ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCE

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Effect of Isoniazid (INH) Prophylaxis on Blood Level of Tacrolimus, Liver Function Test and Renal Function test used for Kidney recipient Patient attending St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia.

By: Yonas Tsehay (BSc, MSc candidate)

Advisor: Mistire Wolde (MSc, PhD)

Co- Advisor: Momina Mohammed (MD, ISNF)

A thesis submitted to the Department of Medical Laboratory Sciences, College of Health Science, Addis Ababa University, in partial fulfillment of Master of Science Degree in Clinical Laboratory Sciences (Clinical Chemistry).

February 2020

Addis Ababa, Ethiopia

Addis Ababa University

School of Graduate Studies

This is to certify that the thesis prepared by Yonas Tsehay, entitled: **Effect of Isoniazid prophylaxes on blood level of Tacrolimus, RFT and LFT used for kidney recipient patients attending St. Paul's Hospital Millennium Medical College in Addis Ababa, Ethiopia** and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Clinical Chemistry) complies with the regulations of the university and meets the accepted standards with respect to originality and quality.

Signed by the Examining Committee:

External Examiner_____ Signature _____Date _____

Internal Examiner_____ Signature _____Date _____

Advisor_____ Signature _____Date _____

Advisor_____ Signature _____Date _____

Advisor_____ Signature _____Date _____

Acknowledgments

First I would like to thank the Holy God and his beloved Mother St. Mary for being with me throughout my life. Secondly my acknowledgement goes to Addis Ababa University (Department of Medical Laboratory Science) to equip me with knowledge in clinical chemistry sciences and professional skill and also for provision of funding for my research work. I would like to thank Dr. Mistire Wolde, Dr. Momina Mohammed and all staffs of St. Paul's kidney transplant center especially, transplant OPD nurses for their scientific advice and technical support. I also thank all study participants for their cooperation during sample collection. Finally, I am grateful to my family, colleagues and individuals who were beside me to encourage and motivate me throughout my study period.

Contents

List of Table

List of figure

List of abbreviations

ALG	Antilymphocyte globulin
ALP	Alkaline phosphates
ALT	Alanine transaminase
AST	Aspartate transaminase
ATG	Antithymocyte globulin
CD	Cluster of differentiation
CKD	Chronic kidney diseases
CMV	Cytomegalovirus
CNIs	Calcineurin inhibitors
CYPP450	Human cytochrome p450
DNA	Deoxyribose nucleic acid
HLA	Human leukocyte antigen
HLMS	Human liver microsomes
IL2 RA	Interleukin 2 receptor antagonists
INH	Isonicotinyl hydrazine or isoniazid
IPT	Isoniazid preventive therapy
KTRs	Kidney transplantation recipients
LTBI	Latent tuberculosis infection
MDR	Multidrug resistance
MIC	Minimum inhibitory concentration

MMF	Mycophenolate mofetil
MTB	Mycobacterium tuberculosis
NAD	Nicotinamide adenine dinucleotide
PTP	Post-transplant period
SOP	Standard operating procedure
SOT	Solid organ transplant
SPHMMC	St. Paul's Hospital Millennium Medical College
SPSS	Statistical package for social science
TB	Tuberculosis
TCL	Tacrolimus
TNF-α	Tumor necrosis factor alpha
TST	Tuberculin skin test
ULN	Upper limit of normal range
VB6	Vitamin B6 or pyridoxine

Abstract

Background: Tacrolimus is a common immunosuppressant, approved for the prophylaxis of organ rejection in renal transplantation .On the other hand INH is a drug used as an antibacterial or prophylaxes for those kidney recipients. In Ethiopia, those individuals who underwent kidney transplantation took both INH and Tacrolimus drugs. Nevertheless the effect of INH on the efficacy of Tacrolimus drugs was not well known in Ethiopia.

Objective: To evaluate the effect of INH on blood level of tacrolimus drug among kidney recipient individuals attending St. Paul Hospital, kidney center.

Methods: An institutional based cross-sectional study was conducted on 14 kidney recipient patients from January to September 2019. The study participants received both tacrolimus drugs as an immunosuppressant and INH as a prophylaxis. Clinical and laboratory data including drug functional tests were recorded at the time of treatment with INH and completion of this treatment after a week, biochemical analysis was also done.

Result: The concentration of Tacrolimus drug level with INH prophylaxis was significantly higher when compared to the concentration after finishing INH prophylaxis; p=0.002. Besides, the liver function parameters were significantly increased when tacrolimus was taken with INH; AST; P=0.001, ALP; P=0.001, ALT; P=0.01. Similar effect of renal function test values on those patients taking tacrolimus with and without INH was seen; as Creatine; p=0.001, Urea; p=0.003 was significantly higher when compared to the values after INH prophylaxis was finished. There were no statistically significance difference observed in Total bilirubin; p=0.933 and Direct bilirubin; p=0.710 during and post treatment values.

Conclusion: Kidney recipient patients who used INH prophylaxis showed alteration of LFT, RFT and blood concentration of tacrolimus (BCT). Biochemical tests except bilirubin were significantly affected by INH drug.

Key words: Isoniazid drug, Tacrolimus drug, Kidney Transplant,

1. Introduction

1.1. Background of study

Kidney transplantation is a surgery done to replace a diseased kidney with a healthy kidney from a donor. A successful kidney transplant can improve many of the complications of kidney failure. A kidney may come from a living donor or from an individual who has died (deceased donor). Its associated problem is blood clots, bleeding, leaking from or blockage of the tube (urethra) that links the kidney to the bladder, infection, failure of the donated kidney and rejection of the donated kidney (1).

The Calcineurin- inhibitor (CNI) Tacrolimus is used to prevent organ rejection following renal transplant (2).Calcineurin inhibitors are medicines which inhibit the action of Calcineurin; Calcineurin is an enzyme that activates T-cells of the immune system.Prograf® is the brand name for immediate release Tacrolimus (ta-KROE-li-mus) and also two extended release Tacrolimus products are available, and they are called Astagraf XL® and Enversus XR® (2, 3).

Commercial tacrolimus capsules are available in three strengths: 0.5mg, 1mg, and 5mg. Tacrolimus capsules can also be specially made into a liquid suspension by the pharmacy. The starting dose of tacrolimus was 0.3mg/kg divided on two daily doses. Doses were subsequently adjusted according to the clinical condition of the patient and to target concentration intervals defined in the study protocol 10-20ng/ml during the first 3 months after transplantation and 5-15ng/ml after the third month and on wards (3).

Tacrolimus (FK506) is a 23-membered macrolide lactone ($C_{44}H_{69}NO_{12}$; for molecular structure see Figure 1) which is isolated from the fermentation broth of Streptomyces tsukubaensis. Additionally, tacrolimus is an immunosuppressive agent belonging to the Calcineurin-inhibitor group that has emerged as a valuable therapeutic alternative to cyclosporine following solid organ transplantation. It is highly effective at preventing rejection in heart, small bowel, pancreas, bone marrow, lung, liver, and kidney recipients and for the therapy of autoimmune diseases (4, 5).

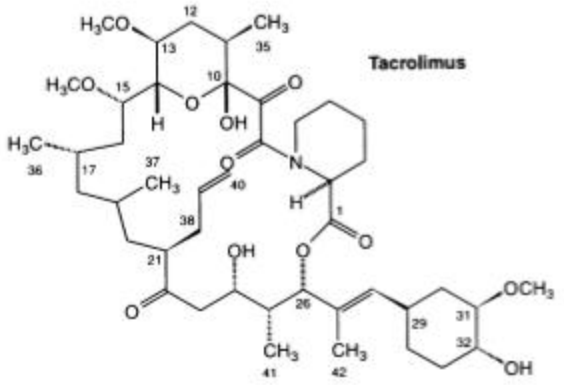


Figure 1Structure of tacrolimus

It is almost always given along with other transplant immunosuppressant's like mycophenolate mofetil (MMF) and prednisone (5).

Systemically available tacrolimus is metabolized by mostly hepatic cytochrome P-450 system. There is also evidence of gastrointestinal metabolism by CYP3A4 in the intestinal wall. Concomitant use of medicinal products or herbal remedies known to inhibit or induce CYP3A4 may affect the metabolism of tacrolimus and thereby increase or decrease tacrolimus blood levels. For example, rifampin induces the P-450 system and INH inhibits the P-450 system. Clinically, interaction with the former drug induces rejection caused by enhanced metabolism of tacrolimus and with the latter drug produces nephrotoxicity by a reduced elimination of tacrolimus (5).It is therefore recommended to monitor tacrolimus blood levels whenever substances which have the potential to alter CYP3A4 metabolism are used concomitantly and to adjust the tacrolimus dose as appropriate in order to maintain similar tacrolimus exposure(5, 6).

This drug is usually taken two times a day, 12 hours apart (either before or after individuals eat) (7).Individuals must take tacrolimus at the same time each day, at the same time before or after he/she eats. **Consistency is very important!** (8)

The mechanism of action of tacrolimus is similar to that of cyclosporine, even though their chemical structures differ greatly. Tacrolimus inhibits T-lymphocyte activation, although the exact mechanism is not known (9). Experimental evidence suggests tacrolimus becomes activated only when complexes with intracellular receptors or cytosine binding proteins known

as immunophilins. The immunophilins - drug complex binds competitively to and inhibits Calcineurin. Inhibition of Calcineurin inhibits transcription, is believed to mediate the immunosuppressive activity of both tacrolimus and cyclosporine. Immunosuppressive agents sometimes result in infection that requires antibacterial agents. Many of these agents are known to interact pharmacologically with TCL (9, 10).

Tacrolimus therapy occasionally produces side effects when used with other drugs such as nephrotoxicity, cardio toxicity, neurotoxicity, and glucose intolerance. Tuberculosis is also a common side effect of tacrolimus therapy (5).

Tuberculosis (TB) is a common infection with high prevalence in developing countries. Recipients of solid organs are more vulnerable than the general population to acquire tuberculosis. Tuberculosis causes significant morbidity imposing significant economic burden on the patient and the healthcare agencies. Also, the emergence of primary and secondary drug-resistant *Mycobacterium* may have a severe impact on the organ transplant setting (11).

The brand name of isoniazid is Nydrazid. It is used for management of tuberculosis. Use this drug this drug is used for organ recipient patients as prophylaxes to prevent those patients from TB infection or reactivation of TB (11).

It is an antibacterial available as 100 mg and 300 mg tablets for oral administration. Each tablet also contains as inactive ingredients: colloidal silicon dioxide, lactose monohydrate, pregelatinized starch (corn), povidone and stearic acid (12).

Isoniazid is chemically known as Isonicotinyl hydrazine or isonicotinic acid hydrazide. It has a molecular formula of C6H7N3O and a molecular weight of 137.14da. It has the following structural formula (13).

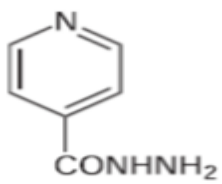


Figure 2 Structure of isoniazid

It is odorless and occurs as a colorless or white crystalline powder or as white crystals. It is freely soluble in water, sparingly soluble in alcohol and slightly soluble in chloroform and in ether. Isoniazid is slowly affected by exposure to air and light (13).

This drug is metabolized by through the hepatic cytochrome P450 (CYP450) 3A enzyme, in particular, CYP3A4 and CYP3A5 (6).

INH is a hydrazide derivative of isonicotinic acid, and is absorbed rapidly from gastrointestinal tract reaching peak levels in 1-2 h. The distribution volume is 0.6-0.7L/Kg. It is excreted in 24 h in subjects with a normal renal function. Metabolism takes place by enzymatic acetylating and hydrolysis in liver. The plasma half-life is 0.5-0.6 h by fast acetylation 2.5 h by slow acetylation (14).

The antimicrobial activity of INH is selectively for mycobacterium, likely due to its ability to inhibit mycolic acid synthesis, which interferes with cell wall synthesis, there by producing a bactericidal effect. INH also disrupts DNA, lipid, carbohydrate, and nicotinamide adenine dinucleotide (NAD) synthesis and/or metabolism (15).

Acute poisoning due to isoniazid overdose is often associated with generalized tonic-clonic seizures, altered sensorium, renal, hepatic dysfunctions, and severe metabolic acidosis (16).

The cause of liver injury due to INH is believed to be accumulation of toxic intermediates of its metabolism. Rates of injury may be somewhat higher in patients with a slow acetylation status. It is known to affect the metabolism of tacrolimus through inhibition of hepatic cytochrome P450 (CYP450) 3A and to a far lesser extent, CYP3A5 (16, 17).

1.2. Statement of the problem

Patients who undergo solid organ (kidney) transplant require lifelong immunosuppressant's to prevent organ rejection. In organ transplantation, the ideal forms of immunosuppressant's are to induce donor specific tolerance without impairing the host defenses or increasing the susceptibility to infection from all types of organisms. The overall rate of acute rejection isapproximately10% within 1year under the current immunosuppressive regimen (8).However, this rate is suboptimal and should be further reduced because acute rejection per second is directly related to the economic burden of kidney transplantation as well as the overall graft outcome (9).

The efficacy and safety of Advagraf and Prograf, both in combination with mycophenolatemofetil (MMF) and corticosteroids, was compared in 667 de novo kidney transplant recipients. The event rate for biopsy-confirmed acute rejection within the first 24 weeks after transplantation was 18.6% in the Advagraf group (N=331) and 14.9% in the Prograf group (N=336). The treatment difference (Advagraf,Prograf) was 3.8% (95% confidence interval [-2.1%, 9.6%]) (18).

Concurrent use of tacrolimus with medicinal products known to have nephrotoxic or neurotoxic effects, so concurrent use of tacrolimus and INH may increase these effects, i.e. I NH inhibits the hepatic CYP3A4 system this induces nephrotoxicity caused by delayed metabolism of tacrolimus (2).INH toxicity manifests with central nervous dysfunction and hepatic dysfunction with metabolic abnormalities such as lactic acidosis, hyperglycemia, and hyperkalemia (3).

Up to 20% of people taking isoniazid experience peripheral neuropathy when taking doses of 6mg/kg of weight daily or higher. Gastrointestinal reactions include nausea and vomiting, headache, poor concentration, weight gain, poor memory, insomnia and depression have all been associated with isoniazid use. INH oral is increase the level or effect of tacrolimus by altering drug metabolism (14).

In 1963, INH was recommended as monotherapy for prevention of active disease in patients with latent tuberculosis, diagnosed on the basis of a positive purified protein derivative (PPD) tuberculin skin test. Cases of sever hepatotoxicity due to INH alone soon appeared and clearly

defined its hepatotoxic potential. Recommendation on use of INH was then modified and restricted (16).

Despite its limited use, INH remains one of the most common causes of serious idiosyncratic liver injury in the United States (14).

Therapy with INH is associated with transient serum aminotransferase (ALT) elevations in 10% to 20% of patients, and levels rising above 5 times the upper limit of the normal range (ULN) in 3% to 5% these enzyme elevations are usually asymptomatic and often resolve even with continuation of therapy with dose adjustment and also minimal increases in alkaline phosphates values (usually <2 times ULN) (13).

In addition, INH can also cause clinically apparent acute liver injury with jaundice, which arises in 0.5% to 1% and is fatal in 0.05% to 0.1% of recipients. Rates of hepatic injury vary greatly in the published literature. Acute liver failure from INH appears to be more common in women than men and in African Americans more than Caucasians (14).

Features of hypersensitivity such as rash, fever and eosinophilia can occur, but are uncommon and usually mild it present at all (19).

INH therapy can induce antinuclear antibodies even without hepatotoxicity or hypersensitivity reactions. For this reason, auto antibodies may be present during acute hepatic injury due to INH, but they are generally low in titer and not accompanied by other features of autoimmune liver injury (hyperglobulinemia, or thralgias) (19).

1.3. Significance of the study

The finding of this study will be used for drug adjustment and/or recommendation for physicians to adjust blood level of Tacrolimus drug. It is also used as a precaution for physicians and reference for further study.

2. Literature review

Tacrolimus is one of the medicines used to stop the body attacking transplanted kidneys. It is available as two different formulations, Prograf or Advagraf. Prograf is taken twice daily and is available in 0.5mg (yellow), 1mg (white) or 5mg (red) capsules. Advagraf is only taken once a day and is available in 0.5mg, 1mg, 3mg or 5ands of tacrolimus available (e.g., Ado port. It is very important that you stick to the same brand the hospital recommends (3).

The efficacy and safety of Advagraf and Prograf, both in combination with mycophenolatemofetil (MMF) and corticosteroids, was compared in 667 de novo kidney transplant recipients. The event rate for biopsy-confirmed acute rejection within the first 24 weeks after transplantation was 18.6% in the Advagraf group (N=331) and 14.9% in the Prograf group (N=336). The treatment difference (Advagraf,Prograf) was 3.8% (95% confidence interval [-2.1%, 9.6%]). The 12-month patient survival rates were 96.9% for Advagraf and 97.5% for Prograf; in the Advagraf arm 10 patients died (3 female, 7 male) and in the Prograf arm 8 patients died (3 female, 5 male). 12-month graft survival was 91.5% for Advagraf and 92.8% for Prograf (18).

The efficacy and safety of Prograf, cyclosporine and Advagraf, all in combination with basiliximab antibody induction, MMF and corticosteroids, was compared in 638 de novo kidney transplant recipients. The incidence of efficacy failure at 12 months (defined as death, graft loss, biopsy-confirmed acute rejection, or lost to follow-up) was 14.0% in the Advagraf group (N=214), 15.1% in the Prograf group (N=212) and 17.0% in the cyclosporine group (N=212). The treatment difference was -3.0% (Advagraf, cyclosporine) (95.2% confidence interval [-9.9%, 4.0%]) for Advagraf vs. cyclosporine and -1.9% (Prograf, cyclosporine) (95.2% confidence interval [-8.9%, 5.2%]) for Prograf vs. cyclosporine. The 12-month patient survival rates were 98.6% for Advagraf, 95.7% for Prograf and 97.6% for cyclosporine; in the Advagraf are 3 patients died (all male), in the Prograf are 10 patients died (3 female, 7 male) and in the cyclosporine arm 6 patients died (3 female, 3 male). 12-month graft survival was 96.7% for Advagraf, 92.9% for Prograf and 95.7% for cyclosporine (18, 20).

The diagnosis and treatment of tuberculosis in organ transplant recipients presents several challenges. Impediments to rapid and accurate diagnosis may lead to treatment delay and include negative

tuberculin skin tests (TSTs), negative sputum smear results in spite of active disease and atypical clinical presentations. Therapeutic challenges arise from drug-related toxicities, metabolic interactions between immunosuppressive and anti-tuberculosis drugs, and side effects from long courses of ant tuberculosis medications. Increasing drug resistance and inadequate immune responses to *Mycobacterium tuberculosis* (MTB) due to exogenous immunosuppressant increase the complexity of treating tuberculosis in this population (21).

Recommendations for the diagnosis and treatment of latent tuberculosis infection and active tuberculosis disease in organ transplant recipients are made based on consensus guidelines formulated by experts in the field. Only a few controlled studies of treatment of latent or active tuberculosis in organ transplant candidates or recipients are available. Case series and epidemiologic surveys of organ transplant patients with tuberculosis are often used for guidance in this area (11).

2.1. Epidemiology of Tuberculosis in renal transplant recipient

It should be noted that the rates of tuberculosis reported in the transplant literature often reflect cumulative rates in populations of patients followed over a number of years and cannot always be compared to or converted to annual incidence rates (11).

The frequency of active tuberculosis disease among solid organ transplant (SOT) patients is estimated to be 20–74 times that of the general population. For active tuberculosis disease, the prevalence among SOT recipients in most developed countries is 1.2%–6.4%, while the prevalence in SOT recipients in highly endemic areas has been reported to be up to 15%. Also, the frequency of the disease appears to be different according to the transplanted organ, with higher frequency in lung recipients (11, 24). About two-thirds of reported cases of active tuberculosis disease in transplant recipients occur in the first post-transplant year with the median time to development of disease reported as 9 months. In most cases, infection is thought to arise by reactivation of old foci of infection, because primary infection has only been documented in a small number of cases (11). Acquisition of MTB from the donated organ has been documented in renal, lung and hepatic transplantation, but appears to account for less than 5% of all active TB cases in transplant recipients. Nosocomial acquisition of MTB has been documented during an outbreak on a renal transplant unit, but fortunately also appears to be uncommon. The rate of primary infection is likely to be greater in developing countries but this has not been carefully evaluated (24).

The incidence and prevalence of TB in India is one of the highest in the world. India contributes 20% of the global incidence of TB which is about 1.8 million new cases of TB in the general population. The incidence in patients on maintenance dialysis is 8.7% and that in renal allograft recipients is 12.3%. The reported incidence from other centers across the country is about the same (24).

In Pakistan, where the incidence of TB in the general population is amongst the highest in Southeast Asia (250/100 000), post-transplant TB infection occurs in 12.4–15.2% of renal transplant recipients in this institution (12).

The reported prevalence of post-transplant TB is 3.1 to 15% in Asia, 1.5 to 8.5% in South Africa, 1.5 to 3.5% in the Middle East, 1.7 to 5% in Europe and 1.5% in the United States. The actual burden may be much higher in the developing countries. Underreporting may be due to poor maintenance of records and follow-ups.Both renal and other solid organ transplant recipients are at a higher risk. Thus, the incidence and prevalence is several fold higher in the transplant population as compared to the general population (11, 21)**.**

2.1.1. Time of onset for Post-transplant Tuberculosis

About 45-60% of TB occurs in the first year after transplantation. A global review on TB estimated the median time for onset at nine months post transplantation. John *et al.*, have shown the median onset to be 26 months for those who received azathioprine and prednisolone as immunosuppressant and 11 months for those who received cyclosporine along with other immunosuppressive agents. An earlier occurrence was noted with non-renal solid organ transplantation, cyclosporine, anti CD3 therapy, malnutrition secondary to prolonged dialysis, relative immunodeficiency and exposure to the organism in hospital setting. Data on the role of newer immunosuppressive is emerging. Immunosuppressant with tacrolimus or mycophenolate has also been associated with the development of TB earlier in the post transplantation period and also in younger patients. Tailored immunosuppressant, with stringent monitoring of the drug levels is expected to decrease the incidence and prevalence in the future (12).

2.1.2. Risk factors for post-transplant Tuberculosis

Few risk factors have been defined for the occurrence of active tuberculosis disease after transplantation. Surprisingly, only 20–25% of all cases of active tuberculosis disease occurring after

transplantation are in patients who had positive TST reactions before transplantation. This may in part be due to energy in patients with end stage organ failure. The precise frequency at which TST positive patients later develop active tuberculosis after transplantation has not been determined. Other reported risk factors for active tuberculosis disease include prior residence outside the United States and the presence of findings on chest radiographs suggestive of healed tuberculosis. A case-control study from Iran revealed that subjects who were on hemodialysis for longer periods of time had greater risk of active tuberculosis disease and the odds of TB were higher with increasing episodes of allograft rejection. It is clear that certain immunosuppressive drugs (e.g., OKT3 and T-cell depleting antibodies) are associated with a greater risk of tuberculosis than others. Reported recently that recipient age is an independent risk factor for post-transplant TB, at least in Spain where TB in the general population has decreased significantly in recent years. This allows for speculation that older persons are more likely to have latent TB and the same might prove to occur in regions where TB control programs have been successful (23)**.**

The major risk factors for TB are chronic liver disease (2 times), other coexisting infections, particularly deep mycoses, pneumocystis, pneumonia and myocardial (1.6 times), OKT3 (1.8 times), and CMV infections (2.25 times). Disseminated disease may also be more common with OKT3 and CMV (24). Cyclosporine use advances the onset to an earlier date and patients on cyclosporine seldom develop TB later than six years. The factors associated with death on univariate analysis were the recipient age, HLA ≤1 antigen match, prednisolone - azathioprine immunosuppressant, pre-transplantation TB, post-transplant TB (after two years), chronic liver disease (>6 years), diabetes mellitus, post-transplant diabetes mellitus (>5 years), and the presence of other coexisting infections. HLA A68 (28)/A69 (28) locus appeared to predispose toward post-transplantation TB in the Indian population. Case reports are available where TB manifests immediately after substituting azathioprine with mycophenolatemofetil (23).

2.2. Use of Isoniazid chemoprophylaxis in renal transplantation

Isoniazid was first made in 1952. It is on the World Health Organization's List of Essential Medicines, the most effective and safe medicines needed in a health system. Isoniazid is available as a generic medication. The wholesale cost in the developing world is about US$0.60–4.75 per month. In the United States a month of treatment costs less than $25 (13).

Isoniazid, also known as **isonicotinylhydrazide** (**INH**), is an antibiotic used for the treatment of tuberculosis. For active tuberculosis it is often used together with rifampicin, pyrazinamide, and either streptomycin or ethambutol. For latent tuberculosis it is often used by itself. It may also be used for atypical types of mycobacterium, such as *M. avium*, *M. kansasii*, and *M. xenopi*. It is usually taken by mouth but may be used by injection into muscle (16).

The use of isoniazid (INH) as chemoprophylaxis for tuberculosis (TB) in renal transplant recipients has not been widely studied or reported from a country where TB is endemic. The results of the largest ever-reported a randomized, prospective study of the use of INH in renal transplant recipient was Four hundred consecutive live related renal transplant recipients between April 2001 and September 2004, from a single center, were randomized to receive or not receive INH for 1 year after transplantation. There were 12 dropouts. Of the remaining 388, 181 recipients received INH for 1 year post-transplant and 207 did not. The primary disease, co morbidities, HLA (human leukocyte antigen) match, immunosuppressant, episodes of rejection, the use of anti-rejection agents, a past history of TB in the donor, the recipients and in family members living in same house and a history of TB in the family were factors compared in the two groups. The only significant difference between the two groups was that there was an increased family history of TB in recipients who received INH ($P = 0.01$). One recipient from the INH group and 16 recipients from the non-INH group developed TB ($P = 0.0003$). Discontinuation of INH for hepatotoxicity was not required in any patient. These results provide evidence that the use of INH following renal transplantation should be considered mandatory in geographical areas where the prevalence of TB is high. Furthermore, these results have important implication in patients from such areas who immunosuppressed are following other kinds of transplantation and for those who are immunocompromised for any other reason (17).

2.2.1. Efficacy of Isoniazid Prophylaxis in Renal Allograft Recipients

The efficacy of isoniazid (INH) prophylaxis in renal allograft recipients who are on long-term immunosuppressant in a region highly prevalent for tuberculosis (TB) was studied. INH (300 mg/d in patients weighing more than 35 kg and 5 mg/kg/d in patients with <35 kg body weight) together with Pyridoxine 50 mg/d for 1 year was started in randomly assigned renal allograft recipients (17). Occurrence of clinical tuberculosis during the initial 2 years post transplantation

was observed in the risk group and patients at no risk. Risks were defined as acute rejection episodes and exposure to ant rejection therapy, past history of TB completely or incompletely treated radiological evidence of past tuberculosis, history of tuberculosis in close contacts. Among 480 patients registered in the study, INH prophylaxis was given to 219 randomly assigned renal allograft recipients. Results were compared among patients developing TB during the initial 2 years post transplantation in both the groups. Risk factors were analyzed for comparison in both groups. No significant difference was observed in terms of past history of TB, TB in close contacts, episodes of acute rejection during the initial 3 months, and co morbidities such as cytomegalovirus infection, hepatitis C virus infection, and post-transplant diabetes. One patient from the INH group and 10 patients from the non-INH group developed TB during the initial 2 years post transplantation ($P < .0001$). None of patients required discontinuation of INH. INH was observed to be safe and effective as a chemo prophylactic agent in renal allograft recipients (17, 18).

2.2.2. Tuberculosis in renal transplant recipients on Isoniazid prophylaxis

The incidence of Tuberculosis (TB) in renal transplant recipients in Pakistan is 15%. Following findings of our randomized controlled trial showing an overwhelming benefit of Isoniazid (INH) prophylaxis, from June 2009 every transplant recipient receives INH for one year post-transplant in unit (18). Its objective is to find out incidence, risk factors and complications of tuberculosis in renal transplant recipients receiving INH prophylaxis. Its method is reviewed the records of all renal transplant recipients on INH prophylaxis from June 2009 to December 2011, followed up till June 2015. This noted details of transplantation and immunosuppressant, Tuberculosis including occurrence after transplantation, site, methods of diagnosis, treatment, drugs side effects and other complications, and outcome (17, 20).

Out of 910 recipients, 91% completed one year of INH. Forty six (5%) developed TB, incidence higher in later than 2 years post transplantation than earlier. Majorities were pulmonary (48%); most were diagnosed on cultures. Out of 14 positive cultures, only one (7%) was INH resistant. Out of 46 patients with TB, majority (84%) were cured, 6 (13%) died while one suffered graft failure. Incidence of hepatotoxicity was 1.42%. Finally they conclude that INH prophylaxis is effective in preventing TB in post-renal transplant recipients. Late development of TB still remains a challenge (17).

2.2.3. Clinical Pharmacology of Isoniazid

Within 1 to 2 hours after oral administration, isoniazid produces peak blood levels which decline to 50 percent or less within 6 hours. It diffuses readily into all body fluids (cerebrospinal, pleural and ascetic fluids), tissues, organs and excreta (saliva, sputum and feces). The drug also passes through the placental barrier and into milk in concentrations comparable to those in the plasma. From 50 to 70 percent of a dose of isoniazid is excreted in the urine in 24 hours (13).

Isoniazid is metabolized primarily by acetylation and dehydrazination. The rate of acetylation is genetically determined. Approximately 50 percent of Blacks and Caucasians are "slow in activators" and the rest are "rapid in activators"; the majority of Eskimos and Orientals are "rapid in activators." The rate of acetylation does not significantly alter the effectiveness of isoniazid. However, slow acetylation may lead to higher blood levels of the drug and, thus, to an increase in toxic reactions (14).

Pyridoxine (vitamin B6) deficiency is sometimes observed in adults with high doses of isoniazid and is considered probably due to its competition with pyridoxal phosphate for the enzyme apo-tryptophanase (20).

2.2.4. Mechanism of Action of Isoniazid

Isoniazid inhibits the synthesis of mycoloic acids, an essential component of the bacterial cell wall. At therapeutic levels isoniazid is bactericidal against actively growing intracellular and extracellular Mycobacterium tuberculosis organisms (15).

The antimicrobial activity of INH is selective for mycobacterium, likely due to its ability to inhibit mycolic acid synthesis, which interferes with cell wall synthesis, there by producing a bactericidal effect. INH also disrupts DNA, lipid, carbohydrate, and nicotinamide adenine dinucleotide (NAD) synthesis and/or metabolism (15).

INH is active against intracellular and extracellular mycobacterium TB. The organism considered most susceptible to INH during its logarithmic phase of growth. The minimum inhibitory concentration (MIC) of MTB for INH ranges from 0.01 to 0.25 microgram/ml (18).

2.3. Adverse effects of isoniazid preventative therapy for latent tuberculosis infection

Isoniazid preventative therapy (IPT) is a widely-used intervention for treatment of latent tuberculosis infection (LTBI). Most commonly consisting of a 6- to 9-month course of daily isoniazid, the use of treatment to prevent tuberculosis infection has previously been focused on household contacts of those with active disease. However, in recent years, an expanding group of patients has been recognized as being at higher risk of LTBI reactivation. Patients within this group are receiving immunosuppressive medications, especially tumor necrosis factor alpha (TNF-α) inhibitors, and are routinely recommended IPT prior to the initiation of therapy (16).

Given the increasing number of patients with significant co morbidities likely to receive IPT in the future, it is important to assess the impact of therapy in real-world cohorts, particularly in relation to adverse effects and treatment completion. Previous reports have tended to emphasize serious treatment limiting adverse effects, particularly hepatotoxicity, which has been well-studied in prospective cohorts. However, a wide variety of less well-documented adverse effects have been observed in association with IPT, including acne form and other rashes, gastrointestinal adverse effects, peripheral neuropathy, drowsiness, and alopecia, many of which have been reported in case reports or series but not prospectively considered in a real-world cohort. The frequency, with which many of these adverse effects occur is poorly defined, yet may have substantial impact on patient willingness to initiate or complete therapy, particularly as patients with LTBI are not symptomatic.Whichisaimed to prospectively study the adverse effects of IPT in a real-world cohort, and consider their frequency, severity and association with failure to complete therapy (14, 16).

. From June 2011, all patients commencing treatment for LTBI at the Royal Melbourne Hospital were prospectively and sequentially enrolled in an ongoing database of LTBI treatment outcomes, particularly related to adverse effects and treatment adherence (16).

2.4. Inhibition of Cytochrome P450 (CYP450) Isoforms by Isoniazid: Potent Inhibition of CYP2C19 and CYP3A

Isoniazid (INH) remains the most safe and cost-effective drug for the treatment and prophylaxis of tuberculosis. The use of INH has increased over the past years, largely as a result of the co epidemic of human immunodeficiency virus infection. It is frequently given chronically to critically ill patients who are co prescribed multiple medications (13). The ability of INH to elevate the concentrations in plasma and/or toxicity of co administered drugs, including those of narrow therapeutic range (e.g., phenytoin), has been documented in humans, but the mechanisms involved are not well understood (18).

Using human liver microsomes (HLMs), effect of INH on the activity of common drug-metabolizing human cytochrome P450 (CYP450) isoforms using isoform-specific substrate probe reactions. Incubation experiments were performed at a single concentration of each substrate probe at its Km value with a range of INH concentrations. CYP2C19 and CYP3A were inhibited potently by INH in a concentration-dependent manner. At 50 mm INH (6.86 mg/ml), the activities of these isoforms decreased by 40%. INH did not show significant inhibition (<10% at 50 μM) of other isoforms (CYP2C9, CYP1A2, and CYP2D6). To accurately estimate the inhibition constants (Ki values) for each isoform, four concentrations of INH were incubated across a range of five concentrations of specific substrate probes. The mean Ki values ($\pm$ standard deviation) for the inhibition of CYP2C19 by INH in HLMs and recombinant human CYP2C19 were 25.4 $\pm$ 6.2 and 13 $\pm$ 2.4 mM, respectively. INH showed potent noncompetitive inhibition of CYP3A (Ki 51.8 $\pm$ 2.5 to 75.9 $\pm$ 7.8 mM, depending on the substrate used). INH was a weak noncompetitive inhibitor of CYP2E1 (Ki 110 $\pm$ 33 mM) and a competitive inhibitor of CYP2D6 (Ki 126 $\pm$ 23 mM), but the mean Ki values for the inhibition of CYP2C9 and CYP1A2 were above $500\mu M$. Inhibition of one or both CYP2C19 and CYP3A isoforms is the likely mechanism by which INH slows the elimination of co administered drugs, including phenytoin, carbamazepine, diazepam, triazolam, and primidone. Slow acetylates of INH may be at greater risk for adverse drug interactions, as the degree of inhibition was concentration dependent. These data provide a rational basis for understanding drug interaction with INH and predict that other drugs metabolized by these two enzymes may also interact (18, 25).

3. Objective

3.1. General Objective

To assess the effect of INH prophylaxis on blood level of Tacrolimus drug, LFT and RFT among people undertaking kidney transplantation at St. Paul Hospital kidney transplantation center, Addis Ababa, Ethiopia.

3.2. Specific Objective

1. To compare blood concentration of Tacrolimus drug level during and after completion of INH prophylaxis among people undertaking kidney transplantation at St. Paul Hospital kidney transplantation center, Addis Ababa, Ethiopia.
2. To compare RFT during and after completion of INH prophylaxis among people undertaking kidney transplantation at St. Paul Hospital kidney transplantation center, Addis Ababa, Ethiopia.
3. To compare LFTduring and after completion of INH prophylaxis among people undertaking kidney transplantation at St. Paul Hospital kidney transplantation center, Addis Ababa, Ethiopia.

4. Hypothesis

4.1. Null Hypothesis

There is no drug interaction between INH and Tacrolimus among people undertaking kidney transplantation at St. Paul Hospital kidney transplantation center, Addis Ababa, Ethiopia.

5. Methods

5.1. Study Area

The study was conducted from January, 2019 to September, 2019 at kidney center of SPHMMC. SPHMMC is one of the biggest referral teaching hospitals, under the administration of Ministry of Health, located in Addis Ababa, Ethiopia. The hospital has more than700 beds and give diagnostic and treatment service for about 370,000-400,000 patients per/year. The kidney department gives service to new and follow-up patients and has 10 beds for kidney transplant candidate patients. Professionally, the unit has 3 nephrologists with palliative care specialist, 2 general practitioners, and more than 30 nurses. Being a public hospital, SPHMMC offers without cost for these services.

5.2. Study design and period

An institutional based cross-sectional study design was conducted from January, 2019 to September, 2019 to determine the effect of INH on blood level of Tacrolimus drug at kidney center of SPHMMC.

5.3. Populations

5.3.1. Source of population

The source populations were all kidney patients who underwent renal transplantation at SPHMMC kidney center during the study period would be included in the study.

5.3.2. Study population

All kidney recipients who satisfied the inclusion criteria and treated with INH as a prophylaxis continuously for nine month at SPHMMC kidney center.

5.4. Inclusion and Exclusion criteria

5.4.1. Inclusion criteria

- Patients who were 18-65 years old.
- Patients, who were Voluntary to answer the questionnaire prepared for this study, had given a written consent and sign to participate in the study.

- Patients who have been treated with INH prophylaxis after transplantation.

5.4.2. Exclusion criteria

- Patients who had sero positive status.
- Patients who were mentally ill and unconscious.
- Patients who have not been finished INH drug prophylaxis after transplantation.

5.5. Study variables

- Tacrolimus drug level
- LFT (AST, ALT, ALP, Direct bilirubin and Total bilirubin)
- RFT (Creatinine and Urea)
- Age
- Sex
- Socio demographic characteristics
- Educational status
- Employment

5.6. Sample size calculation and Sampling method

Convenience sampling method was used to recruit study subjects. In this study all kidney recipients who used INH drug for nine month continuously as a prophylaxis from January2019 to September 2019 were recruited purposefully.

5.7. Data collection tools and procedures

In this study, both primary and secondary data was collected from patient's card by the kidney transplant (OPD) nurses. The first Primary data sources were baseline information about each patient like patient characteristics; current medications, co-morbidities, relevant previous medical and medication histories were recorded using data abstraction format through reviewing patient's medical chart and Blood samples were collected under aseptic conditions.

5.8. Specimen collection and procedure

5.8.1. Whole Blood collection and plasma (suspension) preparation

About3ml of whole blood was collected from kidney recipient patients during INH prophylaxes and after they finish this prophylaxis after using it continuously for nine month. Plasma or suspension was prepared by using pretreatment known as ISD pretreatment. First, about3ml of whole blood with EDTA tube from patients was collected at St. Paul's Hospital by the laboratory personnel. Then by using micropipette, 300µl of both whole blood and pretreatment was added in one empty, clear and sterile test tube, and then mixed by vortex upside down for 5 seconds. After 5 minute centrifugation at 3000 rpm, suspension was appeared; from this suspension Tacrolimus drug level was measured by using **Cobas E analyzer** chemistry machine.

5.8.2. Whole blood collection and serum preparation

About 3ml of whole blood was collected from kidney recipient patients during INH prophylaxes and after they finish this prophylaxis after using it continuously for nine month. After collection of the whole blood, allow the blood to clot by leaving it undisturbed at room temperature. This usually takes 15-30 minutes. Remove the clot by centrifuging at 3000 rpm for 5 minutes in a refrigerated centrifuge. The resulting supernatant is designated serum.

5.9. Data quality assurance

In order to assure its quality, data was collected by two kidney transplant nurses who have basic knowledge on INH prophylaxes care services. The blood samples for biochemical and drug assay was collected by adherence with standard operation procedures (SOP) and measurement of analyses were carried out after running quality control samples by the Investigator.. Every laboratory tests for this study performed by skilled and trained laboratory technologists and the quality of this study was followed by the principal investigator and supervisors. The quality of generated data was assured by double entry. The data is confidential and also maintained at the data base of SPHMMC.

5.10. Data analysis and interpretation

The data was analyzed using statistical Package for Social Sciences (SPSS) Version 20 and parametric data was expressed as mean ± standard deviation. The comparisons of the mean values within the group were done using paired samples t test. The correlation between INH metabolism and Tacrolimus drug metabolism were based on Pearson's correlation analysis. The level of statistical significance was set at $P < 0.05$.

5.11. Ethical consideration

Ethical clearance was obtained from Addis Ababa University department of Medical laboratory science, SPHMMC and ethical review Committees prior to the study. Then a letter informing the center of kidney transplant about the study was written from ethical Committee and permission was obtained from center of kidney transplant to access data from study population. A written informed consent was obtained from the study participants.

5.12. Dissemination of results

The findings of this study will be presented to Addis Ababa University department of MLS and SPHMMC. The information will be presented in different national and international scientific seminars and workshops for dissemination and it will also be submitted to journals for possible publication.

5.13. Definition of Terms

Kidney transplant: is a surgery done to replace a diseased kidney with a healthy kidney from a donor.

Tacrolimus drug: is an immunosuppressive drug belonging to the Calcineurin inhibitor group that has emerged as available therapeutic following solid organ (like kidney) transplant. In this study Tacrolimus drug is used as an immunosuppressant drug.

Isoniazid (INH): is a drug used for management of tuberculosis. In this study INH is used as a prophylaxis to prevent kidney recipient patients from TB infection.

6. Result

6.1.Socio demographic characteristics of study participant

From January to September 2019, a total of 14 kidney transplants were undertaken at SPHMMC. Among 14 kidney recipient patients, there were 11 men and 3 women. The mean age of patients was 32.3. The majority within the age group of 30-39 (9/14), attain at least diploma level of education (7/14) and 2 of them were unemployed (Table 1).

Table 1: Socio-demographic characteristics of study participants, attending St Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia

Variable		N	%
Sex	Male	11	78.6
	Female	3	21.4
Age	20-29	3	21.4
	30-39	9	64.3
	40 and above	2	14.3
Educational status	Elementary	3	21.4
	Secondary	4	28.6
	Diploma and above	7	50
Employment	Government employed	6	42.9
	Self employed	6	42.9
	Non-employed	2	14.3

6.2.Comparison of blood concentration of Tacrolimus during and after completion of INH prophylaxis

Blood concentration of Tacrolimus drug was analyzed in patients with INH prophylaxis and after finishing this prophylaxis. The bivariate analysis showed that there is a significance difference with p-value 0.002. As shown in Table 2, the Tacrolimus level was significantly higher when taken together with INH.

Table 2: Mean blood concentration of Tacrolimus drug with and after completion of INH prophylaxis, January-September 2019, at St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia

Type of prophylaxis drug	Range	P-value
Tacrolimus with INH (ng)	6.31, 8.85	0.002
Only Tacrolimus	5.11, 8.07	

INH= isoniazid drug

6.3.Comparison of serum concentration of RFT and LFT tests with and after completion of INH prophylaxis

Liver enzymes like AST, ALT and ALP were higher in all patients who used INH prophylaxis and renal enzymes like creatinine was lower, but urea was higher almost in all patients.

Table 3: Mean serum concentration of Liver and renal function tests on Tacrolimus with and after completion of INH prophylaxis, January-September 2019, at St Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia

Variable	Type of prophylaxis drug	Mean concentration N=14	SD	Mean ± SD (Range)	P-value

Alkaline phosphates	Tacrolimus with INH	101.34	47.83	53.51, 149.17	0.001
	Only Tacrolimus	85.26	37.71	47.55, 122.97	
Aspartate Aminotransaminas	Tacrolimus with INH	29.44	14.95	14.49,44.39	0.001
	Only Tacrolimus	24.6	13.55	11.05,38.15	
Alanine Aminotransaminas	Tacrolimus with INH	37.25	32.22	5.03,69.47	0.01
	Only Tacrolimus	28.93	26.69	2.24,55.62	
Total bilirubin	Tacrolimus with INH	0.49	0.062	0.43,0.55	0.933
	Only Tacrolimus	0.37	0.068	0.30,0.44	
Direct bilirubin	Tacrolimus with INH	0.18	0.020	0.16,0.2	0.710
	Only Tacrolimus	0.15	0.025	0.13,0.18	
Creatinine	Tacrolimus with INH	1.10	0.24	0.86,1.34	0.001
	Only Tacrolimus	1.14	0.19	0.95,1.33	
Urea	Tacrolimus with INH	29.57	11.8	17.77,41.37	0.003
	Only Tacrolimus	27.88	10.45	17.43,38.33	

INH= isoniazid drug

7. Discussion

To our knowledge, this study is the first study on the clinically significant interaction between Tacrolimus and isoniazid (INH) in our country. This study aims to establish baseline for Tacrolimus drug adjustment when patients on INH prophylaxis. Addition of INH to the medication regimen resulted in an increased in serum Tacrolimus concentrations requiring subsequent dose decreases to maintain desired therapeutic levels (25).

Specifically, marginally inferior immunosuppressive medication regimens were result in substantially better patient outcomes than dialysis. Thus, it is better to perform kidney transplantation even with an inferior immunosuppressive regimen, than to avoid transplantation altogether. Except perhaps for transplantation between identical twins, all kidney transplant recipients (KTRs) need immunosuppressive medications to prevent rejection (26, 27, and 32).

The Calcineurin inhibitor tacrolimus was approved by the United States food and drug administration (FDA) for immunosuppression of liver transplant recipients in 1994 and KTRs in 1997. By 2007, it was administered to more than 80% of KTRs in the United States renal data system. In that year the ELiTE-SYMPHONY study was published comparing 4 different immunosuppressive regimes in kidney transplantation (28, 29).

A personalized approach to the use of Tacrolimus is essential for improving graft outcome and reducing adverse events induced by Tacrolimus (26, 30).

In the present study, we obtained three findings. First, we found that the correlation between INH drug and blood Tacrolimus concentration. Second, INH had statistical significant effect on liver functional tests and third INH also had statistical significant effect on renal functional tests.

In the previous study, a literature search failed to identify published reports of drug interaction between Tacrolimus and INH, which made a drug interaction between rifampin and Tacrolimus more likely (6,).

Rifampin was the previous prophylaxis drug for patients who undergone organ transplant. Addition of rifampin to the medication regimen resulted in a reduction in serum Tacrolimus concentrations requiring subsequent dose increases to maintain desired therapeutic levels. The case reported here followed a similar timeline, with the decrease in Tacrolimus through levels

observed 4 days after initiation of rifampin. For this patient, an approximately 2-fold increase in the Tacrolimus dose was ultimately required to overcome the induction effects of rifampin. In other published cases, the Tacrolimus dose increases ranged from 2-fold to 12-fold (6, 14).

Most of the previous study done on the use of INH following renal transplantation should be considered mandatory in geographically areas where the prevalence of TB is high (17). And Isoniazid preventive therapy is a widely used intervention for treatment of latent tuberculosis infection in spite of rifampin (18).

The present study shows the mean of serum biochemical tests including Tacrolimus test except bilirubin and creatinine were significantly higher in all cases. This finding is not in line with any previous study conducted because there is no previous study conducted directly on this study. This study showed that INH drug significantly affects the metabolism of tacrolimus drug.

In the previous study also strong interaction have been observed with antifungal agents such as ketoconazole, fluconazole, intraconazole and voriconazole the macrolide antibiotic erythromycin or HIV protease inhibitors (e.g. Ritonavir). Concomitant use of these substances may require decreased Tacrolimus doses in nearly all patients (33).

Overall, concomitant use of INH drug and Tacrolimus drug were significantly affects blood concentration of Tacrolimus drug level, liver functional tests and also renal functional tests in nearly all patients.

8. Strength and limitation of the study

8.1. Strength of the study

Up to the knowledge of principal investigator and advisors this study is the first in Ethiopia and it provides baseline information for further study and policy makers.

8.2. Limitation of the study

- Isoniazid drug level was not determined in this study due to lack of budget and time.
- There is no any previous study directly done on this study to compare our findings.
- Insufficient Sample size

9. Conclusion and Recommendation

9.1. Conclusion

Kidney recipient patients who used INH prophylaxis showed alteration of LFT, RFT and blood concentration Tacrolimus (BCT). This may indicates that INH drugs were the determinant factor for developing disorders of liver, then to affect the metabolism of Tacrolimus drugs. Even though, there is no significant effect on bilirubin test. Biochemical tests like BCT, AST, ALT, ALP, CREATININE and UREA were significantly affected by INH drug.

9.2. Recommendations

A longitudinal follow up of kidney recipient patients would be highly valuable to better understand the effect of taking more than one prophylactic drugs with tacrolimus; to adjust the concentration and manage drug associated toxicities.

A large scale longitudinal study is required to confirm and translate this finding into clinically applicable results.

10. Reference

1. National Kidney Foundation. www.kidney.ORG.
2. Woywodt A, Delargy M, Thain Z. Different preparations of tacrolimus and Medication Errors. American Journal of Transplantation. 2008:8; 1962.
3. Bottiger Y, Brattsrom C, Tyden G, Sawe J, Groth CG. Tacrolimus whole blood concentration correlates closely to side effects in renal transplant recipients. Br J Clinpharmacol. 1999; 48(3): 445-448.
4. Dubinsky MC, Seidman EG. Novel immunosuppressive therapies for intestinal and hepatic diseases. CurrOpinPediatr. 1999; 11:390-5.
5. Roy FM. First clinical experience with the new once-daily formulation of tacrolimus. Therapeutic Drug Monitoring. 2008:30; 159-166.
6. Naylor H, Robichaud J. Decreased tacrolimus levels after administration of rifampicin to a patient with renal transplant. Can J Hosp Pharm. 2013; 66(6): 388-392.
7. Han SS, Yang SH, Kim MC, Cho JY, Lee JP, Kim DK, et al. Monitoring the intracellular tacrolimus concentration in kidney transplant recipients with stable graft function. J pone. 2016: 10; 1371.
8. Backman L, Nicar M, Levy M. FK506 trough levels in whole blood and plasma in liver transplant recipients. Correlation with clinical events and side effects. Transplantation. 1994; 57:519–25.
9. Trofe-clark J, Lemonovich TL and the AST infectious diseases community of practice. Interaction between anti infectious agents and immunosuppressants in solid organ transplantation. American J Transplantation. 2013; 13: 318-326.
10. Shreiber S, Crabtree G. The mechanism of action of cyclosporine A and FK506. Immunol. 1992; 13: 136-142.
11. Lattes R, Radisic M, Rial M. Tuberculosis in renal transplant recipients, Transpl infect Dis. 1999; 1: 98-104.
12. Amer J. American Thoracic Society/Centers for Disease Control: Treatment of Tuberculosis and Tuberculosis Infection in Adults and Children. RespirCrit Care Med. 1994; 149: 1359-1374.
13. Schrautnagel DE. Testing for isoniazid. Chest. 1990; 98(2): 314-316.

14. Sridhar A, Sandeep Y, Krishnakishore C, SriramnaVee P, Manjusha Y, Sivakumar V. Fatal poisoning by isoniazid and rifampicin. Indian J Nephrol. 2012; 22(5): 385-387.
15. Bardou F, Raynaud C, Ramos C, Laneelle MA, Laneelle G. Mycobacterium of isoniazid uptake in mycobacterium tuberculosis. Microbiology. 1998; 144: 2539-2544.
16. Denhoim JT, Mcbryade ES, Eisen DP, Penington JS, Chen C, Street AC. Adverse effects of isoniazid preventive therapy for latent tuberculosis infection. Drug Health Patient Saf. 2014; 6: 145-149.
17. Akhtar S, Naseem A, Dodani S, Nagvi R, Ahmad E. Tuberculosis in renal transplant recipients on isoniazid prophylaxis. European J Respiratory. 2016; 48: 2104.
18. Nagiv R, Akhtar S, Mammun H, Saeed T. Efficacy of isoniazid prophylaxis in renal allograft recipients. Transplantation Proceeding. 2006; 38(7): 2057-8.
19. Meier-KriescheHU,Ojo AO, Hanson JA, Cibrik DM, Punch JD, Leichtman AB, etal. Increased impact of acute rejection on chronic allograft failure in recent era. Transplantation. 2000; 70(7):1098–100.
20. John GT. Infections after renal transplantation in India. Indian J Nephrol. 2003; 13:14.
21. Subramanan A, Dorman S. Mycobacterium tuberculosis in solid organ transplant recipient. American J Transplantation. 2009; 59: 1600-6143.
22. Sakhuja V, Jha V, Varma PP, Joshi K, Chugh KS. The high incidence of tuberculosis among renal transplant recipients in India. Transplantation. 1996; 61:211.
23. Hall RG, Leff RD, Gumbo T. treatment of active pulmonary tuberculosis in current standards and recent advances. Insights from the society of infectious diseases pharmacists. Pharmacotherapy. 2009; 29: 1468-81.
24. Melchor JL, Gracida C, Ibarra A. Increased frequency of tuberculosis in Mexican renal transplant recipients. Transplant proce. 2002: 34; 78-79.
25. Desta Z, Soukhova NV, Flockhart DA. Inhibition of Cytochrome P450 (CYP450) Isoforms by Isoniazid: Potent Inhibition of CYP2C19 and CYP3A. Antimicrobial Agents and Chemotherapy. 2001; 45(2): 382-392.
26. KDIGO-Best-Practice-Guideline-for-the-care-of-kidney-Transplant-Recipients-LW-Kopie-2.
27. Wolfe RA, Ashby VB, Milford EL. Comparison of mortality in all patients on dialysis, patients on dialysis awaiting transplantation, and recipients of a first cadaveric transplant. N EngI J Med 1999; 341: 1725-1730.
28. Liu J, Yan J, Wan Q, Ye Q and Huang Y. Bioequivalence of twice daily oral tacrolimus in transplant recipients. Plos Medicine. November 14, 2017.

29. Ekberg H, Tedesco-Silva H, Demirbas A, Vitko S, Nashan B, Gurkan A, et al. Reduced Exposure to calcineurin inhibitors in Renal Transplantation. N EngI J Med. 2007; 357(25):2562-75.
30. Vanhove T, Vermeulen T, Annaert P, Lerut E, Kuypers D. High Intrapatient Variability of Tacrolimus Concentrations Predicts Accelerated Progression of Chronic Histologic Lesions in Renal Recipients. Am J Transplant. 2016; 16(10):2954-63.
31. Liu J, Yan J, Wan Q, Ye Q and Huang Y. The risk factors for tuberculosis in liver or kidney transplant recipients.BMC Infectious Disease 2014; 14:387.
32. Singh N, Paterson DL. Mycobacterium tuberculosis infection in solid-organ transplant recipients: impact and implications for management. Clin Infect Dis 1998; 27:1266-1277.
33. Munoz P, Rodriguez C, Bouza E. Mycobacterium tuberculosis infection in recipients of solid organ transplants. Clin Infect Dis 2005; 40:581-587.

.

11. Annexes

Annex I: English version of Participants' information sheet

Principal Investigator: YonasTsehay

Name of Organization: AAU, College of Health Sciences, , Department of Medical Laboratory Sciences

Name of the sponsors: AAU School of Graduate studies.

Title: Effect of isoniazid (INH) on blood level of tacrolimus drug used on kidney transplant patients, Addis Ababa, Ethiopia.

Aim: INH is part of a prophylaxis against MTB. However, there is a controversial idea on the contribution INH in TB. Therefore we are going to assess the effect of INH on blood level of Tacrolimus drug.

Study Participants: In this study kidney recipient patients used both INH and Tacrolimus drug and healthy control used only Tacrolimus drug age of 18-65 both male and female who fulfill inclusion criteria will participate.

Samples Required: If you agree to participate in the study, you are requested to provide about 3 ml of venous blood, after starting the INH medicine and after accomplish this prophylaxis.

Duration: From Jan. 2019 till stated number of study participants will be recruited.

Procedures to be carried on: In order to perform the indicated study STPHMMC, Addis Ababa, Ethiopia, you are invited to take part in this project. If you are willing to participate, you are expected to understand and sign the informed consent. Then, socio demographic, blood sample and clinical information from kidney recipient patient is important for this study will be taken.

After consent, 6ml blood specimen will be collected from you by specimen collector and face to face interview for additional questions.

Risk: We are asking you to share with us some personal and confidential information, and you may feel uncomfortable talking about some of the topics. There will be minor discomfort during

blood specimen collection. During collection of specimen from you, appropriate precaution will be taken and all samples will be collected by trained health professionals. If anything happened, appropriate medical care will be provided to you.

Benefits: In this study, you are not directly benefited however; the result of this study may provide better management of kidney recipient patients in the future. Hence, you are indirectly benefiting other patients and the society in this respect.

Compensation: Fifty birr will be given for study participants to compensate transport cost.

Confidentiality: All your personal information collected for the purpose of this study will be kept confidential.

Right: The participation is completely voluntary and you have the right not to participate in this study. You may withdraw at any time and place without consequences of any kind. You may also reject to give any sample. You can ask any questions regarding to this study and you have a right to get a laboratory diagnosis result for free.

Whom to contact: If you have any question or description about this study, you can communicate on the following address:

PI: YonasTsehay
Phone: +251913 07 25 82
E-mail: yoloyoni2010@gmail.com

Annex II: Participant information sheet (Amharic version)

የተሳታፊዎች ፈቃድና መተማመኛ ቅፅ

መግቢያ

በአዲስ አበባ ዩኒቨርሲቲ ጤና ሳይንስ ኮሌጅ የሕክምና ላቦራቶሪ ት/ክፍል በማስተርስ ድግሪ ተማ ሪየመመረቂያ ጥናት ላይ እዲሳተፉ ተጋብዘዋል፡፡እባክዎ በዚህ ጥናት ለመሳተፍ ከመስማማትዎ በፊት ከዚህ ቀጥሎ የሚገኘዉን ምንባብ በጥሞና ያንብቡና ግልጽ ያልሆነልዎትን ማንኛዉም ሃሳብ ይጠይቁ፡፡

የጥናቱ ርዕስ: Effect of Isoniazid (INH) prophylaxes on blood level of tacrolimus, RFT and LFT used for kidney recipient patients attending St. Paul's Hospital Millennium Medical College, Addis Ababa; Ethiopia **የጥናቱ ባለቤት፡ YonasTsehay**(BSc, MSc candidate)

የጥናቱ አላማ፡ to determine the effect of Isoniazid prophylaxes on blood level of tacrolimus drug, LFT and RFT used for kidney recipient patients, Addis Ababa; Ethiopia.

እናም እርስዎ በዚህ ጥናት ለመሳተፍ ጠቀሚና ምቹ ሆነው ተመርጠዋል፡፡የእርስዎ በዚህ ጥናት ላይ የሚኖርዎት ተሳትፎ ሙሉ በሙሉ በበጎ ፈቃደኝነት ላይ የተመሰረተ ነዉ፡፡በዚህ ጥና ዉስጥ ላለመሳተፍ ወይም ለመሳተፍ ከወሰኑ በኃላ ለማቋረጥ የሚወስኑ ቢሆንም እንኩዋን በዚህ ሆስፒታል የሚሰጠዉ ማንኛዉም አገልግሎት አይቋረጥም፡፡በጥናቱ ለመሳተፍ የሚስማሙ ከሆነ የስምምነት ቅጹ ላይ በጹሁፍ ወይም በጣት ፊር ማማስቀመጥ ይጠበቅብዎታል፡፡

የጥናቱ ተሳታፊ ለመሆን የሚጠበቅብዎት ምንድን ነው?

በዚህ ጥናት ለመሳተፍ የሚስማሙ ከሆነ የደም ናሙና እንደሚወሰድና ለጥናቱ እንዲሚዉል መስማማት ይጠበቅብዎታል፡፡ ከተወሰደዉ ናሙና ላይ የሚገኙ መረጃዎች ከዚህ ሆስፒታል ዉጭ ለሚገኙና ለስራዉ አግባብነት ላላቸዉ ሰዎች ቢነገር የማይቃወሙ መሆኑን መስማማት ይጠበቅብዎታል፡፡ይሁን እንጅ ይህ አይነቱ መረጃ የርስዎን ማንነት የሚገልጡ መረጃዎችን ማለትም ስም፤አድራሻና የስልክ ቁጥ ርየመሳሰሉትን መረጃዎቸን አይጨምርም፡፡ይልቁንም ለዚህ አገልግሎት ብቻ የሚዉል አርስዎን ለማወቅ የሚያስችል መለያ ቁጥር ጥቅም ላይ እንዲዉል ይደረጋል፡፡በተጨማሪም ስለርስዎ አጠቃላይ የጤና ሁኔታ ለሚቀርቡ አንዳንድ ተጨማሪ ጥያቄዎች መልስ መስጠት ይጠበቅብዎታል፡፡

በዚህ ጥናት መሳተፍ የሚያስከትላቸዉ ቸግሮች ምንድን ናቸዉ?

ናሙና በሚሰበሰብበት ወቅት ምንም አይነት የከፉ ችግር አያጋጥምዎትም፡፡ነገር ግን ደም ሲወሰድ መጠነኛ የህመም ስሜት ሊያስከትል ይችላል፡፡ሆኖም ግን ናሙናዉን ለመሰብሰብ ልምድ ያለዉ ባለሙያ ስለሚመደብና አስፈላጊዉ የጥንቃቄ እርምጃ ስለሚወሰድ የህመም ስሜት አይኖርም፡፡

የህክምና መረጃ በሚስጥር ተጠብቆ መቆየት የሚችለዉ እንዴት ነዉ?

ስለራስዎ የሰጡት ማንኛዉም መረጃና ከተወሰደዉ ናሙና ላይ የተገኘዉ የላቦራቶሪ ዉጤት የሚዉለዉ ለጥናቱ አላማ ብቻ ነዉ፡፡ይህን ማህደር ሊያገኙ የሚችሉት የተወሰኑ የጥናቱ ተባባሪ ሰዎች ብቻ ናቸው፡፡ከዚያም በላይ ስለእርስዎ ያለውን ማንኛውንም መረጃ የተለየ የይለፍ ቃል ባለው የኮምፒውተር የመረጃ ማህደር ውስጥ እንዲቀመጥ ይደረጋል፡፡

በዚህ ጥናት መሳተፍ የሚያስገኛቸው ጥቅሞች ምንድን ናቸው?

ይህ ጥናት የማስተርስ ዲግሪ መመረቂያ እንደመሆኑ መጠን በዚህ ጥናት በመካፈልዎ በገንዘብ የሚያገኙት ጥቅም ባይኖርም ከጥናቱ በሚገኘው ውጤት ግን ተጠቃሚ ነዎት፡፡የእርሶዎ ተሳትፎ የእርስዎንና የወገንዎትን የኩላሊት ህመም ለመመርመርና ለማከታተል ከፍተኛ ጥቅም ይኖረዋል፡፡

በዚህ ጥናት ተሳታፊ የመሆንዎ መብቶች ምንድን ናቸው?

በዚህ ጥናት መሳተፍ ሙሉ በሙሉ በእርስዎ ፈቃደኝነት የተመሰረተ በመሆኑ በማንኛውም ሰዓትና ቦታ የማቋረጥ ሙሉ መብት የተጠበቀ ከመሆኑም በላይ እራስዎን ከጥናቱ በማግለልዎ ምክንያት የሚቀርብዎት ምንም አይነት የሆስፒታል አገልግሎት አይኖርም፡፡ከዚህም በተጨማሪ ጥናቱን በተመለከተ ማንኛውንም አይነት ጥያቄ የመጠየቅና ገለጻ የማግኘት መብት አልዎት፡፡የላብራቶሪ ምርመራ ውጤቱንም በነጻ ማግኘት ይችላሉ፡፡ነገር ግን እርስዎ በሚሰጡን መረጃ የችግሩን ስፋት ለመከላከል እና ለመቆጣጠር ጠቃሚ ስለሆነ ለሚቀርብልዎት ጥያቄ ቀጥተኛ መልስ ይሰጡን ዘንድ በታላቅ አክብሮት እንጠይቃለን፡፡

ጥያቄ ካለኝ ወይም ችግር ቢያጋጥመኝ ምን ማድረግ ይገባል?

ይህንን ጥናት በተመለከተ ወይም ከዚህ ጥናት ጋር በተዛመደ መልኩ ስለሚያጋጥሙ ድንገተኛ አደጋዎች ወይም ጥያቄ ካለዎት በሚመለከተው አድራሻ ይጠቀሙ፡፡

PI: Yonas Tsehay

Phone: +251913 07 25 82

E-mail: yoloyoni2010@gmail.com

AnnexIII: English version of Study Participant informed Consent Form

Name of the participant: _____Age _____Sex___

Code _____Study site/Health center _____

I confirm that I have been given adequate information about the research project; Effect of INH on blood level of Tacrolimus drug used on kidney transplant patients in Addis Ababa, Ethiopia. I have been requested to provide 6 ml of venous blood, after starting the medicine and at end of nine month. The researchers informed that there is no major risk associated with participating in the study or providing the requested samples. I have also understood that the results of clinical and laboratory diagnosis will be used for research purposes and the information related to myself/my family will be kept strictly confidential. I am well informed that participation in the study is fully voluntary and I can withdraw anytime without giving any reason. I confirm that all the information provided to me is very clear and has been conveyed by the language that I fully understand. Finally, I declare that I have been given enough time to deliberate before I agree to participate in the study, and I signed this informed consent.

Name of participant.....Signature.....Date……

Name of the nurse obtaining consent.....signature: …… date ……

Name of witness ……Signature……Date…….

Annex IV: Amharic version of Study Participant informed Consent Form

የተሳታፊዎች ስምምነት ማረጋገጫ

የሚስጥር ቁጥር ----------

የተሳታፊዉ ስም ----------እድሜ----------

እኔ ስሜ ከላይ የተጠቀሰው ተሳታፊ “Effect of Isoniazid prophylaxes on blood level of tacrolimus drug, RFT and LFT used for kidney recipient patients attending St. Paul’s Hospital Millennium Medical College,AddisAbaba,Ethiopia”**ጥናት ላይ በቂ ገለጻ ተደርጎልኛል፡፡ለጥናቱም የደም ናሙና እንደሚያስፈልግ ተገልጾልኛል፡፡የጥናቱንም አላማዎች ተረድቻለሁ፡፡**

በመጠይቁ ላይ የገለጽኳቸው መረጃዎች በሙሉ በሚስጥር የተጠበቁ እንደሚሆኑ ተነግሮኛል፡፡ በጥናቱ ላይ ያለመሳተፍና ማንኛውንም መረጃ ያለመስጠት እንዲሁም በማንኛውም ጊዜ ከጥናቱ ራሴን የማግለል መብቴ የተጠበቀ እንደሆነ ተገልጾልኛል፡፡

ስለዚህ ለዚህ ጥናት መረጃና የስምምነት ቃሌን የሰጠሁት በአጠቃላይ ሁኔታውን በመረዳትና በፍጹም ፍቃደኝነት ነው፡በተጨማሪም ጥያቄ ለመጠየቅ ተፈቅዶልኝ ለማወቅ የፈለኩትን ያህል ማብራሪያ አግኝቻለሁ፡፡የዚህ ጥናት ተሳታፊ በመሆኔ የማገኘው ጥቅም የሁሉንም ምርመራ ውጤት በነጻ ማግኘት እንደሆነ ተረድቻለሁ፡፡

በአጠቃላይ እኔ ከላይ በመተማመኛ ቅፅ የተጠቀሱትን ሁሉ በሚገባና በተረጋጋ መንፈስ አንብቤዋለሁኝ፡፡ስለዚህ በዚህ ጥናት ለመሳተፍ ፈቃደኛ መሆኔን በፊርማዬ አረጋግጣለሁ፡፡

ፊርማ---------- ቀን ----/---/----------

(የስምምነት ቅጹን ማንበብ ለማይችሉ ተሳታፊዎች)

የአማካሪ ነርስ ስም ---------- ፊርማ ----------

ቀን----------

Annex V: English version of questionnaire for study participant

Questioner: Subject identification and Clinical Examination form

STUDY SITE: _____ Date (EC): _____/_____/_____

Instruction: Please try to complete all information

1. Socio demographic data

Patient ID ☐☐☐☐☐

Gender: M ☐ F ☐ Age (years): ☐☐

Education: 1. No formal education ☐ 2.Elementary 3. Secondary ☐
4. Diploma& above ☐

Occupation: 1.Government employed 2. ☐ Self employed ☐
3. Not employed ☐

Table 3: Checklist to record anthropometric data and current medication

S. No	Variables	Value	Remark
1	Blood pressure		
2	Type of clinical diagnosis		
3	Date of diagnosis		
4	Type of current medication		
5	Date of INH started		
6	Date of INH finished		

Table 4: Checklist to record laboratory findings

S. No	Tests	Results	Remarks
1	ALP		
2	AST		
3	ALT		
4	Total Bilirubin		
5	Direct Bilirubin		
6	Creatine		
7	Urea		

Annex VI: Standard operating procedures for laboratory tests

Objective: To describe laboratory tests performed for the present study.

Personnel: Data was collected by principal investigator and health workers. All laboratory tests for this research were performed by skilled and trained researcher.

A. Pre-analytical

Materials and equipment for serum preparation

- ✓ Human blood sample
- ✓ EDTA Tube
- ✓ SST
- ✓ Serological pipette of appropriate volume
- ✓ Centrifuge
- ✓ Nunc tube

First there will have Proper patient identification, and then fasting blood samples before the medicine (tacrolimus drug) will be taken from the anti-cubital vein of the arm by using 10-cc syringes after proper antisepsis with 70% alcohol. Then the blood from each participant will be transferred to serum separator tube (SST), then allowed to stand for 30 minutes till clotted and EDTA tube for drug suspension preparation. Then serum and suspension will be separated by centrifugation at 1500 rpm for 15 minutes and 5000 rpm for 5 minutes respectively.

Procedure for serum separation

1. 4 ml sample whole blood will be drawn into vacationer tubes containing no anticoagulant.
2. Then it will be incubate in an upright position at room temperature for 30-40 minutes (no longer than 60 minutes s) to allow clotting
3. It will centrifuged for 15 minutes (1000-2000rpm)
4. Then it will be inspected for turbidity. Turbidity sample should be centrifuged and aspirate again to remove remaining insoluble matter.
5. Aliquot into Nunc tubes and stored at –20 °C. The Nunc tubes will be labeled with patient identification number.

Procedure for suspension preparation

1. 3-5 ml of whole blood will be collected.
2. Then transferred to EDTA tube and mix.
3. By using micropipette, we will add 300µl of both whole blood and pretreatment (ISD) in one empty, clear and sterile tube.
4. Then mix by vortex upside down for 5-seconds.
5. Then centrifuge at 5000rpm for 5-minutes.
6. After 5-minute we will get the supernatant known as suspension.
7. From this suspension we will measure tacrolimus drug level

B. Analytical

Liver functional test

1. **Alanine Aminotransferase (ALT)**

Test principle

Method according to the International Federation of Clinical Chemistry (IFCC), but without pyridoxal-5'-phosphate. ALT catalyzes the reaction between L‑alanine and 2‑oxoglutarate. The pyruvate formed is reduced by NADH in a reaction catalyzed by lactate dehydrogenase (LDH) to form L‑lactate and NAD+.

L-alanine + 2-oxoglutarate $\xrightarrow{\text{ALT}}$ pyruvate + L‑glutamate

Pyruvate + NADH + H+ $\xrightarrow{\text{LDH}}$ L‑lactate + NAD+

The rate of the NADH oxidation is directly proportional to the catalytic ALT activity. It is determined by measuring the decrease in absorbance at 340 nm.

Test procedure

	Blank	Sample
Reagent	1000 ML	1000 µL
Distilled water	10 ML	-
Sample	-	10 µL
Mix, Incubate 10 min. at 20-25℃ or 5 min. at 37℃. Measure the absorbance of the sample against the reagent blank (ΔA) within 60 min.		
ΔA = [ΔA sample]- [ΔA blank]		

Reagent handling

Ready for use

Storage and stability

Shelf life at 2‑ 8 °C See expiration date on cobas c pack label

On-board in use at 10‑ 15 °C 12 weeks

Calculation

The COBAS INTEGRA 400 plus analyzer automatically calculates the analyte activity of each sample. For more details, please refer to Data Analysis in the Online Help.

Conversion factor: U/L × 0.0167 = µKat/L

Limitations - interference

Criterion: Recovery within ± 10 % of initial value.

Serum, plasma

Icterus: No significant interference up to an I index of 60 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L or 60 mg/dL).

Hemolysis: No significant interference up to an H index of 130 (approximate hemoglobin concentration: 81 µmol/L or 130 mg/dL).

Lipemia (Intralipid): No significant interference up to an L index of 150. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration. Lipemic specimens may cause > Abs flagging. Choose diluted sample treatment for automatic rerun.

Anticoagulants: Citrate and fluoride inhibit the enzyme activity.

Drugs: No interference was found at therapeutic concentrations using common drug panels. Exceptions: Calcium desolate and doxycycline HCl cause artificially low ALT values at the tested drug level. Hydroxocobalamin (Cyanokit) may cause false-low results. Physiological plasma concentrations of Sulfasalazine or Sulfa pyridine may lead to false results. In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.10 for diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Clinical significance

The enzyme alanine aminotransferase (ALT) has been widely reported as present in a variety of tissues. The major source of ALT is the liver, which has led to the measurement of ALT activity for the diagnosis of hepatic diseases. Elevated serum ALT is found in hepatitis, cirrhosis, obstructive jaundice, carcinoma of the liver, and chronic alcohol abuse. ALT is only slightly elevated in patients who have uncomplicated myocardial infarction. Although both serum aspartate aminotransferase (AST) and ALT become elevated whenever disease processes affect liver cell integrity, ALT is the more liver-specific enzyme. Moreover, elevations of ALT activity persist longer than elevations of AST activity. In patients with vitamin B6 deficiency, serum aminotransferase activity may be decreased. The apparent reduction in aminotransferase activity may be related to decreased pyridoxal phosphate, the prosthetic group for aminotransferases, resulting in an increase in the ratio of Apo enzyme to holoenzyme.

2. **Aspartate Aminotransferase**

Test principle

Method according to the International Federation of Clinical Chemistry (IFCC), with pyridoxal-5'-phosphate. AST in the sample catalyzes the transfer of an amino group between L‑ aspartate and 2‑ oxoglutarate to form oxaloacetate and L‑ glutamate. The oxaloacetate then reacts with NADH, in the presence of malate dehydrogenase (MDH), to form NAD+. Pyridoxal phosphate serves as a coenzyme in the amino transfer reaction. It ensures full enzyme activation.

L‑ Aspartate + 2‑ oxoglutarate $\xrightarrow{\text{AST}}$ oxaloacetate + L‑ glutamate

Oxaloacetate + NADH + H+ $\xrightarrow{\text{MDH}}$ L- malate + NAD+

The rate of the NADH oxidation is directly proportional to the catalytic AST activity. It is determined by measuring the decrease in absorbance at 340 nm.

Reagent handling

Ready for use

Storage and stability

Shelf life at 2‑ 8 °C See expiration date on cobas c pack label

On-board in use at 10‑ 15 °C 12 weeks

Calculation

The COBAS INTEGRA 400 plus analyzer automatically calculates the analyte activity of each sample. For more details, please refer to Data Analysis in the Online Help.

Conversion factor: U/L × 0.0167 = µKat/L

Limitations - interference

Criterion: Recovery within ± 10 % of initial value. Serum, plasma

Icterus: No significant interference up to an I index of 60 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L or 60 mg/dL).

Hemolysis: No significant interference up to an H index of 25 (approximate hemoglobin concentration: 16 µmol/L or 25 mg/dL).

Lipemia (Intralipid): No significant interference up to an L index of 150. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration. Lipemic specimens may cause >Abs flagging. Choose diluted sample treatment for automatic rerun.

Anticoagulants: Citrate and fluoride inhibit the enzyme activity.

Drugs: No interference was found at therapeutic concentrations using common drug panels.9, 10

Exceptions: Calcium desolate and doxycycline HCl cause artificially low AST values at the tested drug level. Hydroxocobalamin (Cyanokit) may cause false-low results. Physiological plasma concentrations of Sulfasalazine or Sulfa pyridine may lead to false results. In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results. For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Clinical significance

The enzyme aspartate aminotransferase (AST) is widely distributed in tissue, principally hepatic, cardiac, muscle, and kidney. Elevated serum levels are found in disease involving these tissues. Hepatobiliary diseases, such as cirrhosis, metastatic carcinoma, and viral hepatitis also increase serum AST levels. Following myocardial infarction, serum AST is elevated and reaches a peak 2 days after onset. In patients undergoing renal dialysis or those with vitamin B6 deficiency, serum AST may be decreased. The apparent reduction in AST may be related to decreased pyridoxal phosphate, the prosthetic group for AST, resulting in an increase in the ratio of Apo enzyme to

holoenzyme. Two isoenzymes of AST have been detected, cytoplasmic and mitochondrial. Only the cytoplasmic

3. **Alkaline phosphates (ALP)**

Test principle

Colorimetric assay in accordance with a standardized method In the presence of magnesium and zinc ions, p‑nitro phenyl phosphate is cleaved by phosphatases into phosphate and p‑nitro phenol.

P-nitro phenyl phosphate + H2O $\xrightarrow{\text{ALP}}$ phosphate + p‑nitro phenol

The p‑nitro phenol released is directly proportional to the catalytic ALP activity. It is determined by measuring the increase in absorbance at 409 nm.

Reagent handling

Ready for use

Storage and stability

Shelf life at 2‑8 °C See expiration date on cobas c pack label

COBAS INTEGRA 400 plus system On-board in use at 10‑15 °C 4 weeks

Limitations – interference

Criterion: Recovery within ± 10 % of initial value.

Icterus: No significant interference up to an I index of 42 for conjugated bilirubin and 60 for unconjugated bilirubin (approximate conjugated bilirubin concentration: 718 µmol/L or 42 mg/dL; approximate unconjugated bilirubin concentration: 1026 µmol/L or 60 mg/dL). Hemolysis: No significant interference up to an H index of 250 (approximate hemoglobin concentration: 155 µmol/L or 250 mg/dL). Lipemia (Intralipid):8 No significant interference up to an L index of 2000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Drugs: No interference was found at therapeutic concentrations using common drug panels. In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.11 for diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Renal functional test

1. **Creatinine (CREJ2)**

Test principle

This kinetic colorimetric assay is based on the Jaffé method. In alkaline solution, creatinine forms a yellow-red complex with picrate. The rate of dye formation is proportional to the creatinine concentration in the specimen. To correct for non-specific reaction caused by serum/plasma pseudo-creatinine chromogens, including proteins and ketones, the results for serum or plasma are corrected by -18 µmol/L (-0.2 mg/dL).

Creatinine + picric acid $\xrightarrow{\text{alkaline pH}}$ yellow-red complex

Reagent handling

Ready for use

Storage and stability

Shelf life at 15‑25 °C See expiration date on cobas c pack label

On-board in use at 10‑15 °C 8 weeks

Calculation

The COBAS INTEGRA 400 plus analyzers automatically calculates the analyte concentration of each sample. For more details, please refer to Data Analysis in the Online Help.

Conversion factor: µmol/L × 0.0113 = mg/Dl

Limitations - interference

Criterion: Recovery in the creatinine decision range for adults (90 µmol/L in serum) within ± 10 % of initial value.

Hemolysis: No significant interference up to an H index of 800 (approximate hemoglobin concentration: 497 µmol/L or 800 mg/dL). Do not use the COBAS INTEGRA Creatinine Jaffé Gen.2 test when testing for creatinine in hemolyzed samples from neonates, infants or adults with an HbF level of ≥ 60 mg/dL.

Icterus: No significant interference up to an I index of 5 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 85 µmol/L or 5 mg/dL).

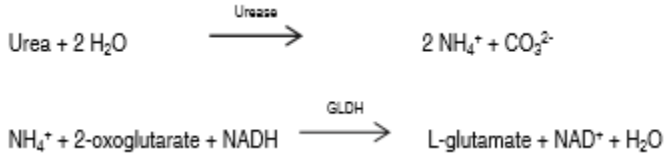
Lipemia (Intralipid): No significant interference up to an L index of 250. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration. Pyruvate: No significant interference from pyruvate up to a concentration of 0.4 mmol/L (3.5 mg/dL).

Ascorbic acid: No significant interference from ascorbic acid up to a concentration of 4 mmol/L (70 mg/dL). Therapeutic drug interference was tested according to the recommendations of the VDGHc). No interferences were found. Exceptions: Antibiotics containing cephalosporin lead to significant false positive values.16, 17 Hydroxocobalamin (Cyanokit) may cause artificially low results. The presence of ketone bodies can cause artificially high results in serum and plasma. In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.18 Values < 0.2 mg/dL (< 18 µmol/L) or negative results are reported in rare cases in children < 3 years and elderly patients. In such cases use the Creatinine plus test to assay the sample.

2. Urea

Test principle

Kinetic test with urease and glutamate dehydrogenase2, 3, 4, 5 Urea is hydrolyzed by urease to form ammonium and carbonate. In the second reaction 2‑oxoglutarate reacts with ammonium in the presence of glutamate dehydrogenase (GLDH) and the coenzyme NADH to produce L‑glutamate. In this reaction two moles of NADH are oxidized to NAD+ for each mole of urea hydrolyzed.



The rate of decrease in the NADH concentration is directly proportional to the urea concentration in the specimen. It is determined by measuring the absorbance at 340 nm.

Reagent handling

Ready for use

Storage and stability

Shelf life at 2‑8 °C See expiration date on cobas c pack label

COBAS INTEGRA 400 plus system On-board in use at 10‑15 °C 8 weeks

Limitations - interference

Criterion: Recovery within ± 10 % of initial value. Serum/plasma

Icterus: No significant interference up to an I index of 60 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L or 60 mg/dL).

Hemolysis: No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L or 1000 mg/dL). Hemolytic specimens may cause high absorbance flagging. Choose diluted sample treatment for automatic rerun.

Lipemia (Intralipid): No significant interference up to an L index of 2000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration. Lipemic specimens may cause high absorbance flagging. Choose diluted sample treatment for automatic rerun.

Anticoagulants: Do not use ammonium heparin as an anticoagulant. Therapeutic drug interference was tested according to the recommendations of the VDGHa). No interferences were found.

Ammonium ions may cause erroneously elevated results. In very rare cases, gammopathy, in particular type IgM (Waldenström’s macroglobulinemia), may cause unreliable results. For diagnostic purposes, the results should always be assessed in conjunction with the patient’s medical history, clinical examination and other findings.

Declaration

I, the undersigned, declare that this M.Sc. research thesis is my original work, has not been presented for a degree in this or any other university and that all sources of materials used for the research proposal have been duly acknowledged.

M.Sc. candidate: Yonas Tsehay (B.Sc.)

Signature: _____

Date of submission: _____

Place Addis Ababa, Ethiopia.

This research thesis has been submitted with our approval as advisors.

Name of advisor: MistireWolde (MSc, PhD,)

Signature _____

Place: Department of Medical Laboratory Sciences, Addis Ababa University

Date of submission_____/_____/_____

Name of advisor: Momina Mohammed (MD, ISNF)

Signature _____

Place: St. Paul's hospital millennium medical college

Date of submission_____/_____/_____