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DEPARTMENT OF MEDICAL LABORATORY SCIENCES

ASSESSMENT OF SELECTED HEMATOLOGICAL AND BIOCHEMICAL PROFILES BEFORE AND AFTER KIDNEY TRANSPLANT AT ST. PAUL'S HOSPITAL MILLENNIUM MEDICAL COLLEGE IN ADDIS ABABA, ETHIOPIA.

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This is to certify that the thesis prepared by Endale Haile, entitled: **Assessment of Selected Hematological and Biochemical Profiles (Urea and Creatinine) among Patients with Kidney Failure Before and After kidney Transplant at 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 (Hematology and Immune-Hematology) complies with the regulations of the university and meets the accepted standards with respect to originality and quality.

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List of Abbreviations

AKI	Acute Kidney Injury
BUN	Blood Urea Nitrogen
CKD	Chronic Kidney Disease
Cr	Creatinine
DM	Diabetis Millitis
ESRD	End Stage Renal Disease
GFR	Glomerular Filtration Rate
HB	HemoglobinD
HTN	Hypertension
HCT	Hematocrite
IRF	Immature Reticulocyte Fraction
K/DOGI	Kidney Dialysis Quality Initiative
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
MPV	Mean Platelet Volume.
MUAC	Mid-upper arm circumference
NRBC	Nucleated Red Blood Cells
PCV	Packed Cell Volume
PLT	Platelet
RBC	Red Blood Cell
RDW	Red Cell Distribution Width
RDW-SD	Red Cell Distribution Width Standard Deviation
RET	Reticulocytes
SOP	Standard operation procedures
SPHMMC	St. Paul's Hospital Millennium Medical College
SPSS	Statistical package for social sciences
SRPU	Student researcher publication unite
TLC	Total Leukocytes cell
WBC	White blood cell

Abstract

Background: Chronic kidney failure is a major worldwide public health problem with greater burden on health care system mostly in developing country. The major risk factors for chronic kidney disease include hypertension, diabetes mellitus and obesity. Kidney diseases are associated with a change in various biochemical and hematological parameter.

Objective: To asses selected hematological and biochemical profiles before and after kidney transplant at St. Paul hospital kidney transplantation center Addis Ababa, Ethiopia.

Methods: A hospital based prospective cohort design was used to conducted a study from November 2020 to May 2021. Convenience sampling techniques was implemented for selecting participants who fulfill the eligibility criteria. Socio-demographic and clinical data was collected using interviewer administered structured questionnaire and data collection format. Approximately 9ml venous blood was collected for hematological and bio-chemical laboratory analysis in to EDTA and serum separate or tube respectively. Beckman Coulter DxH 800 analyzer was used to obtain hematological parameters while COBAS 311 analyzer for Bio-chemical analysis. The data was exported and analyzed using SPSS Version 25 software. Descriptive data was summarized using proportions, means, medians (interquartile range) and standard deviation (± SD) as appropriate, For continuous variables. Paired t-test was used for statistical analysis. P value less than 0.05 was considered as statistically significant.

Result: from the t test analysis, the finding indicated there was a significant difference between a hematological parameters of patients before and after transplantation. Accordingly there significance level of decrement for Pre & post-transfusion RBC count value mean 3.78 & 5.1 ($p < 0.0001$), Hgb mean 11.2 & 15.2 relatively ($p < 0.0001$), HCT mean value 35.2 & 48.9 relatively ($p < 0.0001$), MCV mean value 93.8 & 88.3 relatively (p=0.022), Monocyte mean value 1.5 & 0.89 relatively ($p < 0.0001$), creatinine mean value 7.9 & 1.4 respectively ($p < 0.0001$) & urea mean value 103.7 & 30.2 respectively ($p < 0.0001$) positive correlation between pre and post-operative procedure was observed.

Conclusion: kidney recipient patients were affected to different degree of abnormality in hematological and biochemical parameter before and after kidney transplant that require careful evaluation and management.

Keyword: Chronic Kidney Diseases, Kidney Transplant, Hematological Profile, Biochemical Profile

1. Introduction

1.1. Background

Kidney transplantation is acknowledged as a serious advance of recent medicine which provides high-quality life years to patients with irreversible renal failure (end-stage renal disease, ESRD) worldwide. What was an experimental, risky and really limited treatment option fifty years ago, is now routine clinical practice in more than 80 countries. What was once limited to a couple of individuals during a small number of leading academic centers in high income economies, is now transforming lives as a routine procedure in most high- and middle-income countries – but can do far more[1].

The kidneys are made up of about one million functional unit called Nephrons. Nephrons filter waste product, water and other substance from the blood. The unwanted substance come out of the body as urine. The kidney is responsible to maintain electrolyte and acid-base balance. It also plays vital role in erythropoiesis by producing the hormone erythropoietin. There are 2 main types of kidney disease. These are acute kidney injury and chronic kidney disease. Acute kidney injury (AKI) is reversible depending on your health status, age and the severity. Chronic kidney disease means the slow loss of kidney function over time [2].

Kidney failure is the end stage of renal disease when GFR <15ml/min/1.73m^2 and need permanent renal replacement therapy (transplant) or dialysis [3]. Most of kidney failure cases are found in middle and old age people [4].

Chronic kidney failure (CKD) is a major worldwide public health problem with greater burden on the health care system. The disease is rapidly rising in developing country [5]. The major risk factors for CKD include hypertension, diabetes mellitus and obesity [6]. Kidney diseases are associated with a change in various biochemical and hematological parameter [7].

Creatinine is one of biochemical test used to evaluate kidney failure [8].It is generated from creatine by non-enzymatic degradation. Creatinine is produced at a constant rate and is excreted from the body through kidney glomerular filtration. Decreased kidney function can affect the rate at which creatinine is filtered by the kidneys and can be used as a measure of kidney function. Creatinine levels can be affected by changes in muscle mass, pregnancy, or the use of angiotensin inhibitors or angiotensin receptor antagonists [9].

Urea is the major end product of nitrogen metabolism in most animals and is produced in a series of reactions in the liver by the urea cycle. In the urea cycle, ammonia is converted to urea, which is carried by blood to the kidneys for elimination from the body. High levels of urea in the blood may indicate renal failure [10].

The number and function of platelets and white cells may also be affected in CKD resulting in attendant complications such as increase in susceptibility to infections and coagulopathy[11]. Elevated total white cell counts and granulocyte counts are associated with the increased progression of CKD, cardiovascular morbidity, and mortality [12].

Anemia in CKD is mostly due to reduced erythropoietin production from the kidney leading to decreased erythropoiesis. Other factors for anemia include hemolysis, chronic blood loss, nutritional deficiency, inflammation and hyperparathyroidism. These alterations are due to marrow suppression by retained uremic products and aluminum toxicity associated with hemodialysis [24].

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1.2. Statement of the Problem

Kidney disease is a worldwide public health problem. It is associated with various biochemical and hematological abnormalities that leads to death or chronic illness [13, 14].

The major obstacle to manage CKD is, its asymptomatic nature, the disease may not be recognized until end stage, and commonly called a silent killer [15]. Globally, the total cost of the treatment of the milder forms of chronic kidney disease appears to be much greater than the total cost of treating end-stage kidney disease. In 2015, in the United States of America, for example, Medicare expenditures on chronic and end-stage kidney disease were more than 64 billion and 34 billion United States dollars, respectively [16].

Globally, the number of patients receiving renal replacement therapy (RRT) in 2010 was estimated at 2.618 million, with only 7.2% living in low-and-middle-income countries. African patients with end stage renal disease have the lowest access to RRT, with only 9–16% being treated; in central and eastern Africa, the treatment rate is estimated to be as low as 1–3% [17].

Kidney disease is associated with a tremendous economic burden. High income countries typically spend more than 2–3% of their annual health-care budget on the treatment of end-stage kidney disease, even though those receiving such treatment represent under 0.03% of the total population [18].

Kidney disease imposes disproportionate, incalculable human suffering and a catastrophic economic burden in African; less than 2% of the patients with ESRD have access to renal replacement therapy (RRT), making ESRD a death sentence for most patients[19]. According to the WHO data published in April 2011, kidney disease deaths in Ethiopia reached 12,038 (1.47%) of total deaths [20]

Kidney transplantation is the treatment of choice for most patients with end-stage kidney disease (ESKD) because it prolongs survival, improves quality of life and is less costly than the alternative therapy of dialysis. However, even less than 20% of ESKD patient actually receive a transplant [21].

There are no data on public perception of organ donation and transplantation. While large family size and close bonds within extended families favor living donation, misconceptions about the risks of organ donation prevent some patients from getting transplants [22].

A study to assess changes in the hematological profile in chronic kidney disease, showed some hematological parameters were significantly decreased in the PCV and hemoglobin levels and increase in TLC, platelet count in patients with chronic kidney disease [23].

Most of hematological parameters such as hemoglobin (Hb), hematocrit (Hct), Red blood cell (RBC) count, and Total leukocyte count (TLC) and platelet count are deranged in CKD.

There is less data available globaly & no data in our community on blood parameters result and biochemical (BUN, Cr) parameters in chronic kidney patients before & after kidney transplantation

1.3. Significance of the study

The finding of this study will be used for physicians to give the best treatment for a patient. continuous monitoring and assessment of patients in CKD stage and post transplantation is vital, hence assessment of biochemical and hematological parameters as a prognostic markers would have a profound benefit for such patients. Moreover, this research would serve as a reference for future studies and promote large scale studies in St. Paul and else where in general.

2. Literature Review

Renal diseases are associated with a variety of haemopoietic changes. Anemia parallels the degree of renal impairment and its most important cause is failure of renal erythropoietin secretion. Other factors include chronic blood loss, hemolysis and bone marrow suppression by retained uremic factors [25].

Various hematological parameters such as hemoglobin (Hb), hematocrit (Hct), Red blood cell (RBC) count, and total leukocyte count (TLC) and platelet count are deranged in CKD. These alterations are due to marrow suppression by retained uremic products and aluminium toxicity associated with hemodialysis.[26].

The Global Burden of Disease 2015 study also estimated that, in 2015, 1.2 million people died from kidney failure, an increase of 32% since 2005[27]. In 2010, an estimated 2.3–7.1 million people with end-stage kidney disease died without access to chronic dialysis[28]. Additionally, each year, around 1.7 million people are thought to die from acute kidney injury. [29] Overall, therefore, an estimated 5–10 million people die annually from kidney disease[30].Mortality rate of chronic kidney failure patients had increased annually from 1990- 2013, from 409000 per annum to 956000 [31].The mortality rate in the United State alone is 35 000 per annum[32].

The National Kidney Foundation in India states that, kidney diseases rank 3rd amongst the life-threatening diseases after cancer and heart disease. About 200,000 persons landed into terminal kidney failure every year and millions more suffer from lesser forms of kidney diseases [33].

Suresh M *et al.*, reported that Chronic renal failure patients have lower hematological indices, due to impaired production of erythropoietin, and other factors like increase hemolysis, suppression of bone marrow erythropoiesis, hematuria and gastrointestinal blood loss. The concentration of serum creatinine shows negative correlation with all the hematological parameters. And the degree of changes depends on the severity of renal failure [34].

Afshan Zeeshan Wasti et al., reported that mean of RBCs, Hb and PCV were significantly lowered in chronic kidney disease patients and similarly MCH and MCHC indices also decreased significantly [33]. Naghmi Asif et al., reported that among hematological parameters hemoglobin is the most commonly affected [35].

A cross sectional study was performed during a period from August to November 2016, and included 105 CKD patients (55 hemodialyzed patients undergoing conventional maintenance hemodialysis and 50 pre-dialyzed patients) selected from Center of Kidney Diseases Services – Benghazi and 48 age and sexmatched healthy subjects recruited from Alkeesh Polyclinic to serve as controls. . The most frequent cause of CKD among the studied group was hypertension, followed by diabetes mellitus; then glomerular nephritis, and cardiovascular diseases[36].

Mean RBCs count, hemoglobin concentration and hematocrit levels were significantly lower in both groups of CKD patients than normal healthy controls ($p < 0.05$). On the other hand, WBCs count and platelets counts were significantly higher in pre-dialyzed CKD patients compared to hemodialyzed CKD patients ($p < 0.05$). No significant difference in platelet count was seen between pre-dialyzed CKD patients and normal healthy controls ($p= 0.74$). There was a non-significant difference in WBCs count of pre-dialyzed CKD patients or hemodialyzed-CKD patients when compared with control group ($p= 0.052$; $p= 0.09$, respectively). Similarly, platelet counts were significantly unchanged in both CKD groups compared with the control[36].

A cross sectional study was done on Hematological changes in patients of chronic renal failure and the effect of hemodialysis on these parameters showed that the RBC count, hemoglobin levels and platelets counts are significantly reduced in the patients of chronic renal failure and the process of hemodialysis further decreases the level of all the above mentioned hematological parameters whereas there is slight increase in total leucocyte count but significant increase in ESR was detected[36].

Alghythan, et al.,found a significant negative relationship between platelet count and duration of hemodialysis, and they explained their observation by the finding that megakaryocytopoiesis decreases with years on HD; possibly contributing to their annual decreases in platelet counts[37].

3. Objective

3.1. General objective

- To evaluate selected hematological and biochemical profiles before and after kidney transplant at St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia.

3.2. Specific objectives

- To compare selected hematological parameter before and after renal transplant
- To compare selected biochemical parameter before and after renal transplant
- To assess the magnitude of hematological abnormalities among renal transplant

4. Materials and Methods

4.1. Study area

The study was conducted in SPHMMC National Kidney Transplant Centre, Addis Ababa, Ethiopia. The transplant center was established in 2013 under the Federal Ministry of Health in collaboration with University of Michigan. A multidisciplinary team of specialists was developed that included surgery, nephrology, cardiology, radiology, pulmonology, endocrinology and others. The transplant center has three nephrologists, four transplant surgeons, 20 nurses, and other supportive staffs with a functioning 15-bed dialysis unit, a four-bed Intensive Care Unit (ICU), ward, 24 hour working pharmacy and laboratory [19]. It has so far served more than thousand patients.it is governed by a board under the federal minster of health. Until now, 90 living donor kidney transplants have been performed. The program currently accepts candidates who are 14 years and older.

4.2. Study design and period

- Hospital based retrospective cohort study design was conducted from November to May, 2021.

4.3. Source population

- All patients with end-stage renal disease and on- follow up at the institution

4.4. Study Population

- All patients with end-stage renal disease and selected for renal replacement therapy as well as those who fulfill the Eligibility criteria

4.5. Inclusion and exclusion criteria

4.5.1. Inclusion criteria

- All Patients with end-stage renal failure on renal replacement therapy

4.5.2. Exclusion criteria

- Pregnant women, hematological malignancy.

4.6. Study variables

4.6.1. Dependent variables

- Hematological parameter
- Bio-chemical parameter
    - Urea (BUN)
    - Creatinine

4.6.2. Independent variables

- Age
- Sex
- Residence place
- Educational status
- Marital status
- Duration of disease
- Relationship with the Donor
- Vital sign (Blood pressure)
- Anthropometric measurements
- HTN
- DM

4.7. Sample size calculation and Sampling method

4.7.1. Sample size calculation

In this study all kidney recipients who had their selected hematological and biochemical profile performed before and after transplant from January 2020 to March 2021 were recruited. Seventy two end stage kidney disease (ESRD) patients were waiting to get a renal transplant in the starting of transplant service. However, due to the Covid 19 epidemic most patient were unable to access the services which reduced the sample size to 30.

5.7.2. Sampling Method

- Convenience sampling method was used to recruit study participants.

4.8. Measurement and Data collection

4.8.1. Data collection procedure

In this study, both primary and secondary data were collected from patient's card by the kidney transplant (OPD) nurses. The first data sources were baseline information about each patient like patient age; current medications, educational status, vital sign, anthropometric data medical and medication histories were recorded using data extraction format through reviewing patient's medical chart and blood samples were collected under aseptic conditions. For secondary data collection, a standardized checklist was design to extract all necessary data results from the registration book, based on the variables found in the patient registration book.

Specimen collection and procedure

Approximately **9**ml of whole blood was collected from patients before and after transplant. First, about 4 ml of whole blood was collected into EDTA vacutainer test tube to evaluate hematological parameters. Then 5ml of whole blood was collected into serum separator test tube (SST) for biochemical tests.

4.8.2. Laboratory analysis

Beckman coulter UniCel® DxH 800 for Hematological Analysis

Test principle

Coulter Method (impedance)

Accurately counts and sizes cells by detecting and measuring changes in electrical resistance when a particle (such as a cell) in a conductive liquid passes through a small aperture. Each cell suspended in a conductive liquid (diluent) acts as an insulator. As each cell goes through the aperture, it momentarily increases the resistance of the electrical path between the submerged electrodes on either side of the aperture. This causes a measurable electronic pulse. For counting, the vacuum used to pull the diluted suspension of cells through the aperture must be at a regulated volume. While the number of pulses indicates particle count, the size of the electrical pulse is proportional to the cell volume.

COBAS 311 fully automated clinical chemistry analyzer

1. Urea test principle

Urea was measured by diacetyl monoxime colorimetric method and Berthelot reaction. In this method the urea was converted to ammonia by an enzyme called urease. The ammonia produced was combined with 2-oxoglutarate and NADH in the presence of glutamate dehydrogenase (GDH), which yields L- Glutamate and NAD. The decrease in NADH absorbance is proportional to the urea concentration.

$$\text{Urea} + 2H_2O \xrightarrow{\text{Urease}} 2NH_4^+ + CO_3^{2-}$$

The ammonia formed then reacts with α- ketoglutarate and NADH in the presence of GLDH to yield glutamate and NAD+.

$$\text{2-Oxoglutarate} + NH_4 + \text{NADH} \xrightarrow{\text{GLDH}} \text{L-glutamete} + \text{NAD}^+ + H_2O.$$

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

2. Creatinine test principle

Creatinine was estimated by the Jaffe reaction, a calorimetric procedure in which creatinine forms a yellow orange complex in alkaline solution with picric acid. This colored complex will be determined photometrically. The intensity of produced colored is directly proportional to the amount of creatinine in the sample.

$$\text{Creatinine} + \text{Picric acid} \xrightarrow{\text{alkaline PH}} \text{Yellow-orange complex}$$

In solution creatinine forms a yellow orange complex with picrate. The rate of dye formation is proportional to the creatinine concentration in the specimen.

4.9. Data Quality Assurance

4.9.1 Pre-analytical

Continuous monitoring and orientation of participants on analytical results must reflect all of the pre-analytical and analytical variables that can influence test results. Therefore, all pre-analytical factors, including patient preparation, sample collection and processing, the analytical method, and instrumentation (daily, weekly and monthly preventive maintenances) checkup, must be carefully defined and used for testing. Therefore, samples were collected and processed according to the standard operating procedure (SOP) and protocols.

4.9.2 Analytical

The reliability of the data generated is critical, because both the imprecision and inaccuracy of the method would determine its diagnostic utility. Therefore, in-house or commercial quality control materials was used in the same form at as for patient testing, which not only monitors the analytical protocol used during the process. The participants sample was analyzing after both controls are passed and interpreted using Westgard multi-rule algorithm. Hematology after run complete blood count and peripheral morphology through the whole processes of laboratory works.

4.9.3 Post-analytical

The results was recorded and handled appropriately and secured. In data entry process two persons was involved including principal investigator, after checking for completeness and cleaning. Processing and analysis of the data obtained from laboratory analyses of the blood samples and questionnaires was performed by coding and entering the data into SPSS software version 25 package and the different variables were be tested and analyzed.

4.10. Data analysis and interpretation

Statistical Package for Social Sciences (SPSS) Version 26 software was used for statistical analyses. Descriptive data was summarized using proportions, means, medians (interquartile range) and standard deviation (± SD) as appropriate and compared across using Wilcoxon signed rank test , to evaluate differences before and after transplant for Hematological and Bio-chemical test, to evaluate if the linear trend or ordering increased or decreased across various variables. For continuous variables Pearson's correlation analysis was used to find the interrelation between the various hematological biochemical parameters after and before transplant. P value less than 0.05 will be considered as statistically significant.

4.11. Ethical considerations

Ethical clearance was obtained from Research and Ethics Review Committee of department of Medical Laboratory Science, College of Health Science, Addis Ababa University. Participants were requested to give assent and informed their right to refuse to participate or withdraw from the study at any time. Confidentiality of the data was also maintained throughout the study.

4.12. 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. Result

Part one: - Socio demographic characteristics of study participant

A total of 30 patients had received renal transplant within the study period. Of them, the majority 21 (70%) were males. The mean age of patients was 30.5 years. Most of the participants, 13(43.4%) were within the age group of 18-27 years, Half of the participants have at least diploma level of education and 13(43.3%) of them were unemployed (Table 1)

Table 1: Socio-demographic characteristics of study participants (n=30), attending St Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia.

Variable		Frequency	Percent
Sex	Male	21	70
	Female	9	30
Age	Below 18 years	2	6.6
	18-27	13	43.4
	28-37	8	26.7
	38-47	5	16.7
	48-57	2	6.6
Educational status	Non formal	1	3.3
	Elementary	2	6.7
	Secondary	12	40.0
	Diploma and above	15	50.0
Occupational status	Government employed	7	23.3
	Non-Government employed	10	33.3
	Non-employed	13	43.3
Total		**30**	**100.0**

Part two: - Medical history of participant

This study indicated that majority of the kidney donors were blood related relatives with the patients which accounted for 80% of the donation. In addition the major medical diagnoses were ESRD and HTN, with 40% and 33.3% respectively (table 2).

Table 2: -Shows frequency distribution medical history of participant of study participants attending at St. Paul's Millennium Medical College (n=30), Addis Ababa, Ethiopia,

Variable	Respond	Frequency	Percent
Type of donor	Blood related	24	80
	Spouse	6	20
Current diagnosis	ESRD	10	33.3
	ESRD& HTN	12	40
	ESRD& DM	2	6.7
	ESRD, HTN & DM	3	10
	ESRD, HTN, DM & Cardiac	1	3.3
	Other	2	6.7
	Total	**30**	**100.0**

ESRD: End stage renal disease, HTN: Hypertension DM: Diabetis Millitis

Part three: - Anthropometric measurement data

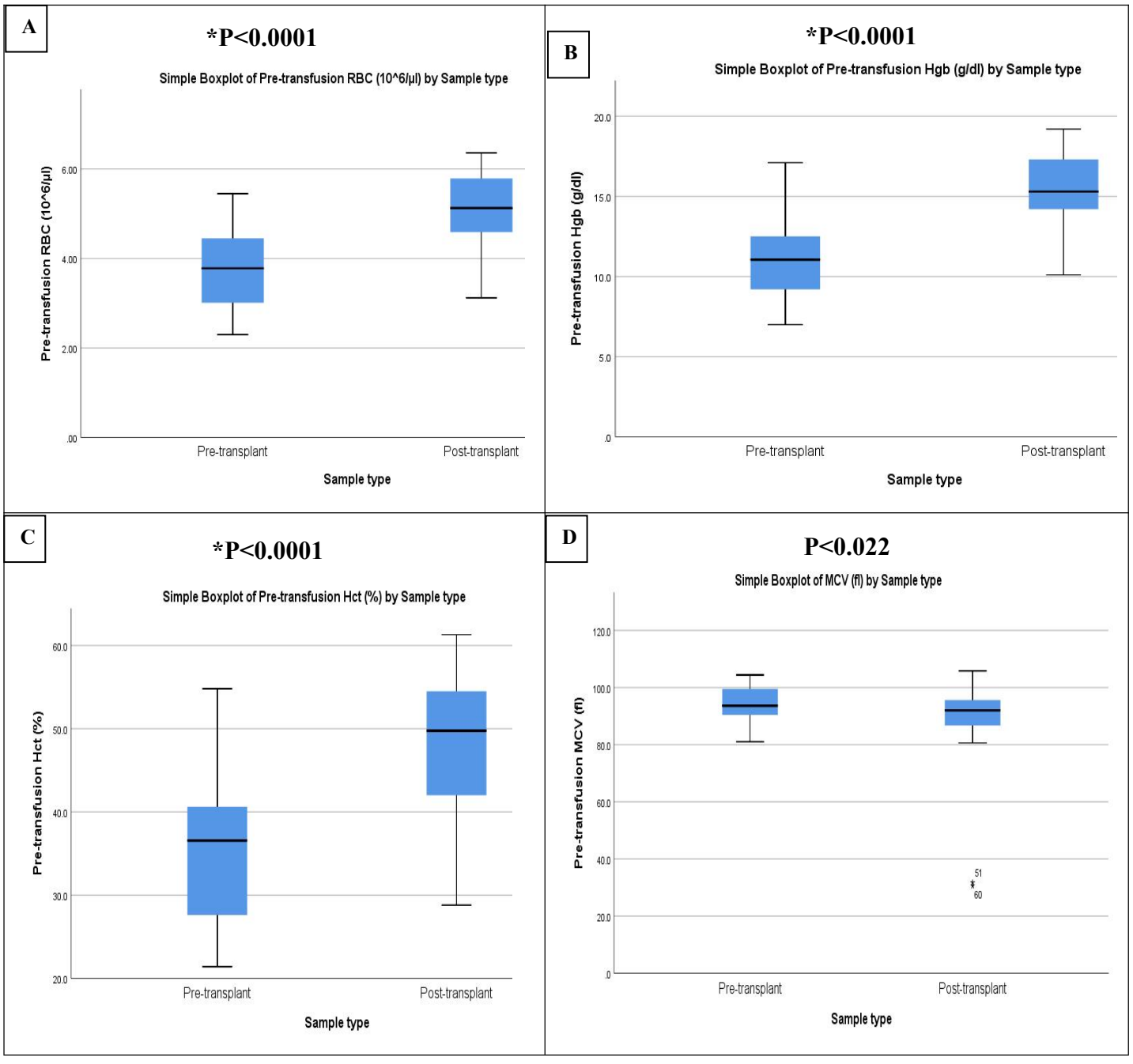
The height of study subjects were 1.65±0.9 meters ranging between 1.48-1.80 meters and the body weight was 62.6±14.8 kilogram with a range of 36.9-89.9 kilogram before kidney transplant and after kidney transplant 67±13.5 kilogram with a range of 44.5-89.9. The body mass index (BMI) showed a mean ± SD value of 22.7±3.9Kg/m^2 varying between 16-30.4 kg/m^2 , was higher pre-transplant than post-transplant value of study participant as illustrated in table 3.

Table 3: - Anthropometric measurement and blood pressure status of study participants (n=30), attending at St. Paul's Millennium Medical College, Addis Ababa, Ethiopia, February, 2021 G.C.

Variables	Pre-transplant (n=30)			Post-transplant (n=30)		
	Mean± SD	Range	Median	Mean± SD	Range	Median
Diastolic blood pressure (mmHg)	83.4(11)	100-160	140	81.3(8.1)	40-60	80
Systolic blood pressure (mmHg	133.6(17.2)	70-100	80	127(14.9)	60-100	130
Weight (kg)	62.6(14.8)	36.9-89.9	64.7	67(13.5)	44.5-89.9	65.4
Height (cm)	165(0.9)	148-180	167	165(0.9)	148-180	167
BMI (kg/m^2)	22.7(3.9)	16-30.4	23.1	24.1(4.1)	16.0-30.45	24.8

Part four: - Selected Hematological& Bio-chemical parameter data

The comparison of pre- & post-transplant with hematological & bio-chemical parameters, using Non-parametric dependent-Samples Wilcoxon signed rank test. Pre & post-transfusion RBC count value mean 3.78 & 5.1 ($p < 0.0001$), Hgb mean 11.2 & 15.2 relatively ($p < 0.0001$), HCT mean value 35.2 & 48.9 relatively ($p < 0.0001$), MCV mean value 93.8 & 88.3 relatively (p=0.022), Monocyte mean value 1.5 & 0.89 relatively ($p < 0.0001$), creatinine mean value 7.9 & 1.4 respectively ($p < 0.0001$) & urea mean value 103.7 & 30.2 respectively ($p < 0.0001$) positive correlation between pre and post-operative procedure was observed.



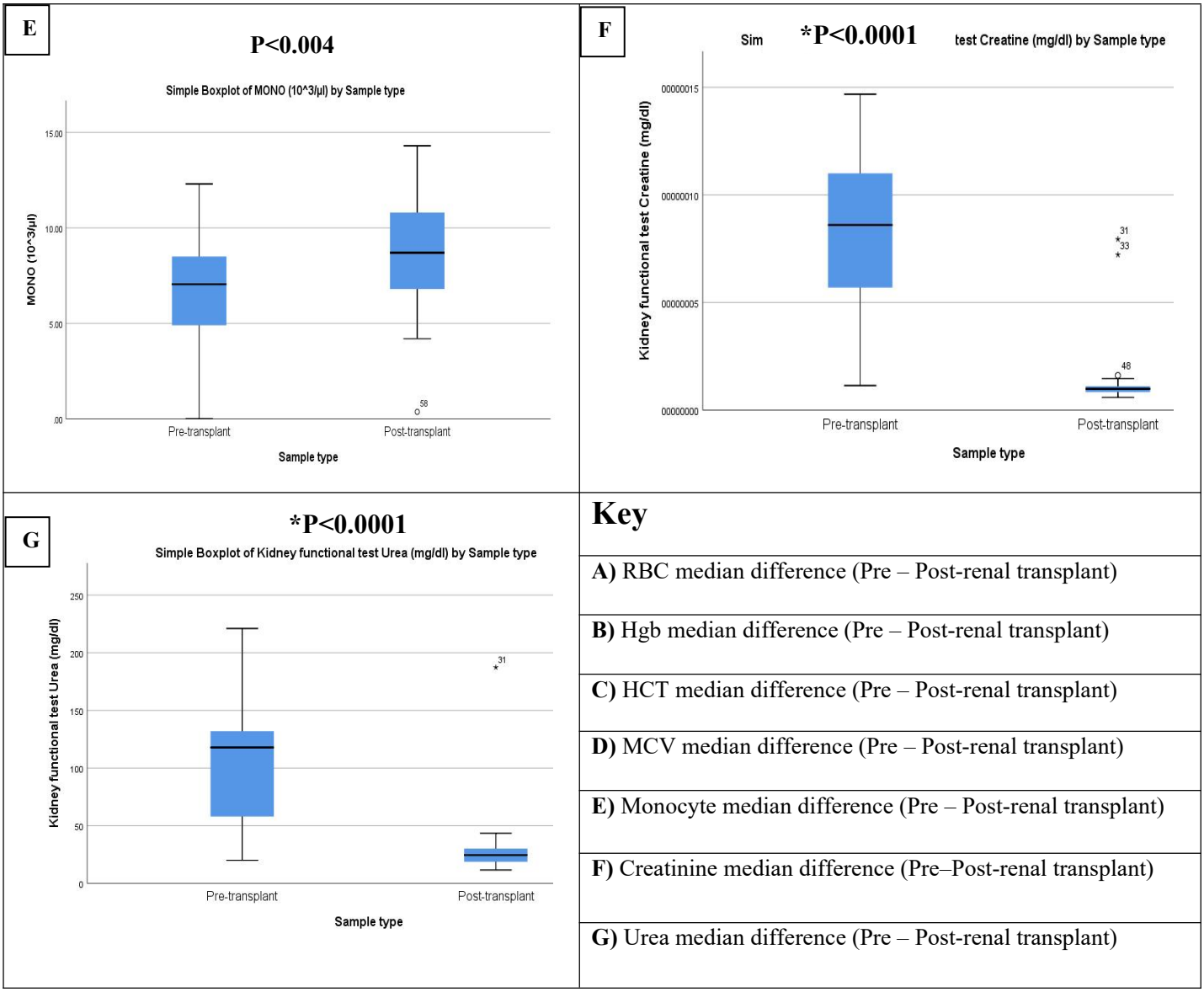


Figure 1: - box plot for pre and post kidney transplantation of selected hematological &bio-chemical profile participants (n=30), attending at St. Paul's Millennium Medical College, Addis Ababa, Ethiopia, February, 2021. The line in the middle represents median, the boxes interquartile range and the whiskers range of values for 30 patients. P value is from Wilcoxon signed rand test.

Table 4: - Median & Range of selected hematological & bio-chemical parameter of study participants (n=30), attending at St. Paul's Millennium Medical College, Addis Ababa, Ethiopia, February, 2021

Variables	Pre-transplant(n=30)		Post-transplant (n=30)		p-value Pre & Post
	Range	Median	Range	Median	
WBC (10^3/µl)	2.54 - 24.82	6.6	2.1-11.3	7.2	0.951
RBC (10^6/µl)	2.30 - 5.45	3.78	6.36-5.1	5.1	**0.0001***
Hgb (g/dl)	7.0 - 17.1	11.1	10.1-19.2	15.3	**0.0001***
HCT (%)	21.4-54.8	36.5	28.8-93.7	49.7	**0.0001***
MCV (fl)	81-104.4	93.6	30.6-105.8	92.0	**0.022***
MCH(Pg)	25.5-34.1	29.9	24.5-93.7	30.3	0.600
MCHC(g/dl)	11.7-36	32.2	29.7-36.2	32.0	0.159
RDW	11.4-164	13.4	11.8-16.1	13.5	0.365
RDW-SD (fl)	8.2-57	47.4	41.4-58.8	45.5	0.943
PLT (10^3/µl)	34-517	218.0	102-561	217.0	0.829
MPV(fl)	6.5-59.5	8.0	7.5-10.2	8.6	0.110
Neutrophil (10^3/µl)	23.3-94.9	63.6	7.87-83.1	61.0	0.084
Lymphocyte (10^3/µl)	3.00-52.50	21.7	2.01-69	23.9	0.052
Monocyte (10^3/µl)	0.01-17.60	7.05	0.37-21.85	8.7	**0.004***
Eosinophils (10^3/µl)	0.00-33.80	3.1	0.10-22	1.75	0.294
Basophil (10^3/µl)	0.00-4.81	0.20	0.00-1.3	0.10	0.542
Neutrophil (%)	1.0-21.7	4.5	0.6-9.1	4.5	0.453
Lymphocyte (%)	0.3-4.3	1.5	0.8-4.4	1.79	0.206
Monocyte (%)	0.1-10.6	0.46	0.3-7.1	0.57	0.619
Eosinophils (%)	0.0-4.3	0.20	0.0-1.0	0.12	0.261
Basophil (%)	0.0-0.2	0.01	0.0-0.1	0.00	0.953
Creatinine (mg/dl)	1.0-15.0	8.6	0.59-7.94	0.99	**0.000***
Urea (mg/dl)	20-221	117.9	11.6-187.3	24.5	**0.000***

Dependent-Samples Wilcoxon signed rank test; ***p values* < 0.05: statistically significant different Comparison of this study before and after renal transplant with current implementation in Ethiopia for decision making hematological abnormalities, lower hematologic parameters indicated significantly different reference value for RBC, Hgb, HCT, & MPV. Other hematological parameter almost similar reference interval value was observed. Blood chemistry analysis was significantly different on creatinine & urea value with current implementation reference value shown table 5.

Correlations of hematological parameter with biochemical parameter of study participants

The correlation of Biochemical markers (Urea & Creatinine) with Pre and Post-transplant hematological parameters, using Pearson correlation, indicated that no correlation in pre-transplant of Biochemical markers (Urea & Creatinine) with Pre-transplant hematological parameters with no statistical significance. But post-transplant value moderate negative correlation in post-transplant creatinine & urea value and red blood cells count with statistical significance (urea: r= -0.420; p=0.021, Creatinine: r= -0.583; p=0.001). Similarly moderate negative correlation in creatinine was observed for hemoglobin amount (creatinine: r= -0.558; p= 0.001). Related to hematocrit, moderate negative correlation with creatinine was observed (creatinine: r= -0.412; p= 0.024).

Table 5: Comparison of hematological values with current laboratory using Beckman coulter UniCel® DxH 800, Addis Ababa, Ethiopia, February, 2021 G.C.

Variables	Currently, lab using	Pre-transplant (n=30)	Post-transplant (n=30)
		Mean± SD	Mean± SD
WBC (10^3/µl)	3.67-11.43	7.53(4.2)	7.1(2.2)
RBC (10^6/µl)	3.00-6.00	3.78(0.9)	5.1(0.8)
Hgb (g/dl)	12.0-18.0	11.2(2.5)	15.2(2.4)
HCT (%)	36.0-50.0	35.25(8.2)	48.9(12.0)
MCV (fl)	85.0-105.0	93.8(6.1)	88.3(16.4)
MCH(Pg)	27.0-36.0	30.1(2.1)	32.2(11.7)
MCHC(g/dl)	32.0-36.0	31.4(4.0)	32.6(1.94)
RDW	11.0-16.0	19.9(7.9)	13.5(1.23)
RDW-SD (fl)	37.0-54.0	45.5(9.6)	47(4.3)
PLT (10^3/µl)	140-450	231(103.7)	231.8(91.5)
MPV(fl)	9.0-13.0	9.93(9.4)	8.65(0.65)
Neutrophil (10^3/µl)	1.27-7.45	5.1(4.1)	4.5(1.9)
Lymphocyte (10^3/µl)	1.08-3.95	1.73(0.88)	1.8(0.73)
Monocyte (10^3/µl)	0.26-0.95	1.5(0.9)	0.898(0.4)
Eosinophils (10^3/µl)	0.03-0.83	0.48(0.86)	0.22(0.27)
Basophil (10^3/µl)	0.01,-0.08	0.023(0.04)	0.009(0.02)
Neutrophil (%)	26.5-75.7	64.4(15.2)	57.4(16.6)
Lymphocyte (%)	14.7-58.5	22.4(10.9)	28.1(13.7)
Monocyte (%)	4.4-13.4	7.1(3.2)	9.4(4.8)
Eosinophils (%)	0.30-9.75	5.3(6.7)	4.1(5.7)
Basophil (%)	0.1-1.2	0.5(1.12)	0.36(0.39)

Assessment of magnitude of hematological abnormalities among renal transplant

From the total 30 participants, anemia (25/30), neutrophilia (10/30), and thrombocytopenia (pre-transplant 25/30 & post-transplant 10/30) were observed compare with current laboratory using reference range value. More than half, (pre-transplant 30/30 & post-transplant 20/30) of the study participants had creatinine value 0.7-1.3mg/dl. Majority of them (pre-transplant 30/30 & post-transplant 20/30) of the study participants had urea value 14-23mg/dl.

Table 1:- Hematological and bio-chemical related characteristics of pre and post-transplant renal patients, Addis Ababa, Ethiopia, 2021.

Hematological abnormalities	Status	
	Pre-transplant	**Post-transplant**
Anemia		
Yes	25	-
No	5	-
Neutrophilia		
Yes	10	-
No	20	-
Thrombocytopenia		
Yes	25	10
No	5	20
Bio-chemical marker (Creatinine)		
High	30	20
Normal	-	10
Bio-chemical marker (Urea)		
High	30	-
Normal	-	30

6. Discussion

To our knowledge, this study is the first study on assessing selected hematological and biochemical profiles before and after kidney Transplant. Hematological abnormalities are usually evident/manifested in CKD stages of 3-5 i.e. estimated glomerular filtration rate (eGFR.) of 30 to 60 mL/minute as stage three; GFR 15 to 30 mL/minute as stage four; and GFR less than 15 mL/minute as stage five of CKD. In stage five level of serum creatinine is greater than 5.0 mg/dl in men, and greater than 4.0 mg/dl in women. [44].

In chronic renal disease, impaired erythropoietin secretion leads to a decrease in platelet count [38]. The detection of receptors for erythropoietin in megakaryocytes is understandable, because erythropoietin levels can affect platelet level and because of extensive homology between erythropoietin and thrombopoietin, erythropoietin act as the major humoral regulator of platelet mass.

Mean RBCs count, hemoglobin concentration and hematocrit levels were significantly lower in CKD patients then those provided by the instrument. On the other hand, WBCs count and platelets counts were significantly higher in pre-dialyzed CKD patients compared to hemo-dialyzed CKD patients ($p < 0.05$) [35]. In this study significant differences were observed for RBC count ($p= 0.000$), Hgb ($p= 0.000$), HCT ($p= 0.000$), Monocyte ($p=0.000$), creatinine ($p= 0.000$) & urea ($p= 0.000$) before and after kidney transplant. Renal diseases are associated with a variety of hematopoietic changes. Anemia parallels the degree of renal impairment and its most important cause is failure of renal erythropoietin secretion. Other factors include chronic blood loss, hemolysis and bone marrow suppression by retained uremic factors [24].

The primary cause of anemia in patients with CKD is insufficient production of erythropoietin by the diseased kidneys. Other factors include iron, folate & B12 deficiency due to nutritional insufficiency or increased blood loss [39]. acute and chronic inflammation with impaired iron utilization, severe hyperparathyroidism with consequent bone marrow fibrosis and shortened red cell survival in the uremic environment [40].

Chronic kidney disease is characterized by continuous reduction in glomerular filtration rate (GFR) that leads to accumulation of various nitrogenous metabolites in the blood including urea and creatinine.

Creatinine is generated from creatine with the aid of non-enzymatic dehydration. Creatinine is synthesized in our body at a continuous manner and is excreted from our body through kidney glomerular filtration unit. Reduced kidney function can have an effect on the rate at which creatinine is filtered by means of the kidneys and may be used as a measure of kidney function. Due to inability of kidney to clear creatinine through urine excretion, kidney function deteriorates which results in increased levels of creatinine in blood serum. Urea is the major end product of protein catabolism in maximum animals and is produced in a sequence of reactions in the liver referred to as the urea cycle. In the urea cycle, ammonia is transformed to urea, which is carried by means of blood to the kidneys for its elimination from the body. Elevated levels of urea within the blood may additionally indicate renal failure [41].

Accumulation of urea and creatinine concentrations in blood of CKD patients leads the body very un-wellness excreted from the blood streams through kidneys [42].

The results show statistically ($P< 0.05$) for urea and creatinine concentration after kidney transplant patients when compared with those before kidney transplant.
Overall, before kidney transplant ESRD was affected blood concentration or abnormal hematological and biochemical value, and also after the transplantation it has returned to a normal reference value for hematological and biochemical kidney marker (urea & creatinine) value. This result indicates that kidney transplantation is an effective mechanism or solution to ESRD management.

7. Strength and limitation of the study

7.1 Strength of the study

- Up to the our literature search goes this study is the first in Ethiopia and it provides baseline information for further study and policy makers.

7.2. Limitation of the study

- The major limitation of the present study was the small sample size.
- There is no any previous study directly done on this study to compare our findings in this country .

8. Conclusion and Recommendation

8.1. Conclusion

- kidney transplant recipient patients had an improved hematological (RBC parameters) and biochemical (creatinine and urea) profiles post transplantation.
- There was different degree of abnormality in hematological and biochemical parameter before and after kidney transplant that require carefull evaluation and management.

8.2. Recommendation

- A longitudinal follow up of kidney recipient patients would be highly valuable to better understand the effect of drug taking for draft or prophylactic drug effect on hematological parameters translate this finding into clinically applicable results. Moreover, the long term outcome of the transplantation and the biochemical and hematological profile should be monitored at a certain interval.

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Annexes

Annex I: English version of Participants' information sheet

Principal Investigator: Endale Haile

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

Name of the sponsors: AAU School of Graduate studies.

Aim: Assessment of selected Hematological and Biochemical Profiles(urea and creatinine) among Patients With Kidney Failure Before And After kidney Transplant in St. Paul hospital kidney transplantation center Addis Ababa, Ethiopia.

Study Participants: In this study to assess selected Hematological and Biochemical Profiles(urea and creatinine) among Patients With Kidney Failure Before And After kidney Transplant who fulfill inclusion criteria will participate.

Sample require: if you agree to participate in the study, you are requested to provide about 3 ml of venous blood, before and after kidney transplant.

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.

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.

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:

You may contact me at

- E-mail address endaleblen@yahoo.com or
- Mobile +251913387509

Department of Medical Laboratory Science research ethics office

Tell: +251 11 275 5170

Annex II: Participant information sheet (Amharic version) የ ተ ሳ ታ ፊ ዎ ች

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መ ግ ቢ ያ

በ አ ዲ ስ አ በ ባ ዩ ኒ ቨ ር ሲ ቲ ጤ ና ሳ ይ ን ስ ኮ ላ ጅ የ ሕ ክ ምና ላ ቦ ራ ቶ ሪ ት /ክ ፍ ል በ ማ ስ ተ ር ስ ድ ግ ሪ ተ ማ ሪ የ መ መ ረ ቂ ያ ጥ ና ት ላ ይ እ ዲ ሳ ተ ፉ ተ ጋ ብ ዘ ዋ ል ፡ ፡ እ ባ ክ ዎ በ ዚ ህ ጥ ና ት ለ መ ሳ ተ ፍ ከ መ ስ ማ ማ ት ዎ በ ፊ ት ከ ዚ ህ ቀ ጥ ሎ የ ሚ ገ ኝ ዉ ን ም ን ባ ብ በ ጥ ሞ ና ያ ን ብ ቡ ና ግ ሌ ጽ ያ ሌ ሆ ነ ሌ ዎ ት ን ማ ን ኛ ዉ ም ሃ ሳ ብ ይ ጠ ይ ቁ ፡ ፡

የ ጥ ና ቱ ር ዕ ስ : **Assessment of Selected Hematological and Biochemical Profiles (urea and creatinine) among Patients with Kidney Failure before and After kidney Transplant in St. Paul hospital kidney transplantation center Addis Ababa, Ethiopia.**

የ ጥ ና ቱ ባ ለ ቤ ት ፡ - Endale Haile (BSc, MSc candidate)

እ ና ም እ ር ስ ዎ በ ዚ ህ ጥ ና ት ለ መ ሳ ተ ፍ ጠ ቀ ሚ ና ም ቹ ሆ ነ ዉ ተ መ ር ጠ ዋ ል ፡ ፡ የ እ ር ስ ዎ በ ዚ ህ ጥ ና ት ላ ይ የ ሚ ኖ ር ዎ ት ተ ሳ ት ፎ ሙ ሉ በ ሙ ሉ በ በ ጎ ፈ ቃ ደ ኝ ነ ት ላ ይ የ ተ መ ሰ ረ ተ ነ ዉ ፡ ፡ በ ዚ ህ ጥ ና ት ዉ ስ ጥ ሊ ለ መ ሳ ተ ፍ ወ ይ ም ለ መ ሳ ተ ፍ ከ ወ ሰ ኑ በ ኃ ላ ለ ማ ቋ ረ ጥ የ ሚ ወ ስ ኑ ቢ ሆ ን ም እ ን ኩ ዋ ን በ ዚ ህ ሆ ስ ፒ ታ ል የ ሚ ሰ ጠ ዉ ማ ን ኛ ዉ ም አ ገ ል ግ ሎ ት አ ይ ቋ ረ ጥ ም ፡ ፡ በ ጥ ና ቱ ለ መ ሳ ተ ፍ የ ሚ ስ ማ ሙ ከ ሆ ነ የ ስ ምምነ ት ቅ ጹ ላ ይ በ ጹ ሁ ፍ ወ ይ ም በ ጣ ት ፊ ር ማ ማ ስ ቀ መ ጥ ይ ጠ በ ቅ ብ ዎ ታ ል ፡ ፡

የ ጥ ና ቱ ተ ሳ ታ ፊ ለ መ ሆ ን የ ሚ ጠ በ ቅ ብ ዎ ት ም ን ድ ን ነ ው?

በ ዚ ህ ጥ ና ት ለ መ ሳ ተ ፍ የ ሚ ስ ማ ሙ ከ ሆ ነ የ ደ ም ና ሙ ና እ ን ደ ሚ ወ ሰ ድ ና ለ ጥ ና ቱ እ ን ዲ ሚ ዉ ል መ ስ ማ ማ ት ይ ጠ በ ቅ ብ ዎ ታ ል ፡ ፡ ከ ተ ወ ሰ ደ ዉ ና ሙ ና ላ ይ የ ሚ ገ ኙ መ ረ ጃ ዎ ች ከ ዚ ህ ሆ ስ ፒ ታ ሌ ዉ ጭ ለ ሚ ገ ኙ ና ለ ስ ራ ዉ አ ግ ባ ብ ነ ት ላ ላ ቸ ዉ ሰ ዎ ች

ቢነገር የማይቃወሙመሆኑን መስማማት ይጠበቅብዎታል፡፡ ይሁን እንጅ ይህ አይነቱ መረጃ የርስዎን ማንነት የሚገልጡ መረጃዎችን ማለትም ስም፤ አድራሻና የስሌክ ቁጥር የመሳሰሉትን መረጃዎችን አይጨምርም፡፡ ይሌቁንም ለዚህ አገሌግልት ብቻ የሚውሌ አርስዎን ለማወቅ የሚያስችል መለያ ቁጥር ጥቅም ሊይ እንዲዉሌ ይደረጋል፡፡ በተጨማሪም ስለርስዎ አጠቃሊይ የጤና ሁኔታ የሚቀርቡ አንዳንድ ተጨማሪ ጥያቄዎች መልስ መስጠት ይጠበቅብዎታል፡፡

በዚህ ጥናት መሳተፍ የሚያስከትሊቸዉቸግሮች ምንድን ናቸዉ?

ናሙና በሚሰበሰብበት ወቅት ምንም አይነት የከፋ ችግር አያጋጥምዎትም፡፡ ነገር ግን ደምሲወሰድ መጠነኛ የህመም ስሜት ሉያስከትል ይችሊል፡፡ ሆኖም ግን ናሙናዉን ለመሰብሰብ ልምድ ያለዉ ባለሞያ ስለሚመደብና አስፈሊጊዉ የጥንቃቄ እርምጃ ስለሚወሰድ የህመም ስሜት አይኖርም፡፡

የህክምና መረጃ በሚስጥር ተጠብቆ መቆየት የሚችሇዉእንዴት ነዉ?

ስለራስዎ የሰጡት ማንኛዉም መረጃና ከተወሰደዉ ናሙና ላይ የተገኘዉ የሊቦራቶሪ ዉጤት የሚዉሇዉሇጥናቱ አሊማብቻነዉ፡፡ ይህን ማህደር ሊያገኙ የሚችሉት የተወሰኑ የጥናቱ ተባባሪ ሰዎች ብቻ ናቸዉ፡፡ ከዚያም በላይ ስለእርስዎ ያለዉን ማንኛዉንም መረጃ የተለየ የይለፍ ቃሌ ባለዉ የኮምፒውተር የመረጃ ማህደር ዉስጥ እንዲቀመጥ ይደረጋል፡፡

በዚህ ጥናት መሳተፍ የሚያስገኛቸዉጥቅሞች ምንድን ናቸዉ?

ይህ ጥናት የማስተርስ ዲግሪ መመረቂያ እንደመሆኑ መጠን በዚህ ጥናት በመካፈልዎ በገንዘብ የሚያገኙት ጥቅምባይኖርምከጥናቱ በሚገኝዉዉጤት

ግን ተጠቃሚነዎት፡ ፡ የእርሶዎ ተሳትፎ የእርስዎንና የወገንዎትን የኩሊሉት ህመም መመርመርና ለማከታተል ከፍተኛ ጥቅም ይኖረዋል፡ ፡

በዚህ ጥናት ተሳታፊ የመሆንዎ ጫቶች 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 አገልግልት አይኖርም፡ ፡ ከዚህም በተጨማሪ ጥናቱን በተመለከተ ማንኛውንም አይነት ጥያቄ የመጠየቅና ገለጻ የማግኘት መብት አለዎት፡ ፡ የላብራቶሪ ምርመራ ውጤቱንም በነጻ ማግኘት ይችላሉ፡ ፡ ነገር ግን እርስዎ በሚሰጡን መረጃ የችግሩን ስፋት ለመከላከል እና ለመቆጣጠር ጠቃሚስለሆነ ለሚቀርብሌዎት ጥያቄ ቀጥተኛ መልስ ይሰጡን ዘንድ በታላቅ አክብሮት እንጠይቃለን፡ ፡

ጥያቄ ካለኝ ወይምችግር ቢያጋጥመኝ ምን ማድረግ ይገባሌ?

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

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Annex III: 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; **Assessment of selected Hematological and Biochemical Profiles(urea and creatinine) among Patients With Kidney Failure Before And After kidney Transplant in St. Paul hospital kidney transplantation center Addis Ababa, Ethiopia.**

I have been requested to provide 6 ml of venous blood, before and after transplant. 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

የ ተ ሳ ታ ፊ ዎ ች ስ ም ም ነ ት ማ ረ ጋ ገ ጫ

የ ሚ ስ ጥ ር ቁ ጥ ር ----------

የ ተ ሳ ታ ፊ ዉ ስ ም ----------እ ድ ሜ----------

እ ኔ ስ ሜ ከ ላ ይ የ ተ ጠ ቀ ሰ ው ተ ሳ ታ ፊ “**Assessment of selected Hematological and Biochemical Profiles(urea and creatinine) among Patients With Kidney Failure Before And After kidney Transplant in St. Paul hospital kidney transplantation center Addis Ababa, Ethiopia.**

”ጥ ና ት ላ ይ በ ቂ ገ ለ ጻ ተ ደ ር ጎ ል ኛ ል ፡ ፡ ለ ጥ ና ቱ ም የ ደ ም ና ሙ ና እ ን ደ ሚ ያ ስ ፈ ል ግ ተ ገ ል ጾ ል ኛ ል ፡ ፡ የ ጥ ና ቱ ን ም አ ላ ማ ዎ ች ተ ረ ድ ቻ ለ ሁ ፡ ፡ በ መ ጠ ይ ቁ ላ ይ የ ገ ለ ጽ ኳ ቸ ው መ ረ ጃ ዎ ች በ ሙ ሉ በ ሚ ስ ጥ ር የ ተ ጠ በ ቁ እ ን ደ ሚ ሆ ኑ ተ ነ ግ ሮ ኛ ል ፡ ፡ በ ጥ ና ቱ ላ ይ ያ ለ መ ሳ ተ ፍ ና ማ ን ኛ ው ን ም መ ረ ጃ ያ ለ መ ስ ጠ ት እ ን ዲ ሁ ም በ ማ ን ኛ ው ም ጊ ዜ ከ ጥ ና ቱ ራ ሴ ን የ ማ ግ ለ ል መ ብ ቴ የ ተ ጠ በ ቀ እ ን ደ ሆ ነ ተ ገ ል ጾ ል ኛ ል ፡ ፡

ስ ለ ዚ ህ ለ ዚ ህ ጥ ና ት መ ረ ጃ ና የ ስ ም ም ነ ት ቃ ሌ ን የ ሰ ጠ ሁ ት በ አ ጠ ቃ ላ ይ ሁ ኔ ታ ው ን በ መ ረ ዳ ት ና በ ፍ ጹ ም ፍ ቃ ደ ኝ ነ ት ነ ው፡ በ ተ ጨ ማ ሪ ም ጥ ያ ቄ ለ መ ጠ የ ቅ ተ ፈ ቅ ዶ ል ኝ ለ ማ ወ ቅ የ ፈ ለ ኩ ት ን ያ ህ ል ማ ብ ራ ሪ ያ አ ግ ኝ ቻ ለ ሁ ፡ ፡ የ ዚ ህ ጥ ና ት ተ ሳ ታ ፊ በ መ ሆ ኔ የ ማ ገ ኘ ው ጥ ቅ ም የ ሁ ለ ን ም ም ር መ ራ ው ጤ ት በ ነ ጻ ማ ግ ኘ ት እ ን ደ ሆ ነ ተ ረ ድ ቻ ሁ ሁ ፡ ፡

በ አ ጠ ቃ ላ ይ እ ኔ ከ ላ ይ በ መ ተ ማ መ ኛ ቅ ፅ የ ተ ጠ ቀ ሱ ት ን ሁ ሉ በ ሚ ገ ባ ና በ ተ ረ ጋ ጋ መ ን ፈ ስ አ ን ብ ቤ ዋ ለ ሁ ኝ ፡ ፡ ስ ለ ዚ ህ በ ዚ ህ ጥ ና ት ለ መ ሳ ተ ፍ ፈ ቃ ደ ኛ መ ሆ ኔ ን በ ፊ ር ማ ቼ አ ረ ጋ ግ ጣ ለ ሁ ፡ ፡

ፊ ር ማ---------- ቀ ን ----/---/----------

(የ ስ ም ም ነ ት ቅ ጹ ን ማ ን በ ብ ለ ማ ይ ች ሉ ተ ሳ ታ ፊ ዎ ች)

የ አ ማካ ሪ ነ ር ስ ስ ም ---------- ፊ ር ማ ----------ቀ ን ----------

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 ☐

Date of diagnosis ☐☐☐

Relationship with the donor

1. Blood related 2. Spouse 3. Non-blood related 4. Other ……..

Table 3: Checklist to record anthropometric data and current medication

S.No	Variables	Value	Remark
1	Blood pressure	_____mmHg	
2	MUAC	_____cm	
3	Height	_____cm	
4	Weight	_____Kg	
5	BMI		
6	Type of clinical diagnosis		
7	Type of current medication		

Table 4: Checklist to Record Biochemical Profile Laboratory Findings

S.No	Variables	Value	Remark
1	**Creatine**		
2	**Urea**		

Table 5: Checklist to record Hematological Profile Laboratory Findings

S.No	Variables	Value	Remark
1	WBC		$x10^3/\mu L$
2	RBC		$x10^6/\mu L$
3	HGB		g/dL

4	HCT		%
5	MCV		fL
6	MCH		pg
7	MCHC		g/dL
8	RDW		%
9	RDW-SD		fL
10	PLT		$x10^3/\mu L$
11	MPV		fL
12	NE		%
13	LY		%
14	MO		%
15	EO		%
16	BA		%
17	NE#		$x10^3/\mu L$
18	LY#		$x10^3/\mu L$
19	MO#		$x10^3/\mu L$
20	EO#		$x10^3/\mu L$
21	BA#		$x10^3/\mu L$
22	NRBC		/100WBC
23	NRBC#		$x10^3$/Ml
24	RET		%
25	RET#		/100WBC
26	IRF		

Annex VI: Standard operating procedures for BUN & Creatinine tests

Objective: To describe laboratory tests to perform selected renal function test (RFT) study.

Personnel: Data will be collected by principal investigator and trained health workers. All laboratory tests for this research were performed by skilled and trained researcher (PI).

A. Pre-analytical

Materials and equipment for serum preparation

- Human blood sample
- EDTA Tube
- SST
- Micro-pipette of appropriate volume
- Centrifuge

First there will have Proper patient identification, and then fasting blood samples before and after kidney transplant we 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 hematological tube.

B. Analytical

1. Urea test

Purpose

To measure the concentration of BUN in serum using Chemistry Analyzer.

Clinical significance

Urea is the major end product of nitrogen metabolism in most animals and is produced in a series of reactions in the liver called the urea cycle. In the urea cycle, ammonia is converted to urea, which is carried by blood to the kidneys for elimination from the body. High levels of urea in the blood may indicate renal failure. Urea levels may also be elevated in response to treatment with certain drugs such as corticosteroids or in response to decreased kidney filtration due to dehydration or congestive heart failure. Decreased blood urea levels can occur in response to liver disease or malnutrition.

Principle

$$\text{Urea} + 2H_2O \xrightarrow{\text{Urease}} 2NH_4^+ + CO_3^{2-}$$

$$\text{Oxoglutarate} + 2NH_4^+ \xrightarrow{\text{GLDH}} \text{L-glutamate} + H2O + NAD^+ + NADH$$

Reagents

- Buffer /Enzyme and substrate reagents kits

Reagents preparation

- Mix 40 volume of Buffer/Enzyme reagent with 10 volume of substrate reagent. Reagents stability and storage:
    1. Stock reagent, stored at 2 – 8 °C, until expiry date.
    2. Working reagent, stored at 2 – 8 °C for 5days

Limitations

Gross hemolysis, lipemic and icterus specimen

2. Creatinine test

Clinical significance

Creatinine is generated from creatine by nonenzymatic dehydration. Creatinine is produced at a constant rate and is excreted from the body through kidney glomerular filtration. Decreased kidney function can affect the rate at which creatinine is filtered by the kidneys and can be used as a measure of kidney function. Decreased kidney function can result in increased serum creatinine levels due to the inability to clear creatinine through urine excretion. Creatinine levels can be affected by changes in muscle mass, pregnancy, or the use of angiotensin inhibitors or angiotensin receptor antagonists.

Principle

Creatinine + Picric acid $\xrightarrow{NaOH}$ Creatinine-picrate complex

Reagents

- Enzyme and substrate kits

Reagents preparation

- Mix 4unite of NaOH with 1unite of picric acid**.**

Reagents stability and storage:

1. Stock reagent, stored at 15- 30°C, until expiry date.
2. Working reagent, stored at 15 - 25°C for 2days

Annex VII: Standard operating procedures Complete blood count by Coulter DxH 800 Hematology analyzer

Principle of test

Coulter Method (impedance)

Accurately counts and sizes cells by detecting and measuring changes in electrical resistance when a particle (such as a cell) in a conductive liquid passes through a small aperture. Each cell suspended in a conductive liquid (diluents) acts as an insulator. As each cell goes through the aperture, it momentarily increases the resistance of the electrical path between the sub merged electrodes on either side of the aperture. This causes a measurable electronic pulse. For counting, the vacuum used to pull the diluted suspension of cells through the aperture must be at a regulated volume. While the number of pulses indicates particle count, the size of the electrical pulse is proportional to the cell volume.

Equipment:

- ✓ UniCel® DxH 800 hematologyanalyzer
- ✓ Printer
- ✓ Test tube cassette
- ✓ Barcode reader

Reagent	**consumable supplies**	
UniCel® DxH 800 Diluent (10L)	Distilled water	container for a bleach solution
UniCel® DxH 800 Cell lyse (5 L)	Ethanol (70%)	container for DI water
UniCel® DxH 800 diff pack (3L)	Alcohol resistant marker	5 to 6% solution of sodium hypochlorite
UniCel® DxH 800 Retic pack (3L)	Plastic dispensing bottles	soft cloth or tissue, lint-free swab or tissue
UniCel® DxH 800 clean (5 L)	Gauze	

Reagent stability and storage:

- ✓ Stable up to expiry date and, up to 60 days after opening and store at room temperature except for cleaner it's stable up to 90 days after opening.

Specimen requirements: - About 3-4 ml of venous blood collected into EDTA tubes.

Procedure

1. Turn ON the power switch on the right side of the unit. Self-check, auto rinse, and background check will be automatically performed, and the "Ready" (ready for analysis) will appear.
2. When auto rinse and background check are normally completed, "Ready" is displayed.
3. If the result of quality control in acceptable range run the blood samples.
4. Press Run Samples

Cassette Presentation

1. Ensure your specimens have been collected and stored properly.
2. Load the specimens into the cassettes.
3. Place the cassettes into the input buffer to the right of the SPM.
4. The SPM automatically begins cycling the cassettes.
5. After the SPM cycles the samples, review the sample results at the System Manager.

Data Extract form

S.No	Variables	Value		Measurement unit	Remark
		After	Before		
1	WBC			$x10^3/\mu L$	
2	RBC			$x10^6/\mu L$	
3	HGB			g/dL	
4	HCT			%	
5	MCV			fL	
6	MCH			pg	
7	MCHC			g/dL	
8	RDW			%	
9	RDW-SD			fL	
10	PLT			$x10^3/\mu L$	
11	MPV			fL	
12	NE			%	
13	LY			%	
14	MO			%	
15	EO			%	
16	BA			%	
17	NE#			$x10^3/\mu L$	
18	LY#			$x10^3/\mu L$	
19	MO#			$x10^3/\mu L$	
20	EO#			$x10^3/\mu L$	
21	BA#			$x10^3/\mu L$	
22	NRBC			/100WBC	
23	NRBC#			$x10^3$/Ml	
24	RET			/100WBC	
25	RET#			$x10^3/\mu L$	
26	IRF				
Clinical Chemistry analysis					
27	BUN			mg/dl	
28	Creatinine			mg/dl	

Letters for declaration

I, the undersigned, declare that this M.Sc. 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 thesis have been duly acknowledged.

M.Sc. candidate: Endale Haile (B.Sc.)

Signature: - _____

Date of submission: -_____

This thesis has been submitted with our approval as advisors.

Advisor: **Mikyas Negash (MSc, Ph.D. fellow)**

Signature: - _____

Date: - _____

Place: Addis Ababa, Ethiopia.

Advisor: **Moges Wordofa (MSc)**

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.