Alushi Hospital was analysed using Pearson correlation and the results are displayed in table 4.2. The results showed a significant ($p < 0.01$) positive correlation (correlation coefficient of 0.852); a near-perfect correlation between the blood group of TB patients attending Alushi Hospital and the fast acetylators. Also, a significant ($p < 0.05$) positive correlation was obtained between blood group and the slow acetylators, with a correlation coefficient of 0.7

Table 4.2: Correlation Coefficients of Blood Group and the Acetylator Status of Tuberculosis Patients Attending Alushi Hospital

	Blood group	Fast acetylator	Slow acetylator
Blood group	1	0.852**	0.767*

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

4.8: Correlation Coefficients of each Blood Group and the Acetylator Status of Sampled Population.

The correlation coefficients of each blood group and the acetylator status of sampled population showed negative correlations between each blood group and acetylator status. The result is presented on table 4.3.

Table 4.3: Correlation Coefficients of each Blood Group and the Acetylator Status of Sampled Population

Blood group	Fast acetylators	Slow acetylators
A+	-0.500	-0.500
A-	-1.00**	-1.00**
B+	-1.00**	-1.00**
B-	-1.00**	-1.00**
O+	-1.00**	-1.00**
O-	-1.00**	-1.00**
AB+	-1.00**	-1.00**
AB-	-	-1.00**

**Correlation is significant at the 0.01 level (2-tailed).

4.9: Blood Groups and the Ability to Taste Phenylthiocarbamide of Sampled Population

Table 4.4 represents the results of various blood groups tests and their ability to taste PTC. The table revealed a total of 16 A^+ blood group with 11 (7.30%) tasting positive for PTC and 5 (3.30%) tasting negative for PTC. Blood group A^- blood group had a total of 5 samples representing 3.30% tested positive and 0.00 (0.00%) tested negative for PTC. B^+ blood group shows 39 positive representing 26.00% and 0.00 negatives. B^- blood group had 4 positives (2.70%) and 1 (0.70%) negative test. A total of 66 O^+ blood group samples were tested with 58 (38.70%) positive and 8 (5.30%) negatives while O^- had 6 (4.00%) positives and 2 (1.30%) negatives. A total of 10 AB+ samples were tested and the results showed 4 (2.70%) positives and 6 (4.00%) negatives to PTC while 1 (0.70%) sample for AB^- blood group which tested negative for PTC was obtained for the

Table 4.4: Blood Groups and Phenylthiocarbamide Taste Sensitivity of TB Patients

Blood group	No. of samples %	No. of tasters (%)	No. of tasters (%)
A^+	16 (10.70 %)	11.00 (7.30 %)	5.00 (3.30 %)
A^-	5 (3.30 %)	5.00 (3.30 %)	0.00 (0.00 %)
B^+	39 (26.00 %)	39.00 (26.00 %)	0.00 (0.00 %)
B^-	5 (3.30 %)	4.00 (2.70 %)	1.00 (0.70 %)
O^+	66 (44.00 %)	58.00 (38.70 %)	8.00 (5.30 %)
O^-	8 (5.30 %)	6.00 (4.00 %)	2.00 (1.30 %)
AB^+	10 (6.70 %)	4.00 (2.70 %)	6.00 (4.00 %)
AB^-	1 (0.70 %)	0.00 (0.00 %)	1.00 (0.70 %)

4.2.1 Phenylthiocarbamide (PTC) Taste Sensitivity among Male and Female TB Patients

The Phenylthiocarbamide (PTC) Taste Perception among Male and Female TB Patients accessing Medicare in Alushi Medical Centre is presented in figure 4.5. The result clearly showed that 48 of the patients were female which correspond to 32% while 102 of them were male which correspond to 68% of the population.

Table 4.5: Phenylthiocarbamide (PTC) Taste Sensitivity among Male and Female TB Patients .

Gender	Tasters	Non-tasters	Total
Females	41(27.3%)	7(4.7%)	48(32%)
Males	39(26.0%)	63(42.0%)	102(68%)
Total	80(53.3%)	70(46.7%)	150(100%)

4.2.2: Correlation Coefficients of Blood Group and the Ability to Taste PTC of Sampled Population

The correlation coefficients of Blood group and the ability to taste PTC by tuberculosis patients attending Alushi Hospital was analyzed using Pearson correlation and the results are displayed in appendix C. The results showed a significantly ($p < 0.01$) strong positive correlation (correlation coefficient of 0.99) or near-perfect correlation between the blood group of TB patients attending Alushi Hospital and sensitivity (positive) to PTC. However, the correlation coefficient between blood group and inability (negative) to taste PTC was 0.527, indicating a weaker and non-significant($p > 0.01$) correlation.

Table 4.6: Correlation Coefficients of Blood Group and the Ability to Taste PTC of Sampled Population.

	Blood group	+ve to PTC	-ve to PTC
Blood group	1	0.992**	0.527

**. Correlation is significant at the 0.01 level (2-tailed).

4.2.3: Correlation Coefficients of each Blood Group and Ability to Taste PTC of Sampled Population.

Table 4.7 represents the correlation coefficients of blood groups and ability to taste PTC by tuberculosis patients attending Alushi Hospital. The result showed negative correlations between each blood group and the ability to taste PTC.

Table 4.7: Correlation Coefficients of Each Blood Group and the Acetylator Status of Sampled Population.

Blood group	Positive to PTC	Negative to PTC
A+	-0.500	-0.500
B-	-1.00**	-1.00**
B+	-1.00**	-1.00**
B-	-1.00**	-1.00**
O+	-1.00**	-1.00**
O-	-1.00**	-1.00**
AB+	-1.00**	-1.00**
AB-	-	-1.00**

**Correlation is significant at the 0.01 level (2-tailed).

4.2.4 Discussion of Findings

Figure 4.5 shows the gender of the TB patients in the study area. In this study, the male gender was more prevalent 102 (68 %) than the female gender 48 (32 %). This is similar to the report of Borgdorff *et al.,* (2000), which states that male TB prevalence exceeded female TB prevalence in 27 (93%) of 29 prevalence surveys conducted in 14 countries between 1953 and 1997. World Health Organization, 2011 also states that over the years, tuberculosis (TB) case notifications among men have exceeded those among women in most settings. Katherine *et al.* (2016) in their study involving 28 countries also provides strong evidence that TB prevalence is higher among men than women. The excess of notified cases among men has often been explained as a result of barriers faced by women in seeking care for and being diagnosed with TB. These barriers could be caused by certain cultural beliefs. (Dodd *et al.*, 2016).

Figure 4.2 shows the state of the TB patients accessing the medicare in Alushi Medical Centre. Nasarawa state has the highest number of patients 123 (82 %) followed by Benue state as well as some neighbouring states. This can be attributed to the fact the centre is located in Nasarawa state and so individuals within this location as well as neighbouring states will find it closer and easier to seek medical attention there.

In this study, blood group O (O^+ and O^-) was found to be the most common 74 (49.3 %). This is similar to earlier reports from other parts of Nigeria with prevalence of 56.3% in Port Harcourt (South-South) and 47.1% in Jos (North-Central) Nigeria (Worledge *et al.*, 1974; Onwukeme *et al.*, 1990; Chima *et al.*, 2012) who reported 57% in their study. Blood group B 44 (29.3 %) was slightly more prevalent than blood group A 21 (14 %) in this study. This support earlier report of 24.6% in Port Harcourt and 20.5% in Lagos where blood group B was

also found to be the second most prevalent in the population (Odeigah *et al.*, 1990; Chima *et al.*, 2012. While reports from Caucasian population show that blood group A is the next most common after blood group O (Chima *et al.*, 2012). This clearly points to the fact that more research iss still required on the percentage distribution of ABO blood group phenotype across the country.

The result obtained showed a higher percentage of 80 (53 %) of slow acetylator than the fast acetylator 70 (47 %). Lee *et al.*, (2010) in their study also reported higher percentage of slow acetylator. The implication of this is that such individuals have the greatest risk of experiencing drug-induced toxicities during INH-based tuberculosis therapies (Henrik *et al.*, 2016). This can be taken care of by reducing the daily INH doses (300 mg) in order to maintain high treatment efficacies and simultaneously reduce the exposure to the toxic metabolites Hz and AcHz in the liver. For fast acetylators, standard daily INH dose administered can be increased, in order to maintain therapeutic plasma concentration of the drug for high treatment efficacy (Pasipanodya *et al.*, 2012).

A higher percentage of tasters were obtained 80 (53.3%). This may imply that the ability to taste PTC is associated with some diseases like TB and also that individuals who are sensitive to PTC are at higher risk of contracting TB than those who are not sensitive to it.

Table 2 shows the blood groups and the acetylator status of the sampled population. The highest percentage of slow acetylators was observed in blood group B^+ (24.00%) followed by blood group O^+ (15.30%). The lowest percentage acetylator (0.70%) was observed in blood group A^- and AB^-. However, the blood group with the highest percentage of fast acetylators is

the O^+ (28.70) followed by blood group A^+ (7.30%). The blood group with the least percentage of fast acetylators is the AB^- (0.00%).

In this study, the cut-off point for fast acetylation was taken at 70% acetyl-isoniazid (AcINH) according to the method of Ellard & Gammon (1973). The bimodality gave 53.30% slow acetylators and 46.70% fast acetylators in the sampled population. This is similar to the findings of Mukanyangezi *et al.*, 2015. In many studies, the acetylation phenotype of isoniazid has been found to be bimodal although some discrepancies had been raised over the proportions of slow and fast acetylators. In some population, the slow acetylator group was dominant while in others the dominant was fast acetylator. Bouayad *et* al., 1981 in their study obtained 41% slow acetylator as compared to 59% fast acetylator; Bach *et al* (1976) also obtained 41% slow acetylator against 59% fast acetylator. Differing results have been obtained concerning the influence of age, sex, and TB or HIV illness on the pharmacokinetics of isoniazid and on the acetylator profile of individuals. Some data demonstrated that HIV/AIDS status or gender had no significant effect on the concentrations of isoniazid in plasma (Kimerling *et al.*,1998), (Comte *et al.*, 2002), while other studies showed a significant reduction of isoniazid and rifampin concentrations in serum of non-HIV-infected patients with tuberculosis who received directly observed therapy (Peloquin *et al.*, 1997). However, there is no study on the influence of blood group and ability to taste PTC on the acetylator status of tuberculosis patients.

Table 4.4 shows the blood groups and the PTC taste sensitivity of the sampled population. Blood group O^+ has the highest percentage (38.70 %) of positive PTC taste sensitivity, which is similar to that of Chibuisi *et al.*, 2010. The data obtained showed that 84.70 % and 15.30 % of the study population were tasters and non-tasters of PTC respectively. The high incidence of

tasters among this study population is in line with reports of Christopher *et al.*, 2014 (81.3 %), in their studies carried out in southwestern Nigeria. Alimba *et al.*, 2010 reported 70.6% tasters and Bakare *et al.*, 2009 reported 77.4 % tasters. A higher percentage of tasters from this study compared to others could be as a result of the presence of certain health conditions such as tuberculosis, diabetes, HIV/AIDS, gastric ulcers which have been reported to be associated with the ability to taste PTC (Guo & Reed, 2001).

The result obtained from PTC taste perception of the male and female gender in the study population showed that women have higher PTC sensitivity (85.4%) than men. According to Guo & Reed (2001), many studies had shown women to be more sensitive tasters than men. Igbeneghu *et al.*, 2014 also reported 70.0% PTC sensitivity in women. The slight increase in the percentage of female tasters in this study (85.4%) compared to that of Igbeneghu *et al.*, 2014 (70.0%) could be as a result of the fact that PTC taste sensitivity is associated with some diseases including TB (Pal *et al.*, 2004). The female sex hormones had been linked with influencing PTC sensitivity and it is thought that the modifier loci that increase PTC taste sensitivity might be on the X chromosome or might be autosomal genes regulated by sex hormones, this accounts for the high incidence of female tasters (85.4%) compared to the male tasters (14.6%). (Igbeneghu *et al.*, 2014).

This study revealed strong positive correlation ($p<0.01$) with a correlation coefficient of 0.99 which is near perfect correlation between the blood group of TB patients and positive PTC taste sensitivity while correlation coefficient between blood group and inability (negative) to taste PTC was insignificant ($p>0.01$). A positive correlation between blood group and acetylation of tuberculosis was obtained ($p<0.05$) with a correlation coefficient of 0.85.

However, a negative correlation coefficient was obtained between each blood group and positive and negative PTC taste ability as well as between each blood group and fast acetylators and slow acetylators. This negative correlation implies that there is no relationship between blood groups and the ability or inability to taste PTC as well as the isoniazid acetylation status of TB patients.

CHAPTER FIVE

SUMMARY, CONCLUSION AND RECOMMENDATION

5.1 SUMMARY

Tuberculosis is the deadliest infectious disease after HIV/AÏDS, affecting about one third of the world population (WHO, 2017). The current internationally accepted therapy by Directly Observed Treatment, Short-Course (DOTS) for drug-susceptible TB consists of multiple expensive antibiotics and is lengthy (Tousif *et al.,* 2015). Adherence and compliance are critical for optimal efficacy of these drug regimens. However, hepatotoxicity is the most serious complication arising from the first line of TB treatment. Isoniazid, rifampicin and pyrazinamide are potentially hepatotoxic drugs (Saukkonen *et al.,* 2006). Significantly, about 0.5% patients show hepatotoxicity to INH mono-therapy. The percentage was higher for combination therapies. It is not INH levels in the serum that is toxic but rather its metabolites, especially hydrazine. The enzyme N-acetyltransferase 2 (NAT-2) in humans acetylates INH to form acetylisoniazid, which further undergoes hydrolysïs to generate acetylhydrazine. Polymorphic variants of NAT-2 redirect some INH towards an alternative oxidative pathway via P450 to generate hydrazine (Sinha *et al.,* 2014). The polymorphism profile of isoniazid acetylation varies with race and ethnicity and its knowledge has been found useful in tailoring individual dosing to minimize side effects and maximize clinical outcomes (Patin *et al.,* 2006). It therefore beholds that new or alternative approach at determining individual acetylator status is necessary to streamline individual treatment regime to the person's ability to acetylate the drugs.

The aim of the study was to determine the correlation of blood groups, acetylator status and phenylthiocarbamide taste sensitivity in TB patients accessing medicare in Alushi Medical

Centre, Akwanga. In order to generate a common or an alternative test that can be used to determine individual's acetylator status, which will help to fast track diagnosis on individuals that are undergoing tuberculosis treatment as well as provide useful information on the option of using PTC taste sensitivity or blood groups by clinicians in predicting individual acetylator status in prescription.

The study was carried out by determining the blood group, acetylator status and the PTC threshold of the sampled population. The correlation of the blood group, acetylator phenotype and PTC taste sensitivity and in the sampled population was also examined. The reults obtained shows a negative correlation between blood groups, ability or inability to taste PTC as well as fast and slow acetylators.

5.2 Conclusion

This study reveals that there is a significant correlation between acetylator phenotype with the blood group of Tb patients. However, there is no correlation between blood group and inability to taste PTC. The correlation between individual blood group with both negative and positive PTC taste as well as fast and slow acetylators was negative. This implies that there is no relationship between an individual TB patient's blood group with the ability or inability to taste PTC as well as acetylator status.

5.3 Recommendation

The study should be carried out using a larger sample size from different TB centres across the country to provide room for easy generalization.

5.4 Limitations of the study

I. A small sample size of 150 participants was used; the study was carried out using TB patients receiving treatment in just a TB centre. This may limit the generalization of the findings.

II. There was a challenge of poor epileptic power supply.

5.5 Suggestions for Further Studies

Further studies should be carried out on the correlation of genotypic polymorphism of *n-acetyltransferase-2* and blood groups as well as PTC taste sensitivity in tuberculosis patients.

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APPENDICES

Appendix E: Preparation of Reagents

1. Hydrochloric acid (0.5moldm^{-3}): 18.2 cm^3 of the concentrated acid was measured up to 1000cm^3 with distilled water under an ice bath.
2. Solution of chloramine T^C was prepared by dissolving 3.52 grams of chloramine T^c in 100 cm^3 of distilled water.
3. Sodium hydroxide (7 moldm^{-3}) was prepared by dissolving 28grams of sodium hydroxide pellets in 100 cm^3 of distilled water.
4. Sodium hydroxide (0.5 moldm^{-3}) was prepared by dissolving 2 grams of sodium hydroxide pellets in 100 cm^3 of distilled water.
5. Aqueous solution of potassium cyanide: the solution was prepared by dissolving 1.3grams of potassium cyanide in 100 cm^3 of distilled water.
6. Potassium dihydrogenphosphate (0.5 moldm^{-3}) was prepared by dissolving 6.8 grams of potassium dihydrogenphosphate in 100 cm^3 of distilled water.
7. Potassium hydrogenphosphate (0.5 moldm^{-3}) was prepared by dissolving 8.7 grams of potassium hydrogenphosphate in 100 cm^3 of distilled water.
8. potassium phosphate buffer (0.5 moldm^{-3}), pH 6 was prepared by mixing 87.7 cm^3 of 0.5 moldm^{-3} potassium dihydrogenphosphate and 12.3 cm^3 of 0.5 moldm^{-3} potassium hydrogenphosphate, the pH was then set at 6.