ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

DEPARTMENT OF BIOCHEMISTRY

ASSESSMENT OF HEMODIALYSIS-ASSOCIATED THROMBOCYTOPENIA AND ASSOCIATED FACTORS AMONG PATIENTS ON RENAL REPLACEMENT THERAPY IN ADDIS ABABA ETHIOPIA

By: Seblework Abeje (BSc)

A THESIS SUBMITTED TO THE DEPARTMENT OF BIOCHEMISTRY, COLLEGE OF HEALTH SCIENCES, ADDISABABA UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN MEDICAL BIOCHEMISTRY

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ADVISORS

1. Solomon Genet (Associate Professor, PhD)Department of Biochemistry, College of health science, Addis Ababa University, Ethiopia.
2. Menakath Menon (Associate Professor, PhD) Department of Biochemistry, College of health science, Addis Ababa University, Ethiopia.

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APPROVAL SHEET

ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

DEPARTMENT OF BIOCHEMISTRY

I, the undersigned MSc student, declare that I have submitted my original work titled "Assessment of hemodialysis-associated thrombocytopenia and other factors among patients on renal replacement therapy in Addis Ababa Ethiopia" for examination

Submitted by;

Seblework Abeje (BSc) ……... ……..

Name of student signature Date

This thesis is accepted in its present form by the board of examiners as satisfying for degree of Master of Science in Medical Biochemistry

Approved by;

Advisors;

Main advisor Solomon Genet (Associate Professor, PhD) …… ……

Signature Date

Co-advisor Menakath Menon (Associate Professor, PhD) …… ……

Signature Date

Statement of Declaration

With my signature put below, I declared that this thesis is my own work and I have followed all the ethical principles of research during preparation, data collection, data analysis and compilation of this thesis. This thesis is submitted to Addis Ababa University in partial fulfillment of the requirements for the degree of Master of Science in Medical Biochemistry.

Approval by the board of examination

This thesis is accepted in its present form by the board of examiners as satisfying thesis for degree of Master of Science in Medical Biochemistry at Addis Ababa University

External Examiner

……. ……. …… …….

Name Rank Signature Date

Modulator

……. …… …….

Name Rank Signature Date

Advisers

Solomon Genet (Associate Professor, PhD) …….. …… ……

Rank Signature Date

Menakath Menon (Associate Professor, PhD)…….. …… ……

Rank Signature Date

Department Head

……. ……. …… …….

Name Rank Signature Date

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Table of content: **Page Number**

Contents

List of Tables

List of Figures

List of Abbreviations

AHA	American Heart Association
AKF	Acute Kidney Failure
CKD	Chronic Kidney Disease
CRF	Chronic Renal Failure
CVS	Cardio Vascular Disease
ELISA	Enzyme-Linked Immunosorbent Assays
ESRD	End Stage Renal Disease
ETB	Ethiopian Birr
HD	Hemodialysis
HD-HIT	Hemodialysis related Heparin Induced Thrombocytopenia
HIT	Heparin Induced Thrombocytopenia
MI	Myocardial Infarction
MPV	Mean Platelet Volume
PD	Peritoneal Dialysis
PDW	Platelet Distribution width
PLT	Platelet
PT	Prothrombin Time
PTT	Partial Thromboplastin Time
RBC	Red Blood Cell
SPSS	Statistical Package for the Social Science
SSA	Sub-Sharan Africa
WHO	World Health Organization

Abstract

Background; Hemodialysis is one of the renal replacement therapies, in addition to peritoneal dialysis and renal transplantation. Hemodialysis can improve morbidity and mortality in renal patients; however it has different multifactorial idiosyncratic complications that occur during the dialysis and/or during long term use of hemodialysis. Dialysis as treatment modality is extremely scarce in Ethiopia and affordable to only the rich.

Objectives: The objective of this study was to assess the prevalence of thrombocytopenia and associated factors among ESRD patient under hemodialysis in Addis Ababa Ethiopia from May 1st to June 1st, 2019:

Method: Institution based cross sectional study was conducted in Addis Ababa Ethiopia from May 1st to June 1st, 2019. 100 patients who were on hemodialysis during the study period were involved in the study. Platelet number was determined by flow cytometry. The collected data was checked for completeness, entered to computer and statistically analyzed using SPSS version 25.0

Results: The total number of patients in the study was 100. The mean age of the subjects was 40.25± 12.84 years, with a range of 18-76 years and 70% (70) of the study subjects were male. 85% of the study subjects had hypertension, and 19% had diabetes mellitus. Only 5% had co-morbidities likely to contribute to CKD, such as chronic glomerulonephritis and renovascular disease. The mean duration of hemodialysis was 0.78 ±0.42 years, with a range of 2 months to 3.5 years. The mean pre dialysis and post dialysis platelet count was 233.5×10^3±72.13cells/µl and 178×103 ±71.8 cells/µl respectively and thought it is in the normal range PT and PTT showed slight increase post HD (16.66±3.19) seconds compared to pre HD (15.4±5.69) seconds and 34±6.76 seconds compared to pre HD 33.8±5.34 seconds respectively. Age of patients COR=0.4(0.12, 1.356) and duration of treatment COR=0.43(.118, 1.531) were statistically insignificantly associated with thrombocytopenia.

Conclusion and recommendation: Over all prevalence of thrombocytopenia was higher in the study area and coagulation parameters; prothrombin time and partial thromboplastin time showed slight increase after hemodialysis among ESRD patient.

Key words; Hemodialysis, Thrombocytopenia

1. Introduction

1.1 Back ground

Renal failure is a condition in which the kidneys are unable to adequately filter toxins and waste products from the blood. It is described as a decrease in glomerular filtration rate (GFR) and can be determined by measuring the plasma clearance of different glomerular filtration markers like inulin and ethylene-diamine-tetra-acetic-acid (Russell *et al.*, 1985, Prasad *et al.*, 2012). Almost every organ system is influenced by the development of renal failure, and these derangements are modulated by the type of treatment. Basic scientific studies include the effects of renal hormones, the effects of fluid and electrolyte derangements on cell and organ function, the organ specificity of these changes, the indicators of normal and abnormal body function which might have clinical utility, and the rate and type of change in body fluid exchange necessary for homeostasis. Chronic renal failure (CRF) is a progressive loss in kidney function over a period of time. It is identified by higher amount of creatinine and lower glomerular filtration rate. In the early stages, there may be no symptoms but it gets worse gradually. The final stage of chronic kidney disease is an irreversible debilitating condition of kidney which requires intensive treatments of dialysis or transplantation (Dor *et al.*, 2007)

Chronic renal failure (CRF) is a growing problem worldwide leading to increasing incidence of life threatening complications and mortalities. Indication of dialysis in CRF is end stage renal disease which is defined as a state of low G.F.R. i.e.: 15ml/min per 1.73 meter square. The option of treatment in this condition is renal replacement therapy which includes both kidney transplantation and dialysis. Although Kidney transplantation remains the gold standard for the treatment of this condition, dialysis (both hemo and peritoneal) is the most common and most practiced modality of treatment in spite of high cost for renal transplantation (Farhad *et al.*, 2009).

Dialysis involves the removal of urea and other toxic substances from the plasma as well as the correction of electrolyte imbalance. Of the two methods of dialysis, hemodialysis (HD) is the most commonly used method in which, blood is passed through an extra corporeal circuit and pumped across an artificial semi permeable membrane to bring the blood into contact with the dialysate (Bishop *et al.*, 1996). During hemodialysis, blood is removed from a vein. It is run through filters to remove waste products. The blood is then returned to the body. Hemodialysis

usually is done at a dialysis center. The treatments are done three times a week, in three- to four-hour session (Chakravarti *et al.*, 2017).

The second method is the intermittent and continuous ambulatory peritoneal dialysis (PD). This method utilizes the peritoneal membrane, as the semi permeable membrane, with capillaries on one side and high osmotic fluid infused into the peritoneal cavity on the other side. The peritoneal cavity is drained and the cycle is repeated after a suitable time to allow the equilibration of diffusible substances. Peritoneal dialysis can be done at home. It takes longer time than hemodialysis and must be done four to five times a day. It can be automated to occur during sleep (Malyszk and Myliwiec 2001).

Both types of dialysis are known to have side effects on the variable blood components. These effects vary with several physiological and non-physiological factors such as age, sex, race, and muscular activity, position of patient during dialysis as well as the duration and type of dialysis. It has been reported that dialysis lowers the hemoglobin (Hb) level and red blood cell (RBC) count; this is more pronounced in females than males and in patients in advanced age because of the reduced erythropoietin concentration in these patients (Mohamed *et al.*, 2008).

ESRD becomes financial burden on family and health care sector due to a high morbidity and mortality associated with it. Cardiovascular complications remain the most common cause of death among ESRD patients and those undergoing hemodialysis (HD). Patients on Hemodialysis behave in a distinct way that they are relatively more prone for bleeding than thrombotic manifestations. In recent days abnormalities in platelet parameters are found to be an effective tool in risk stratification of patients with chronic kidney disease (CKD) to develop coronary artery disease (Lokesh *et al.*, 2016). Unlike CRF in which the bleeding tendency is due to platelet functional defects, the patients on dialysis particularly hemodialysis tend to have bleeding or thrombotic tendency due to disturbances in the function of platelets as well as coagulation and fibrinolytic systems. The bleeding tendency can also be attributed to the use of anticoagulants in the procedure. This is observed by measuring coagulation parameters after dialysis (Milburn *et al.*, 2011, Anatole *et al.*, 2012, Daugirdas and Bernardo, 2012, Schoorl. *et al.*, 2013). Coagulation parameters, prothrombin time (PT) and activated partial thromboplastin time (APTT) are screening tests for extrinsic, intrinsic and common pathway clotting factors respectively (Attika and Lubna, 2015).

Platelets are extremely small and discoid 3.0 x 0.5 micrometer in diameter. With mean volume 7-11 fimtoliter, they are the smallest of the three major types of blood cells is only about 20% of the diameter of red blood cells. Platelets are produced in the bone marrow by fragmentation of cytoplasm of megakaryocytes, one of the largest cells in the body. The dominant hormone controlling megakaryocytes development is thrombopoietin. Platelets are actually not true cells but merely circulating fragments of cells. But even though platelets are merely cell fragments, they contain many structures that are critical to stop bleeding. They contain proteins on their surface that allow them to stick to breaks in the blood vessel wall and also to stick to each other. They contain granules that can secrete other proteins required for creating a firm plug to seal blood vessel breaks. Also platelets contain proteins similar to muscle proteins that allow them to change shape when they become sticky. The main function of platelets is formation of mechanical plugs during normal homeostatic response to vascular injury. In the absence of platelets, spontaneous leakage of blood through small vessels may occur. Immobilization of platelets at the sites of vascular injury requires specific platelets to vessel wall (adhesion) and platelets to platelets (aggregation) interaction (Remuzzi G, 1995)

Thrombocytopenia is a known potential side effect of hemodialysis. In pre-dialysis patients, as well as in hemodialysis patients, platelet number tends to be reduced. Platelets have been known to interact with dialysis membranes since the 1970's; dialysis membranes have been shown to cause platelet adhesion, aggregation, and activation (Katz, 2005).

End-stage renal disease patients mostly suffer from various hemostatic disorders. Bleeding tendency in uremic patients is high, due to abnormalities of primary hemostasis. This is particularly attributed to platelet dysfunction. In this regard, the most important abnormalities are related to decreased availability of platelet factor-3, defective platelet aggregation, adhesiveness, and prolongation of the bleeding time (Krawczyk *et al.*, 2004). The implicated mechanisms include: increased vessel wall prostacyclin abnormal platelet arachidonic acid, increased levels of parathyroid hormone, platelet (PLT) inhibition by various plasma metabolites, such as urea, guanidinosuccinic acid, and phenolic acid metabolism (Henning *et al.*, 2002, Ozdemir *et al.*, 2004, Mahmoud *et al.*, 2012). Substantial activation of platelets can occur in the course of hemodialysis and Platelet surface markers show evidence of platelet degranulation. Some activation occurs due to exposure of blood to the roller pump segment and microbubbles may play a role (Gafter *et al.*, 1987).

Platelet activation seems to be reduced with reused dialyzers or with those containing synthetic versus cellulosic membranes. Nevertheless, a substantial degree of platelet activation can be demonstrated with polysulfone and other synthetic membranes; the amount of activation may differ substantially among polysulfone membranes, depending on the manufacturer and the polyvinylpyrrolidone content. Platelet–platelet and platelet–leukocyte aggregates have been detected in the dialyzer blood outflow line and the consequences of these to the microcirculation are unknown (John *et al.*, 2012). Typically, the platelet count decreases slightly during the first hour of dialysis, but mostly returns to initial values by the end of dialysis. A number of chronic hemodialysis patient cases have been reported in which a marked decrease in platelet count (50% or more) during dialysis was observed, resulting in mild degrees of predialysis thrombocytopenia (Gawa and Schmidt, 2004). In only one case was the decrease in platelet count associated with bleeding. Dialyzer hypersensitivity symptoms are infrequently associated with a fall in platelet count. Most recent cases of dialysis-associated thrombocytopenia have been with polysulfone membranes, especially polysulfone membranes sterilized by electron beam (Martinovic *et al.*, 2016).

HD-associated thrombocytopenia has been also described in patients undergoing HD with cellulosic (cuprophane) membranes. However, this event has rarely been described when using the highly biocompatible membranes, such as the optiflux membranes. There have been multiple reports describing HD-associated thrombocytopenia in patients undergoing HD with bioincompatible membranes, such as the cellulosic (cuprophane) membrane, Exposure of blood to hemodialysis membranes results in numerous interactions between the blood elements and the membrane. Transformation and adsorption of plasma proteins (such as complement) and activation of blood cells (such as neutrophils and monocytes) have been studied most extensively by nephrologists in recent years (Knudsen *et al.*, 1985). There is no consensus on the definition of biocompatibility for dialyzer membranes. An operational definition of biocompatibility is the lack of any perturbation of blood constituents. According to this “inert surface” definition, a membrane (for example, one that adsorbs/32microglobulin) can be considered as bio incompatible and yet desirable. Because of the multitude of blood-membrane interactions that may occur during hemodialysis, multiple criteria for biocompatibility need to be applied in the classification of membranes. A certain bio incompatible phenomenon can be further classified as beneficial or deleterious depending on its biological effects as well as its acute and chronic

impacts on the dialysis patient (Bishop *et al.*, 1996). This has been clearly demonstrated by Hakim and Schaffer (1995) who reported HD-associated thrombocytopenia when using the cuprophane membrane, which did not occur when using the highly biocompatible, non-complement activating dialyzer membrane, polymethylmethacrylate. Biomaterials have been regularly used in different types of artificial tissues and organs, such as the plasmapheresis equipment, hemodialysers, catheters, and prostheses; biocompatibility has always been a concern with these devices. Several bio-incompatible events have been described, such as in HD where the dialysis membranes can trigger whole body inflammation leading to accelerated arteriosclerosis (Hakim and Schafer, 1985). The contact of blood with the dialyzer membrane triggers an inflammatory reaction similar to what occurs during infection. Some of the proposed mechanisms are that this inflammatory reaction triggered by the dialyzer membrane leads to the activation of the coagulation cascade, which causes the release of activated Factor 10. This continuous state of activation might assist in the chronic inflammatory state which would lead to the several HD associated co-morbidities. It is the membrane itself, along with the biochemical characteristics and composition that determine the extent of complement activation (Nasr. *et al.*, 2013)

Similarly, Schoorl *et al* (2013) also showed that HD-induced PLT activation occurred at different time in CKD patients; first, already before dialysis both activation markers on the PLT surface area and the PLT granule content are markedly decreased below the reference range. PLTs from chronic HD patients are chronically exhausted due to repeated stimulation and activation in the course of HD treatment and recurrent release of PLT degranulation products. Second, during HD treatment, several alterations of the hemostatic process additionally occur. Besides deviations of coagulation parameters during HD, PLTs are activated and a concomitant reduction in the granule content is observed. Because changes occur three times a week, chronic HD patients reveal persistent coagulation defects and PLT activation in addition to alterations induced by a severe uremic state itself. Third, during HD treatment, PLT activation combined with thrombin and fibrin generation occurs. However, fibrinogen or fibrin depositions on clot devices could not be demonstrated. Micro-aggregates occur in the blood circulation.

1.2 Statement of the problem

According to WHO comprehensive and integrated action of the means to prevent and control chronic diseases in developing countries, chronic diseases are growing problem in developing countries like Ethiopia. The incidence of chronic renal disease in Ethiopia is rising because of increased risk factors such as high blood pressure and diabetes mellitus (Judd, 2009).

Over 1.1 million patients are estimated to have renal failure worldwide with an annual increase at a rate of 7% (Gilbertson *et al.*, 2015). Chronic renal failure (CRF) is a growing problem worldwide leading to increasing incidence of life threatening complications or death(Sukru *et al.*, 2004, Attika and Lubna, 2015). The prevalence of CKD in Ethiopia is high and has increased in the last few years to be 12.2% with an increased prevalence of diabetes and hypertension which shows it is becoming one of the public health problems(Cheru *et al.*, 2018). Dialysis as treatment modality is extremely scarce in Ethiopia and affordable to only the rich. Survival pattern in those on the treatment is less satisfactory and short of usual standards in the developed world and needs further investigation (Shibiru *et al.*, 2013).

Hemodialysis is one of the renal replacement therapies, in addition to peritoneal dialysis and renal transplantation in end stage renal disease (ESRD) patients(Elmukhtar Habas *et al.*, 2012). In developing countries and it still remains a primary modality of treatment due to growing numbers of ESRD and lack of adequate donors and transplantation centers (Murat *et al.*, 2012, Subhanud *et al.*, 2013, Pal *et al.*, 2014). Patients on hemodialysis have an increased risk of thrombotic events. The most common thrombotic Complication in hemodialyzed patients is thrombosis of vascular access, which is a major cause of hemodialysis-associated morbidity, while cardiovascular and cerebrovascular incidents are major causes of dialysis-associated mortality(Martinovic *et al.*, 2016, Naumnik *et al.*, 2002).

Hemostatic disorders are common complications in patients with end-stage renal disease (ESRD), the principal cause of morbidity and mortality in hemodialysis patients remains mainly in the form of bleeding diathesis, but also as an increased risk of thrombotic events. In terms of abnormalities of primary hemostasis, platelet dysfunction and impaired interaction between platelets and vessel wall are considered as the main factors responsible for bleeding tendencies(Boccardo *et al.*, 2004, Kaw and Malhotra, 2006, Jalal *et al.*, 2010, Jnny *et al.*, 2014). There are more than 1.7 million patients who undergo hemodialysis in about 28,500 dialysis

units worldwide, some of these complications are related to the bio-incompatibility of the dialyzer membranes used. These bio-incompatible events range from leukopenia to complement activation and thrombocytopenia and might lead to an increased tendency for bleeding (Chow *et al.*, 1999).

According to the latest WHO data published in April 2011, kidney disease deaths in Ethiopia reached 12,038 or 1.47% of total deaths and renal diseases accounted for 1.26 % of adult hospital medical admissions in reports from various parts of the country. Diseases of the genitourinary system were 5th in rank among the 10 leading causes of outpatient visits (4.45% of visits) (Phillip, 2011).

1.3 Significance of the study

This study was primarily designed to investigate changes in platelet count that may occur in renal failure patients after HD. The possible effect of the duration of dialysis, age, sex and number of dialysis per week on hematological parameters that may occur in renal failure patients was also investigated at SPHMMC, Menelik II Referral hospital and Zewditu memorial hospital Addis Ababa Ethiopia.In addition, the findings from this study provide new insight to see the problem for better intervention and important to shape clinical as well as public health care of the patients and the general population. Results from this study enable us to determine whether precautions need to be taken before and after HD sessions to minimize and avoid complications in HD patients .

Moreover, the results obtained from this study may be used as baseline or reference to pave the way for conducting further related studies and used as input for stake holders (health policy makers).

2. Literature Review

2.1 Epidemiology and risk factors of renal failure

Disease morbidity and mortality patterns all over the world are changing, both in the developed and the emerging world. Throughout the 20 century, infectious diseases were the major cause of death and disability. Nonetheless in these days, non-communicable, noninfectious diseases have become the main cause of mortality and morbidity around the world. This change is mirrored in the type of diseases causing chronic kidney failure and in their presentation and progression (Ababio *et al.*, 2017, Omari *et al.*, 2019). Kidney disease is evaluated in terms of overall renal function (glomerular filtration rate, GFR) and the presence of kidney damage established by either kidney biopsy or other markers of kidney damage and it is a common condition in which there is a loss of kidney function over time (Adekunle *et al.*, 2018).

Chronic kidney disease is an emergent worldwide public health problem and Chronic Kidney Disease (CKD) is defined as kidney damage or Glomerular filtration rate (GFR) <60 ml/min/1.73 m2 for more than 3 months with implications for health(Cheru *et al.*, 2018). Several reports have shown that chronic kidney disease (CKD) is a world-wide public health problem associated with adverse outcomes of kidney failure, cardiovascular disease (CVD), and premature death(Miso *et al.*, 2005, Martina *et al.*, 2019). It has been estimated that more than 500 million individuals globally have CKD, defined by either kidney damage or glomerular filtration rate (GFR) < 60 ml/min/1.73 m2 for ≥ 3 months, regardless of the cause(Boccardo *et al.*, 2004). In developing countries, the growing prevalence of chronic diseases such as Chronic Kidney Disease has severe implications on health and economic output. The rapid rise of common risk factors such as diabetes, hypertension, and obesity, especially among the poor, results in even greater and more profound burdens that developing nations are not equipped to handle(Nugent *et al.*, 2011).

In developing countries, common causes of CKD also include glomerular and tubule-interstitial diseases due to infections and exposure to drugs and toxins. Sub-Saharan Africa is a vast heterogeneous region of roughly 47 countries and more than 900 million people. By 2030, more than 70% of patients with end-stage renal disease are estimated to be living in low-income countries, such as those in sub-Saharan Africa. There are many potential causes of CKD in sub-Saharan Africa, making kidney disease especially burdensome in the region. In addition to non-communicable diseases, communicable diseases such as infectious glomerulonephritis,

schistosomiasis, leishmaniasis, and HIV infection are common and can cause CKD. Because more than 22 million people in sub-Saharan Africa have HIV, the potential for an overwhelming burden of CKD in the region is high (Sanyaolu *et al.*, 2018). Though there is lack of renal registries and reliable statistics about the prevalence of CKD in the majority of African countries, few studies indicate that the increasing burden of chronic kidney disease and end-stage renal disease presents a challenge for the continent. CKD affects mainly young adults aged 20–50 years in Sub-Shran Africa (SSA) and it is primarily due to hypertension and glomerular diseases, unlike developed countries, where CKD presents in middle-aged and elderly patients and is predominantly due to diabetes mellitus and hypertension. Presentation is usually late, with 47.5% in ESRD requiring dialysis at presentation and severely ill with co-morbidities (Naicker, 2013). Although there is limitation of data about prevalence of CKD at national level in Ethiopia, few studies indicate that the disease has become a significant public health problem in Ethiopia (Temesgen *et al.*, 2014, Alemayehu *et al.*, 2018). A cross sectional study showed that the prevalence of CKD among Ethiopian population was estimated to be 12.2%. The prevalence of CKD is higher among age <35 years (41.02%) and males than females 62% and 38% respectfully (Cheru *et al.*, 2018). This is supported by WHO data published in April 2011, kidney disease deaths in Ethiopia reached 12,038 or 1.47% of total deaths and renal diseases accounted for 1.26 % of adult hospital medical admissions in reports from various parts of the country. A disease of the genitourinary system is the 5th in rank among the 10 leading causes of outpatient visits (4.45% of visits)(Phillip, 2011). The complex interplay between modifiable and non-modifiable factors or conditions, including both environmental and genetic factors, are reported to play a role in the development of CKD. On top of this the three most important modifiable risk factors are: unhealthy diet and excessive energy intake, physical inactivity and tobacco use. Globally these risk factors are increasing as people's dietary habits change to foods high in fats and sugars, and people's work and living situations are much less physically active. Increased marketing and sales of tobacco products in low and middle income countries mean greater exposure to the risks of tobacco. Elimination of the preventable risk factors would prevent 80% of heart diseases, 80% of strokes, 80% of type 2 diabetes and 40% of cancer (W.H.O, 2013).

Several reports have shown that the most frequent causes of CKD are diabetic nephropathy, hypertension, glomerulonephritis, interstitial nephritis, pyelonephritis, polycystic kidney disease

and obstructive nephropathy. CKD can also be the final result of untreated acute kidney injury (AKI) caused by infections, medicines, toxic substances heavy metals including lead, cadmium, mercury and chromium (Eleftheria *et al.*, 2014, Temesgen *et al.*, 2014). Several studies have shown that essential hypertension along with diabetes is associated with abnormalities in hemostatic imbalance caused by rheological, hemostatic, endothelial and platelet abnormalities that may act synergistically to increase the risk of thrombogenesis and atherosclerosis and thereby to the thrombotic complications in CKD patients (Dell'omo *et al.*, 2013).

2.2Complications of Hemodialysis

In this era of enhanced technology, complications due to HD are few compared to other procedure; patient group represents complex medical problems, is increasing in number at an annual rate of 8%, and suffers from very high morbidity and mortality (Dorgalaleh *et al.*, 2013). Different studies reported cardiovascular disease (CVD) is the major cause of death in ESRD patients on regular HD. Atherosclerosis is present in most if not all long-term dialysis patients. CVD in HD patients is 5 to10 times higher than in general population, and it accounts for at least half of all patients' deaths. The death in these patients were mainly due to coronary heart disease and predisposes patients to blood born infections, partly due to an abnormal immune system function in CKD patients and in dialysis dependent ESRD patients. In dialysis patients, white blood cells count may be normal, but the white blood cells are typically not functioning normal. This abnormality has led to more risk of staphylococcus infection (Elmukhtar Habas *et al.*, 2012). Hemodialysis vascular access infection is common causes for hospitalization in HD patients, and can cause serious complications. In less serious catheter and graft infections, it is sometimes possible to eradicate the infection with several weeks antibiotics course. The persistence of fevers, an elevated number of white blood cells in the blood, or constitutional symptoms, all can indicate antibiotic treatment failure, and that indicates the vascular access should be removed. Under nutrition is often common in HD dependent patients, and time duration on dialysis is a strong predictor of malnutrition. Patients on HD more than ten year are tending to loss their body weight despite adequate protein intake. Although the underlying cause of malnutrition is unclear, it is may be due to chronic metabolic acidosis, or to decreased physical activity as a result of β Mamyloidosis rather than the reduction in energy expenditure. It had been reported that the survival rate is correlated with serum albumin and prealbumin levels (Omari *et al.*, 2019).

Some of the complications are also related to anticoagulant therapy and the bio incompatibility of the dialyzer membranes used. These bio incompatible events range from leukopenia to complement activation and thrombocytopenia and might lead to an increased tendency for bleeding (Nasr. *et al.*, 2013).

Several reports have shown that Patients with mild-to-chronic kidney disease exhibit a variety of hemostatic disorders ranging from an increased clotting tendency and reductions in the levels of natural inhibitors of coagulation to defective fibrinolysis. Hemostasis is a dynamic physiological condition that serves to coordinate the balance between bleeding and clot formation by collaborating the activity of the blood vessel, platelet, and plasma proteins (Elmukhtar Habas *et al.*, 2012, Chinwuba and Ngozi, 2010, Bhatta *et al.*, 2011). Second Leading Cause of death in Hemodialysis Patients is infection-related mortality that accounts about 12% to 22% in patients with end-stage renal disease. Septicemia responsible for 75% of infectious deaths and Sepsis-related mortality is 100- to 300-fold greater in dialysis patients than general population (Tokars *et al.*, 2004).

According to the 2017 American Heart Association AHA guideline, persons With CKD should be regarded as the highest risk group for subsequent CVD and CKD is an independent risk factor for cardiovascular disease (CVD), kidney dysfunction should be an additional target for intervention and prevention of CVD (Carey and Whelton, 2018).

A Comparative cross sectional study has shown that HD is related with reduced efficiency and ability to perform activities, social isolation, immobility, reduced self-confidence and, finally, disappointment at the future and continuation of the treatment. In addition to encountering many physiological changes, these patients face many mental and psychological stresses each of which in turn disturb their mental status and sleep quality. As such, most of these patients are not adapted to problems and tensions, and develop disorders such as anxiety, depression and isolation which affect their health status and weaken their health promoting behaviors over time (Alireza *et al.*, 2019). Health promoting behaviors refer to those behaviors that make people able to improve their own and their society's health. The areas of health promoting behaviors include nutrition, physical activity, stress management, health responsibilities, interpersonal relationships, and spiritual growth (Lippke *et al.*, 2012). These behaviors are important as they have the potentiality to prevent the complications of illness and treatment, reduce pathogenicity, improve quality of life, maintain the function and independence of individuals, and reduce the

burden of care on society. These behaviors are considered to be important determinants of health, and promoting them can prevent one third of deaths and the risk of heart disease in these patients (Alizadeh *et al.*, 2018). Several studies have shown that health promoting behaviors in patients undergoing hemodialysis are affected by renal disease and hemodialysis treatment. Studies conducted by (Mavel *et al.*, 2013) demonstrated that health promoting behaviors in hemodialysis patients are not in a desirable level and some interventions are needed to improve these behaviors.

2.3 Hemodialysis and Hemostatic parameters

Disturbances in hemostasis are common complications of kidney disease. Both bleeding diathesis and thromboembolism have been identified. The principle cause of these abnormalities is the uraemic state the pathogenesis of uraemic bleeding is multifactorial. The most important determinants of pathogenesis is increased levels of clotting factors, decreased levels of clotting inhibitors, diminished fibrinolytic activity and platelet hyperaggregability (Subhanud *et al.*, 2013). At present the incidence of bleeding declining, where thrombotic complications have become the predominant cause of mortality. Several reports have shown that end stage renal disease patients who are undergoing hemodialysis tended to show a disturbance in platelet parameters and tendency towards a hypercoagulability and frequent thrombotic complications as compared to healthy controls (Miso *et al.*, 2005, Abdullah *et al.*, 2012, Schoorl. *et al.*, 2013). It is widely believed, mainly based on results from relatively old studies, that not only chronic renal disease but also the hemodialysis process by itself activates platelets, coagulation and fibrinolysis. Hemodialysis procedure could influence hemostasis by two distinct pathways: the first is by the effect of the dialysis membrane, the composition of the dialysis circuit, and changed rheology, and second, by the effect of added anticoagulants. Noticeably, these factors have been significantly changed in the last decade. In the above-mentioned studies, it was mainly shown that activated platelets, coagulation and fibrinolysis contribute to the occurrence of atherothrombotic events in hemodialysis patients, one could speculate that the hemodialysis procedure itself facilitates the development of atherothrombotic events (Miso *et al.*, 2005).

Studies have shown that thrombotic complications are thought to be a net outcome of both underlying kidney disease, complicated by renal failure, and its management through dialysis or renal transplantation and different factors that may contribute to hemorrhagic complications in

HD patients include platelet dysfunction of uremia; reduced activity of several coagulation factors, including factors II, IX, X, and XII; and heparin anticoagulation during HD(Paola *et al.*, 1999). In pre-dialysis patients, as well as in hemodialysis patients, platelet number tends to be reduced .in the range of 175–180,000/mm3 compared with 250,000/mm3 in healthy controls. In continuous ambulatory peritoneal dialysis patients, platelet counts have been reported to be closer to the normal range. Platelet survival in hemodialysis patients is thought to be of normal duration, although the only paper examining this was published in 1967. The megakaryocyte number in bone marrow is normal but the reticulated platelet count, a measure of thrombopoiesis, is reduced despite elevated thrombopoietin levels. Platelets have been known to interact with dialysis membranes since the 1970's; dialysis membranes have been shown to cause platelet adhesion, aggregation, and activation. Platelet activation has been demonstrated by elevated levels of platelet factor 4 as well as thromboxane following hemodialysis. Accordingly, thrombocytopenia is also a well-known complication of hemodialysis treatment as thromboxane following hemodialysis (Yasir *et al.*, 2016).

Hakim and Schafer (2016) suggested that thrombocytopenic episodes occurring with hemodialysis were associated with complement activation, specifically C3a, in addition to activation of platelets themselves Complement activation occurred specifically in the setting of cuprophane membranes, and thrombocytopenia was only observed in the presence of complement activation.

Thrombocytopenia is defined as platelet count less than 15,0000cell/µl (Gauer and Braun, 2012) Dialysis patients, especially those in the intensive care unit, but even those treated as outpatients in dialysis units, often can be affected by other medical conditions associated with thrombocytopenia. In acutely ill patients with sepsis, thrombocytopenia is common, with or without disseminated intravascular coagulation. Platelet consumption due to thrombotic thrombocytopenic purpura or idiopathic thrombocytopenic purpura, due either to immunologic diseases, especially various forms of vasculitis, or drugs, is not uncommon in renal patients. Physical destruction of platelets may occur because of intravascular catheters. Thrombocytopenia and platelet dysfunction are commonly seen in liver disease, paraproteinemia, myeloproliferative disorders, and myelodysplastic syndrome. Thrombocytopenia may be found in patients receiving nicotinamide for treatment of cholesterol abnormalities or hyperphosphatemia, and many drugs sometimes taken by hemodialysis patients, including

clopidogrel and other antiplatelet agents, as well as quinine, for example, can cause drug-induced thrombocytopenia. Finally, in actively bleeding patients, dilutional thrombocytopenia can occur when transfusing packed red blood cells (RBCs) only, as functional platelets are not present in packed RBC transfusions(George *et al.*, 2015).

In addition Heparin-induced thrombocytopenia (HIT) is a serious and life-threatening complication that occurs in 5% of patients exposed to heparin. It should be considered in patients with a platelet count <100 × 109 cells/l or a >50% decrease from baseline count in association with heparin therapy. Thromboembolic complications develop in 50% of patients. Bleeding is rare as the platelet count typically does not drop below 20 × 109 cells/l. Up to 12% of dialysis patients develop HIT, named hemodialysis-related-heparin-induced thrombocytopenia (HD-HIT), as they are a risk group with continuous exposure to heparin. The definition of HDHIT is less strict, in the range of a platelet count decrease of 30% and below 150 × 109 cells/l due to the intermittent use of heparin. Heparin cessation and alternative anticoagulation are the key interventions in patients with HIT. In dialysis patients, citrate anticoagulation, heparin-free dialysis or peritoneal dialysis are options that must be considered. A number of studies have examined the effect of HIT on mortality in dialysis patients, and the results are varied, with some studies showing an adverse effect, whereas others show little risk(Joana *et al.*, 2018).

The diagnosis of HIT is not always straight forward. ELISA antibody kits to detect IgG, IgA, and IgM PF4-heparin antibodies exist, but sometimes the antibodies detected are not associated with manifestations of HIT. A functional test involving the release of radiolabeled serotonin by heparin is available and is considered a 'gold standard'. The prevalence of HIT antibodies in dialysis patients ranges from 0 to 17%, although functional HIT antibody assays are positive in only about 3% of patients. Because heparin seems to increase platelet stickiness in most if not all patients, and also can degranulate platelets, as discussed above, one would expect that, in the general population, the use of heparin for dialysis might be expected to 'amplify' dialyzer-associated effects on platelets such as activation and thrombocytopenia. In a substantial minority of patients with HIT, one might expect a 'high volume amplification' of any dialyzer-associated platelet effects (Wardle, 2002).

A study by Matsuo and Wanaka (2011) however, argues against this. In 50 chronic dialysis patients, six of whom had HIT antibodies, they examined the decrease in platelet count during dialysis. In the six patients with HIT antibodies, the mean decrease in platelet count (pre versus

postdialysis) was 12%, which was not markedly different from the 8% decrease in platelets in the entire group of 50 patients. Interestingly, in 10 out of 50 patients in this particular study, the postdialysis platelet count decreased by more than 35%, which was a greater drop than in most of the other studies where platelet count during dialysis was measured; One possible explanation for the thrombocytopenia is that Platelet surface markers measured in dialyzer outlet blood give evidence for both platelet activation and degranulation in the course of dialysis (Malyszko and Myliwiec, 2001). In addition, there is evidence for formation of platelet–platelet and platelet–leukocyte aggregates. The clinical consequences of this effect of dialysis on platelets are unknown. Some of the activation and aggregation of platelets during dialysis may be due to exposure of blood to the roller pump segment of the dialysis tubing or to microbubbles and does not depend on exposure to the dialyzer membrane platelet activation occurs almost universally when blood is subjected to dialysis and even to extracorporeal circulation only when using a blood roller pump. This platelet activation may be accompanied by formation of platelet–platelet and platelet–leukocyte aggregates, which might then lodge in and initiate harmful changes in the microcirculation. Renal replacement therapies that require prolonged exposure times to extracorporeal blood circuits, such as frequent long nocturnal hemodialysis, various forms of continuous renal replacement therapy, and wearable kidneys, may endanger as yet unrecognized harmful effects related to chronic platelet activation. Platelet number, survival, and function in chronic kidney disease: Several studies have demonstrated that elevated levels of Mean Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT) among End Stage Renal Disease Patients after dialysis (Attika and Lubna, 2015). Several reports have shown that the presence of significant positive correlation between duration of dialysis and APTT and PT levels while, there is a negative significant correlation between duration of dialysis and platelets count (Abdullah *et al.*, 2012). Similar study has shown that higher decrease in Platelet count is present among the age group 45-54 followed by group 75-84 (Yasir *et al.*, 2016). In contrast to these; several studies have shown that the duration of HD and PD did not affect the hematological parameters with the exception of the red cell count, which is significantly decreased as the duration of HD increase (Mohamed *et al.*, 2008). This is supported by Study conducted in USA on Relationship between platelet count and hemodialysis membranes indicate that there is no statistically significant difference between sex, age and post dialysis platelet count (Nasr. *et al.*, 2013).

2.4 Hypothesis

1. Post hemodialysis platelet count of ESRD patients is less than Pre hemodialysis platelet count
2. Post hemodialysis prothrombin time /PT and partial thromboplastin time / PTT of ESRD patientsare prolonged than Pre hemodialysis prothrombin time /PT and partial thromboplastin time / PTT.

3. Objective

3.1 General objective:

- To assess hemodialysis-associated thrombocytopenia and associated factors among patients on renal replacement therapy at St. Paul's Hospital and millennium medical college, Menelik II Referral Hospital and Zewditu memorial Hospital, Addis Ababa, Ethiopia from May 1st to June 1st2019

3.2 Specific Objectives:

- To determine platelet number of patient under hemodialysis
- To compare the platelet number pre and post hemodialysis
- To determine prothrombin time and partial thromboplastin time of patients under hemodialysis
- To identify factors associated with hemodialysis-associated thrombocytopenia

4. Materials and Methods

4.1 Study area and period

This study was conducted in Addis Ababa, public Hospitals from May 1st to June 1st, 2019. Addis Ababa is the capital city of Ethiopia and it has a population size of 3,048,631 of whom 1,595,968are females and 1,452,663 are males. The city is divided in to 10 sub-cities (City Government of Addis Ababa, 2013). There are about 13 public Hospitals in the city which are distributed throughout ten sub cities (5 Federal, 6 under Addis Ababa health bureau, 1 owned by police force and 1 armed force hospital). Among these St. Paul's Hospital and millennium medical college, Menelik II Referral Hospital and Zewditu memorial hospital have renal dialysis centers and were used to collect patient data.

St. Paul's Hospital and millennium medical college has currently 33 dialysis machines providing dialysis for more than 80 patients per week, Menelik II Referral Hospital has 10 dialysis machines providing dialysis for more than 19 patients and Zewditu memorial hospital has currently 6 dialysis machines providing dialysis for about 20 patients per week.

4.2 Study design

- Institution based cross sectional study was conducted on patients who are under hemodialysis

4.3 Population

4.3.1 Source population

- Source populations were all CKD patients who visit St. Paul's Hospital and millennium medical college, Menelik II Referral Hospital and Zewditu memorial hospital during the study period.

4.4.2 Study population

- Study populations were CKD patients who undergo hemodialysis in St. Paul's Hospital and millennium medical college, Menelik II Referral Hospital and Zewditu memorial hospital during the study period and fulfill the eligibility criteria.

4.4 Inclusion and exclusion criteria

4.4.1 Inclusion:

- All patients who were undergoing hemodialysis at St. Paul's Hospital and millennium medical college, Menelik II Referral Hospital and Zewditu memorial hospital during the study period and volunteers were included.
- Those patients age eighteen and above were included.

4.4.2 Exclusion criteria

- Patients with known cases of liver disease, and Malignancy were excluded.

4.5 Sample size and Sampling Technique

4.5.1 Sampling size

- 100 patients who were undergoing hemodialysisinSt. Paul's Hospital and millennium medical college, Menelik II Referral Hospital and Zewditu memorial hospital during the study period.

4.5.2 Sampling Technique

- Convenient sampling technique was used; all patients who were undergoing hemodialysis in St. Paul's Hospital and millennium medical college, Menelik II Referral Hospital and Zewditu memorial hospital and those who were volunteers during the study period were involved

4.6 Study Variables

4.6.1 Dependent variables

- Platelet count
- Prothrombin Time /PT and
- Partial Thromboplastin Time /PTT

4.6.2 Independent variables

- Age
- Sex
- Duration of treatment
- Number of dialysis per week and
- Platelet number before dialysis

4.7 Operational definition

- Thrombocytopenia is a platelet count below 150×103 cells per microliter (Gauer and Braun, 2012) Mild Thrombocytopenia is platelet count of 100×10^3 to 150×10^3cells per microliter
- Moderate Thrombocytopenia is platelet count of 50×10^3 to 99×10^3cells per microliter
- Sever Thrombocytopenia is platelet count below 50×10^3cells per microliter
- Normal values; PT =11.0 to 12.5 seconds and on anticoagulant therapy >1.5 to 2 times
- PTT =30 to 40 seconds and on anticoagulant therapy >1.5 to 2.5 times (Pagana *et al.*, 2010)
- Renal replacement therapy includes hemodialysis, peritoneal dialysis and renal transplantation (Martinovic *et al.*, 2016).
- Age <50 younger age
- >50 elder age

4.8 Data and Specimen collection procedure

4.8.1 Data collection

- After informed consent was obtained from patients, all necessary information regarding socio-demographic characteristics, medical history and laboratory investigations was collected from the patients using well-structured and pretested questioner and Patients' card were reviewed.
- Pre-HD sample was taken before connecting patients to HD and post-HD sample was collected after 4 hours session of hemodialysis by trained Nurse under the supervision of the investigator.

4.8.2 Principle of laboratory test

- Platelet count: it is the number of thrombocytes derived from the platelet histogram and multiplied by a calibration constant. This number is expressed as: n x 10^3 cells/µl Procedures: After obtaining informed consent, Nurses collected 4-mL of blood in K3EDTA tube, and thoroughly mixed with anticoagulant. Blood samples were collected with double syringes technique for minimal clots contamination. Sample volume is 185 µl of whole blood in the closed-vial mode. The minimum sample volume per tube in the closed-vial mode is 1-mL with the proper proportion of blood to anticoagulant. Run the CBC as soon as possible
- To do PT test a sample of the patient's blood is obtained by venipuncture. The blood is decalcified (by collecting it into a tube with oxalate or citrate ions) to prevent the clotting process from starting before the test. The blood cells are separated from the plasma by centrifugation.
- The PT test is performed by adding the patient's plasma to some source of Tissue Factor (e.g.: a protein, thromboplastin, from homogenized brain tissue) that converts prothrombin to thrombin. The mixture is then kept in a warm water bath at 37°C for one to two minutes. Calcium chloride (excess quantities of ionized calcium) is added to the mixture in order to counteract the sodium citrate and allow clotting to start. The test is timed from the addition of the calcium chloride until the plasma clots.
- The prothrombin test specifically evaluates the presence of factors VII, V, and X, prothrombin, and fibrinogen. A prothrombin time within the 11 -15 second range (depends on the source of thromboplastin used) indicates that the patient has normal amounts of the above clotting factors.

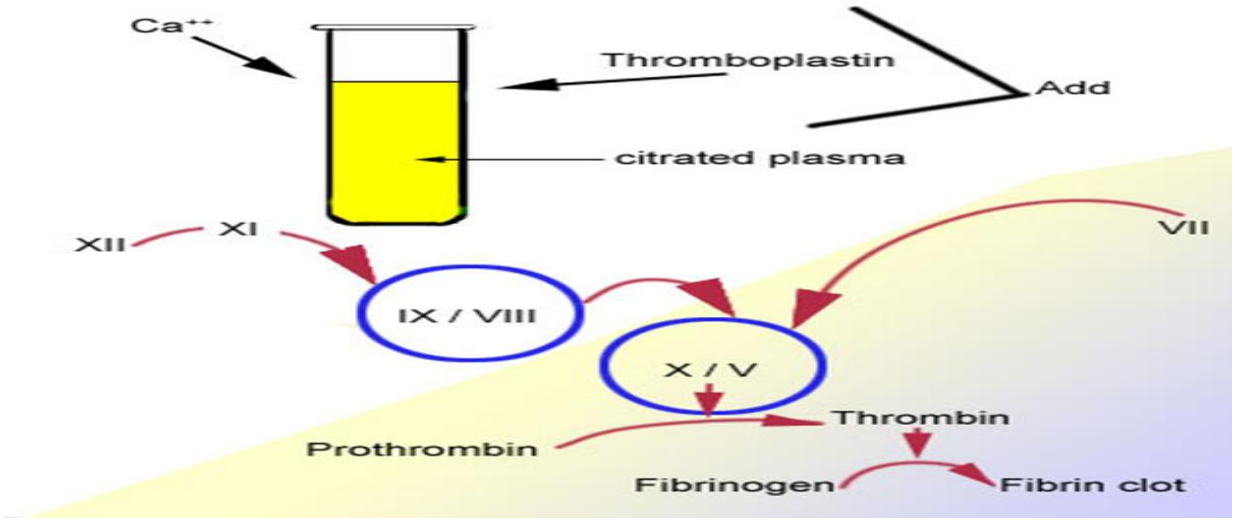

Figure 1: Laboratory Principle of Prothrombin Time (PT) Test

PTT test uses blood which is decalcified to prevent clotting before the test begins. The plasma is separated by centrifugation. (Ionized) Calcium and activating substances are added to the plasma to start the intrinsic pathway of the coagulation cascade. The substances are: kaolin (hydrated aluminum silicate) and cephalin. Kaolin serves to activate the contact-dependent Factor XII, and cephalin substitutes for platelet phospholipids. The partial thromboplastin time is the time it takes for a clot to form, measured in seconds. Normally, the sample will clot in 35 seconds.

4.9 Quality Control

- Data quality management started during questionnaire development by translating the questionnaires prepared in English language by reviewing different literatures into the local Amharic language. Before data collection, training was given for data collectors regarding the objective of the study, inclusion and exclusion criteria's, which group of patient, was involved in the study. Assigning these trained data collectors improve the data quality during data collection. Data quality was also assured in pre-analytic stages during blood sample collection by strictly following the standard aseptic operational procedure, labeling and transport of the sample, during analytic stages, in post-analytic stages data was checked for documentation and completeness. It was also checked that instruments used were properly calibrated before sample analysis.

4.10 Data entry and analysis

- Data entry, cleaning and screening was done exclusively by the principal investigator. The edited and cleaned data was entered to SPSS version 25 for analysis. Descriptive statistics was performed using Frequency tables, graphs, percentages, means and standard deviations and the association between dependent and independent variables as well as the correlation was determined by using logistic regression, paired t-test and parsons correlation. The 95% CI or and p-value less than 0.05 was used to determine a significant association.

4.11 Ethical consideration

- Ethical clearance and official letter was obtained from, Department of Biochemistry research ethics committeewith reference number SOM/BCHM/102/2011 to Addis Ababa public health research and emergency management directorate. Formal letters from Addis Ababa University department of biochemistry was written to,St. Paul's Hospital and millennium medical college, Menelik II Referral Hospital and Zewditu memorial hospital and study was carried out after obtaining permission from hospital authorities. The whole objective of the study was briefly explained to the laboratory head as well as those assigned in the dialysis unit and nephrology department.

4.12 Dissemination of results

- The study findings will be submitted to the Department of Biochemistry, College of Health Sciences and School of Medicine in Addis Ababa University and Addis Ababa public health research and emergency management directorate. Apart from this, effort will be made to disseminate the results through publications in local or international journals, presentation on annual scientific meeting and copy of the result will be offered to Tikure Anbesa specialized hospital library, Federal Ministry of Health (FMOH) and all other concerned bodies.

Result

The total numbers of patients in the study were 100 and mean age of the subjects was 40.25± 12.84 years, with a range of 18-76 years. Seventy percent of the study subjects were male. Almost about two third of the patients were married 61 (61%) and 34 (34%) were single. Majority of the patients77 (77%) reached secondary school and university, 21 (21%) completed elementary school and 2 (2%) were illiterate. The majority of the patients 94 (94%) were from Urban and 8 (8%) were from Rural areas**. (Table 1)**

Table 1: Socio-demographic characteristics of ESRD patient under hemodialysis in Addis Ababa Ethiopia from May 1st to June 1st, 2019: (N=100).

Independent Variables	Category	Total	
		Frequency	%
Sex of respondents	Male	70	70
	Female	30	30
Age of respondents	18-50	91	91
	>50	9	9
Place of residence	Urban	92	92
	Rural	8	8
Respondents education	No education	2	2
	Primary	21	21
	Secondary	30	30
	Higher	47	47
Marital status	Single	34	34
	Married	61	61
	Divorced	5	5

Clinical characteristics of respondents and factors associated with thrombocytopenia

All patients were treated by hemodialysis with hollow-fiber synthetic membrane and received regular doses of heparin before hemodialysis. The average time of one hemodialysis procedure was 4 hours.

The platelet count shows statically significant decrease (P=0.001) post –HD 178×103±71.8 cell/µl when compared to that of pre HD 233.5×103±72.13 cell/µl. Thrombocytopenia (platelet count <150,000cell/µl) was present in 14 % of patients and the study found that there is decrease in platelets count in (99%) of patients almost in all age intervals with only one patient showing stable count after dialysis (0.1%), The higher decrease range was among the age group (>60), followed by age group (35-49) .

Eighty-five percent of the study subjects had hypertension, 19% (19) had diabetes mellitus and only 5% had comorbidities likely to contribute to CKD, such as chronic glomerulonephritis and renovascular disease. The mean duration of hemodialysis was 0.78 ±0.42 years, with a range of 2 months to 3.5 years.

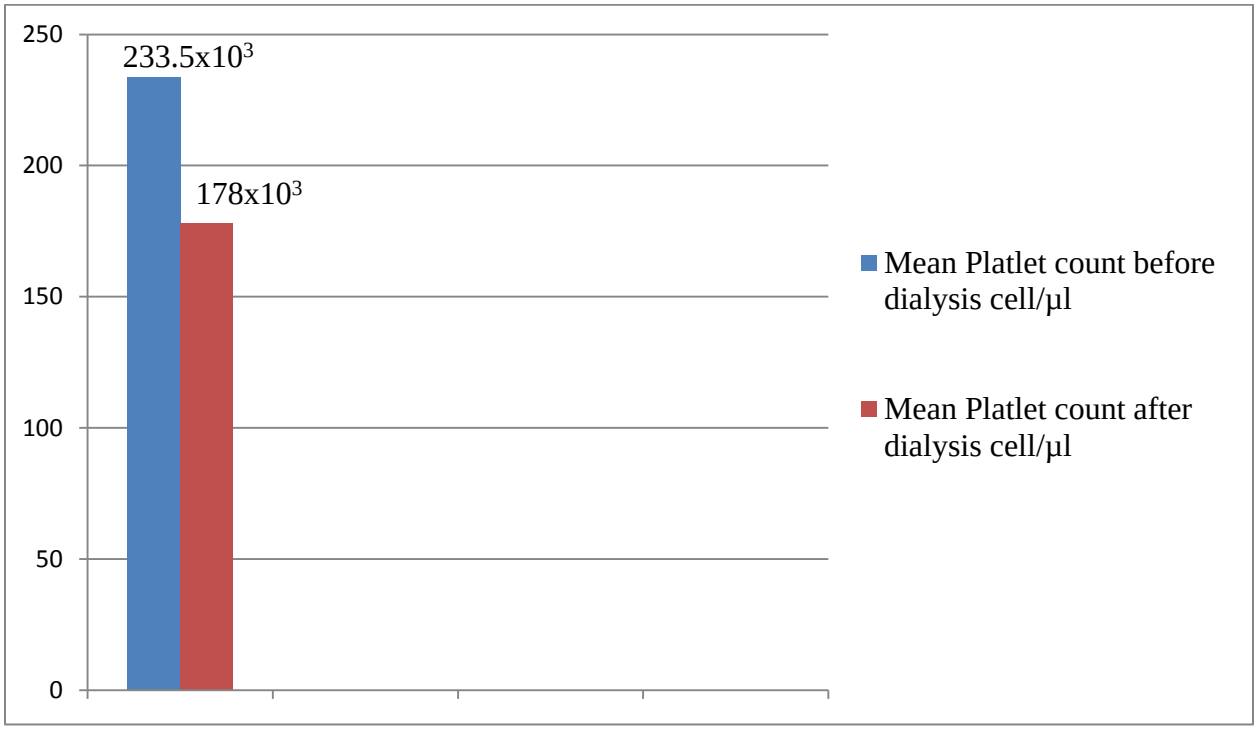

Figure 2: Mean platelet count before and after hemodialysis of ESRD patient under hemodialysis in Addis Ababa Ethiopia

In our study we have found that 2% (2), 8% (8) and4% (4) of the study participants had mild, moderate and sever thrombocytopenia respectively. (**Figure 3)**

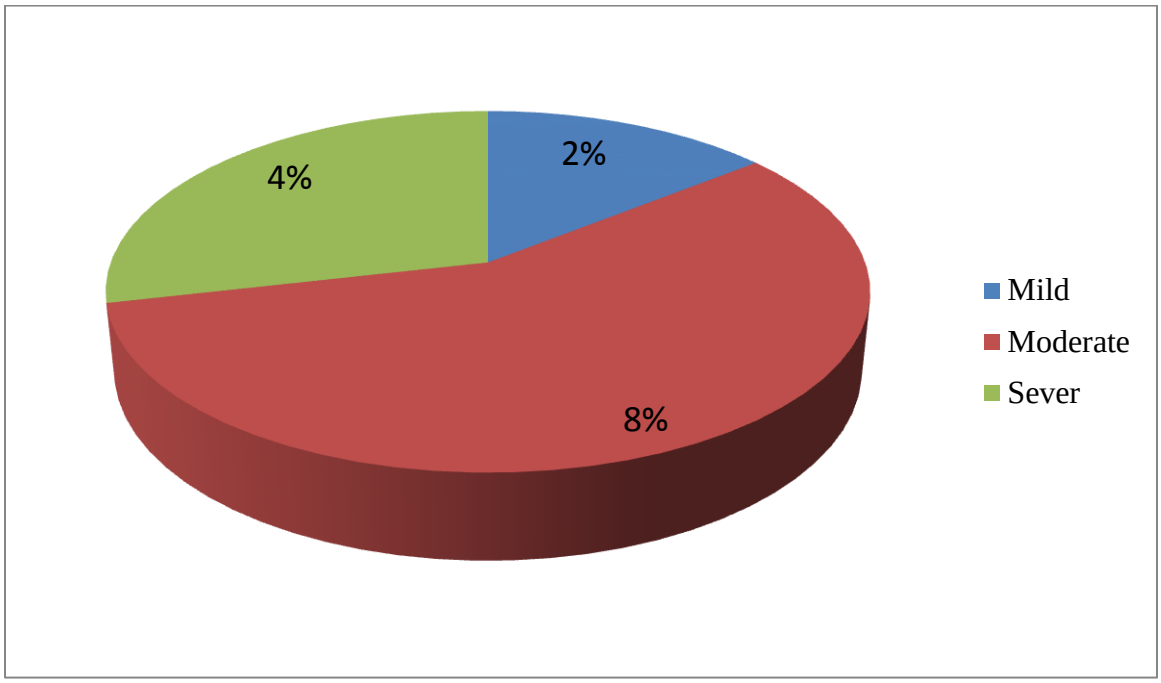

Figure 3: Degree of thrombocytopenia in ESRD patient after hemodialysis in Addis Ababa Ethiopia, from May 1st to June 1st 2019 (N= 100).

Pearson correlation analysis showed no correlation between platelet count and number of dialysis per week($r = 0.06$, $p = 0.6$), negative correlation was obtained between age of respondent and platelet count after dialysis ($r = -0.042$, $p=0.5$) while there was significant negative correlation between duration of treatment and platelet count after dialysis. Paired t-test also showed statically significant association between platelet count before dialysis and platelet count after dialysis ($p= 0.01$). (**Table 2**)

Table 2: Pearson correlation between of dependent and independent variables among ESRD patient under hemodialysis in Addis Ababa Ethiopia from May 1st to June 1st, 2019:

Correlations

		Age	Sex	Duration of treatment	Number of dialysis per week	Platelet count before dialysis (×103µ/L)	Platelet count after dialysis (×103µ/L)
Age	Pearson Correlation	1	-.020	.097	-.035	.070	-.042**
	Sig. (2-tailed)		.846	.337	.732	.491	.479
	N	100	100	100	100	100	100
Sex	Pearson Correlation	-.020	1	.007	.113	.016	-.099
	Sig. (2-tailed)	.846		.947	.265	.876	.328
	N	100	100	100	100	100	100
Duration of treatment	Pearson Correlation	.097	.007	1	-.088	-.017	-.519**
	Sig. (2-tailed)	.337	.947		.383	.863	.000
	N	100	100	100	100	100	100
Number of dialysis per week	Pearson Correlation	-.035	.113	-.088	1	.012	.059
	Sig. (2-tailed)	.732	.265	.383		.902	.563
	N	100	100	100	100	100	100
Platelet count before dialysis (×103µ/L)	Pearson Correlation	.070	.016	-.017	.012	1	.339
	Sig. (2-tailed)	.491	.876	.863	.902		.001
	N	100	100	100	100	100	100
Platelet count after dialysis (×103µ/L)	Pearson Correlation	-.042**	-.099	-.519**	.059	.339	1
	Sig. (2-tailed)	.479	.328	.000	.563	.001	
	N	100	100	100	100	100	100

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

Bivariate analysis showed that sex (COR=1.38), duration of treatment (COR=0.43) and age of patients (COR=0.40) were not significantly associated with hemodialysis associated thrombocytopenia. **(Table 3)**

Table 3: Factors associated with thrombocytopenia among Patients on renal replacement therapy in Addis Ababa Ethiopia from May 1st to June 1st, 2019: (N= 100)

Variables	**Platelet count after dialysis ($\times 10^3$µ/l)**		**COR (95%CI)**	**P-Value**
	<150	>=150		
Sex				
Female	9(12.9%)	61(87.1%)	1.38(0.4,4.702)	0.608
Male	5(16.7%)	25(83.3%)		
Age				
18-49	9(21.4%)	33(78.6%)		
≥50	5(8.6%)	53(91.4%)	0.4(0.12, 1.356)	0.142
Duration of treatments				
<12 weeks	5(26.3%)	14(73.7%)		
≥ 12weeks	9(11.1%)	72(88.9%)	0.43(.118, 1.531)	0.191

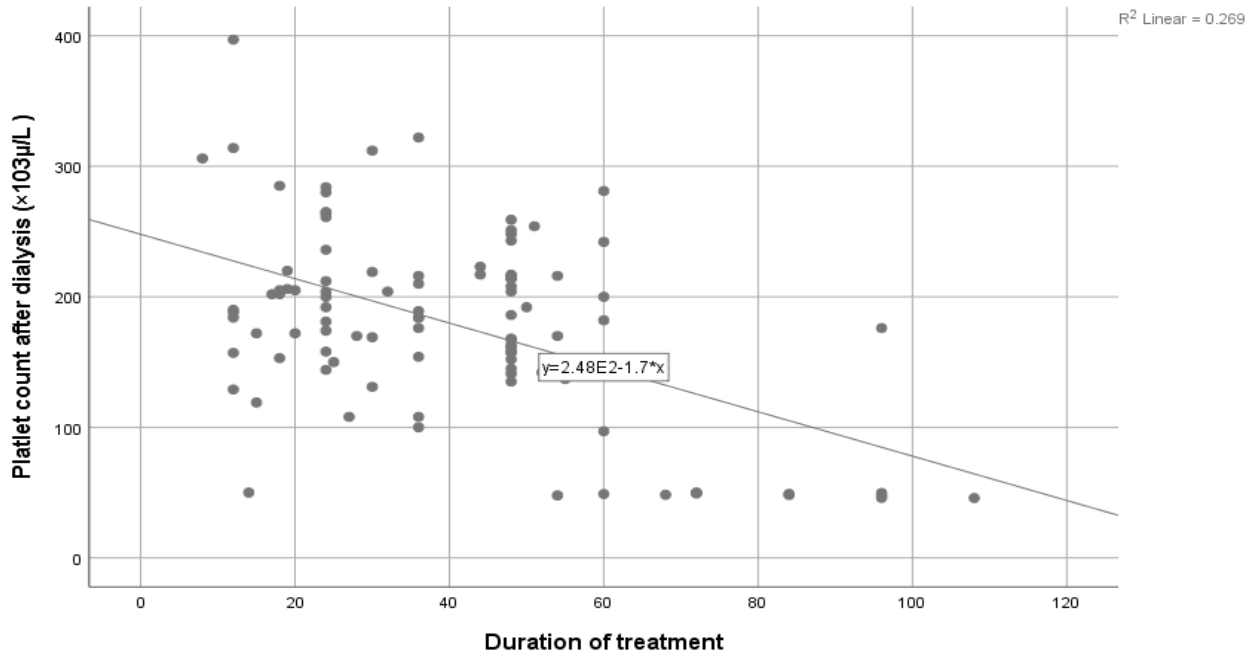

Figure 4: Scatter plot shows inverse relation of duration of hemodialysis and platelet count after hemodialysis of ESRD patients before and after hemodialysis in Addis Ababa Ethiopia.

Prothrombin time/ PT showed slight increase post HD (16.66±3.19) seconds compared to pre HD (15.4±5.69) seconds. Likewise PTT level showed slight increase post HD. 34±6.76 seconds compared to pre HD 33.8±5.34 seconds. **(Figure 5)**

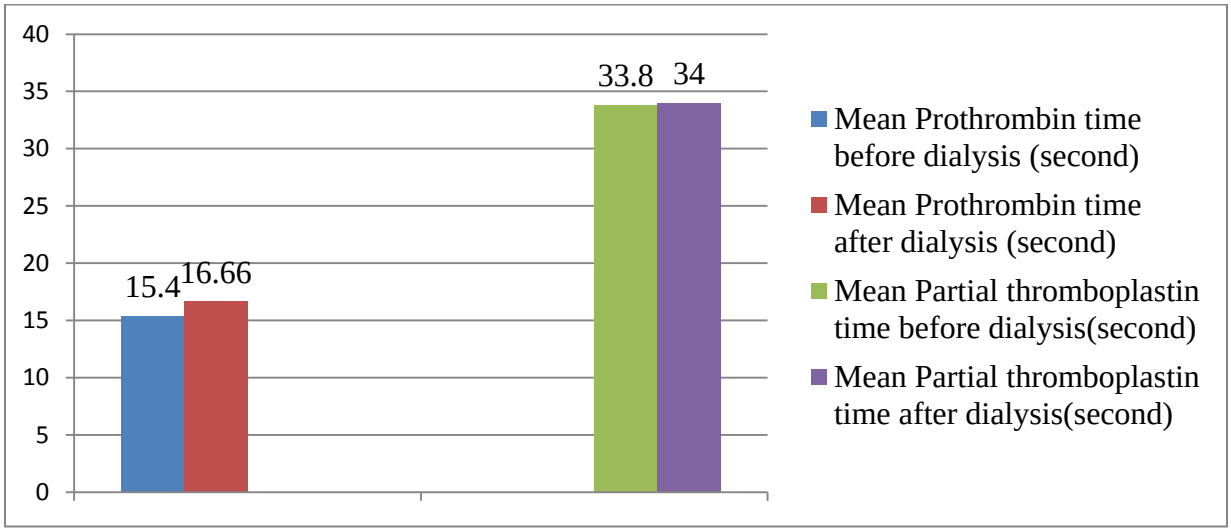

Figure 5: Mean Prothrombin time and partial Thromboplastin time of ESRD patients before and after hemodialysis in Addis Ababa Ethiopia

6. Discussion

6.1 Hemodialysis associated thrombocytopenia among patients on renal replacement therapy.

Hemodialysis procedure could influence hemostasis by two distinct pathways: the first is by the effect of the dialysis membrane, the composition of the dialysis circuit, and changed rheology, and second, by the effect of added anticoagulants(Miso *et al.*, 2005). Hakim and chafer (1995) also suggested that thrombocytopenic episodes occurring with hemodialysis were associated with complement activation, specifically C3a, in addition to activation of platelets themselves Complement activation occurred specifically in the setting of cuprophane membranes, and thrombocytopenia was only observed in the presence of complement activation. Another possible explanation for the thrombocytopenia is that Platelet surface markers measured in dialyzer outlet blood give evidence for both platelet activation and degranulation in the course of dialysis. In addition, there is evidence for formation of platelet–platelet and platelet–leukocyte aggregates (Attika and Lubna, 2015).

This study therefore tried to explore the effect of hemodialysis on platelets count, coagulation parameters and associated factors among ESRD patients and we first investigated, the effect of hemodialysis on platelet count by comparing patients' platelet count before hemodialysis (Patients' card were revived) and after hemodialysis.

All patients were treated by hemodialysis with hollow-fiber synthetic membrane and received regular doses of heparin before hemodialysis. The average time of one hemodialysis procedure was 4 hours and 92% of patients were treated by hemodialysis three times per week whereas 8% of patients were treated two times per week. In this study the mean platelet count before hemodialysis was found to be 233.5×103±72.13 cell/µl which is statistically significant (P=0.001) when compared to that of mean platelet count after hemodialysis (178×103±71.8cell/µl) and 14% of the patients had thrombocytopenia (platelet count<150×103cell/µl). These findings may be clinically relevant since decrease in platelets count after hemodialysis may contribute to an increased bleeding tendency in some susceptible hemodialysis patients. Similarly, Alghythan AK *et al* (2012) and his collogue observed that the mean platelet count in pre HD patients was 199.19±0.5674×103 cell/µl and they also observed further fall of platelet count reaching 176.86±0.5608×103cell/µl after HD. Another previously

conducted study by Dorgalaleh *et al* (2013) found that the mean platelet count among patients on renal replacement therapy to be 172±0.9×103cell/µl which was low compared to controls. Similarly, Chakravarti *et al* (2017) also observed fall of platelet count and 13.16% of the patients had thrombocytopenia and another similar study conducted by Akbar et al (2013) observed 8% thrombocytopenia and witnessed a drop of platelet count after hemodialysis. Gafter *et al* (1987) also found that the mean platelet count among patients on renal replacement therapy to be significantly reduced (175±0.06×103cell/µl) compared to controls. Knudsen F *et al* (1985) also reported a decrease in the number of platelets after HD. Schoorl *et al* (2013) observed that chronic hemodialysis patients had lower range of platelet counts below the reference limits.

Another similar study done by Abdullah *et al* (2012), revealed that the patients with renal failure are at high risk of bleeding due to thrombocytopenia and platelet dysfunction and another study conducted by Yenicerioglu *et al* (2000) also revealed that there was a statistically significant decrease in the mean platelet counts, though still within the normal range, ESRD patients, pre- and post-HD when compared to the results of the control group. The mean platelet counts in the study showed a significant decrease in patients' after-HD when compared to pre-HD procedures. Yasir *et al* (2016) also reported that 99.5% of patients have reduced platelets count after hemodialysis, while 0.5% has stable count of platelets. Lokesh *et al* (2016) although observed fall of platelet count to 239±0.74×103cell/µl and in contrast of the present study none of the cases had thrombocytopenia. This could also be explained by the reduction of the number of HD sessions.

In contrast to this study, Gawaz and Wardle (2004) reported that Platelet counts in the hemodialysis group were not different from those in the control group and arterial platelet counts changed significantly (P =0.01) during the time of dialysis. The magnitude of the change was small, however, being no greater than 5% at any sampling time. There was no change in platelet count between the inlet and outlet of the dialyzer at any sampling time. Similarly, Nasr *et al* (2013) showed that there were no differences in the mean platelet count before, mid, and post dialysis.

This difference may be because of inclusion of patients with all the three types of renal replacement therapies including renal transplant and peritoneal dialysis in their study which generally tends to improve bleeding tendency while in this study only hemodialysis patients were involved. In contrast to the above findings, Mohamed *et al* (2008) reported that moderate

increase in the number of platelets after HD and, although without statistical significance. This difference may be because of longer duration of HD in the current study and difference in the type of membrane used.

6.2 Factors associated with thrombocytopenia among Patients on renal replacement therapy

During hemodialysis, platelets, coagulation and fibrinolytic systems could be importantly affected due to several known and unknown factors Miso *et al* (2005). In this study there was no association between platelet count after hemodialysis and number of dialysis per week. This is supported by Nasr *et al* (2013).

The study results also have shown that sex (COR=1.38), duration of treatment (COR=0.43) and age of patients (COR=0.40) were not significantly associated with hemodialysis associated thrombocytopenia. Similarly, another study had shown that the duration of HD and PD did not affect the hematological parameters with the exception of the red cell count, which is significantly decreased as the duration of HD increase Mohamed *et al* (2008). This is supported by Nasr et al (2013) who reported that age does not affect the results of platelets count post dialysis. Similarly Lokesh S *et al* (2016) also reported that there is no correlation between blood platelets count and the time period patients are on dialysis Whereas Abdullah *et al* (2012) and Yasir *et al* (2016) got higher decrease in Platelet count among the age group 45-54 followed by group 75-84 and negative significant correlation between duration of dialysis and platelets. Abdullah *et al* (2012) also indicate that most of the hematological parameters measured in HD patients, pre- or post-HD were either elevated or lowered compared to the control groups. Furthermore, it was noticed that there were changes in these parameters between post-HD and pre-HD levels. More significantly, PT, APTT and fibrinogen were found to increase post-HD compared to pre-HD procedures while there was a concurrent decrease of platelet counts. A significant positive correlation was noticed between the time periods that patients have been on dialysis and each of fibrinogen, APTT and PT levels. However, there was a significant negative correlation between the time periods the patients have been on dialysis and platelets count. Thus, the longer the time period the patients are on dialysis, the more the coagulation profile is affected, that is, fibrinogen, APTT and PT levels increase with longer duration of HD whereas the platelet count decreases with longer duration of HD. Similarly, Ando *et al* (2001) indicated

that there was a significant negative correlation between the time period the patients have been on dialysis and platelets counts.

This difference may be because of the difference in the socio-demographic characteristics of the study participants and the type of membranes used.

In developing countries, common causes of CKD also include glomerular and tubule-interstitial diseases due to infections and exposure to drugs and toxins. In addition to non-communicable diseases, communicable diseases such as infectious glomerulonephritis, schistosomiasis, leishmaniasis, and HIV infection are common and can cause CKD. Because more than 22 million people in sub-Saharan Africa have HIV, the potential for an overwhelming burden of CKD in the region is high Sanyaolu *et al* (2018). However, Hypertension and diabetes mellitus were identified as the main risk factors for CKD in this study; 85% (85) of the patients had hypertension and 19% (19) had diabetes mellitus in consistence with this study hypertension is a cause of chronic kidney failure in Africa, especially in Black patients. Hypertension affects <25% of the adult population and is the cause of chronic kidney failure in 21% of patients on renal replacement therapy in the South African and hypertension was the most common cause of end-stage renal disease (ESRD) in Black South Africans and accounted for 34.6% of ESRD in that racial group.

In contrast, hypertension was reported to be the cause of ESRD in 4.3% of Whites, 13.8% of Indians, and 20.9% of people of mixed ancestry (George *et al.*, 2015). In a study to determine the pathologic basis of ESRD in Black South Africans, essential malignant hypertension was the single most common cause of ESRD, occurring in 49% of patients (Yasir *et al.*, 2016). Another study reported that about 44% of ESRS patients are diabetics. Glomerulonephritis and cystic kidney disease also remain relatively steady as a cause of ESRD. Similarly Diabetes is the major cause of ESRD worldwide, in both developing and developed countries; In Australia the incidence of ESRD due to diabetes is about 25% as well in European Union registry, the number of diabetics with ESRD is about 15% - 33% while due to glomerulonephritis is about 9% - 20%. This was similar to studies by Bhatta S *et al* (2011) and Chakravarti *et al* (2017).

However, Chinwuba et al (2010) found the prevalence of hypertension (22.5%) and diabetes mellitus (15.1%) to be lower than that of chronic glomerulonephritis (37%) among CKD patients. This could also be explained by the difference in the socio-demographic characteristics of the study participants.

6.3 Hemostatic parameters of Patients on Renal Replacement Therapy

Disturbances in hemostasis are common complications of kidney disease. Both bleeding diathesis and thromboembolism have been identified. The principle cause of these abnormalities is the uraemic state. The pathogenesis of uraemic bleeding is multifactorial; The most important determinants of pathogenesis is increased levels of clotting factors, decreased levels of clotting inhibitors, diminished fibrinolytic activity and platelet hyperaggregability (Subhanud *et al.*, 2013).

The present study results show a comparable trend in mean PT and PTT to regional study done by Shoaib M *et al* (2008) on 61 patients receiving renal replacement therapy. The study suggested that mean PT and PTT to be 15.6 and 34 seconds respectively. Similarly, Ulusoy et al (2004) measured the PT and PTT in ESRD patients undergoing HD, before and after HD sessions and they found increase in PTT value while there was an insignificant difference in PT levels in pre and post HD patients. Romeo JE *et al* (1997) also observed that post HD PT and PTT to be 15.0±0.81 seconds and 87.30±27.96 seconds respectively. The prominent increase in mean PTT in these patients is probably because of the inclusion of patients with already prolonged PTT in pre dialysis samples with a mean pre dialysis PPT 39.70±4.30 seconds in contrast to this study where the pre dialysis mean PTT was 33.8±5.34 seconds. In the present study, conventional hemostasis parameters (PT and APTT) were slightly increased after-HD when compared to the before-HD level; though it is in the normal range. This finding can be explained by using systemic anticoagulation (heparin) during conventional HD for extracorporeal procedures which binds to the enzyme inhibitor antithrombin III. This result in the inactivation of thrombin and other proteases involved in blood clotting, most notably FXa (Wardle *et al*., 2002). Another possible factor that has been reported by Naumnik *et al* (2002) could account for the increased hemostasis parameters would be the increase in the level of tissue factor path way inhibitor (TFPI). It is potent inhibitor of the extrinsic coagulation pathway that results in the reduction of activity of several coagulation factors during HD, including factor II, IX, X, and XII.

In contrast to this study, Alghythan AK *et al* (2012) studied 100 patients' pre and post HD for hemostatic parameters that is PT and PTT; the reported mean PT was found to be 23.10±5.69 whereas, mean PTT was 64.64±13.61 seconds and Mohammad *et al* (2008) also showed that mean PT and PTT post HD as 23.2 and 69.8 seconds respectively. The results of studies

mentioned above are analogous to the results of this study with the general tendency of increase in mean PT and PTT after HD. The striking difference in post HD mean of both parameters which are higher from this study may be explained by the difference in the type and dose of anticoagulant used as different anticoagulants in different dosage are preferred in various setups and have a significant effect on the bleeding tendency and change in coagulation parameters post HD. Similarly the individual variation in the condition of the patient, reduction in the time duration of dialysis and decrease in number of previous dialysis may cause the discrepancy. Another study conducted by Malyszko J *et al* (2001) also reported that the dissimilarity could be explained by the difference in the dose of heparin as well as the reduction of the number of HD sessions.

7. Strength and Limitation

7.1. Strength of the study

- This study could be an input for further studies and policy makers as it identified higher prevalence of thrombocytopenia among patients on renal replacement therapy.

7.2. Limitation of the study

- The study did not show cause and effect relationship
- The study was conducted only in public hospitals in Addis Ababa Ethiopia
- Using limited parameters was also a limitation

8. Conclusion and Recommendation

8.1. Conclusion

Over all prevalence of thrombocytopenia was higher and there was decrease in mean platelet count after hemodialysis in ESRD patients undergoing hemodialysis.

Coagulation parameters; prothrombin time and partial thromboplastin time showed slight increase after hemodialysis among ESRD patients.

Bivariate analysis showed that sex, duration of treatment and age of patients were not significantly associated with hemodialysis associated thrombocytopenia

8.2. Recommendations

- It is recommended to have research on large number of patients and different parameters.
- It is also advisable to conduct research that can incorporate hospitals from different regions.

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Annex I: English Version Information Sheet

Addis Ababa University, School of Medicine, Department of Medical Biochemistry

Research project: Assessment of hemodialysis-associated thrombocytopenia and associated factors among patients on renal replacement therapy in Addis Ababa Ethiopia

Principal Investigator: Seblework Abeje (BSc)

Advisors: 1-Dr. Solomon Genet 2-Dr. Menakath Menon

Sponsoring organization: Addis Ababa University

Introduction: Good morning/afternoon dear participant! My name is _____. I am working as a data collector for the study conducted in St. Specialized Hospital by Seblework Abeje who is studying for MSc degree in Medical Biochemistry at Addis Ababa University College of Health Sciences. I kindly request you to lend me your attention to explain about the study and being you selected as the study participant.

Study Objective: The purpose of this study is to assess hemodialysis-associated thrombocytopenia and associated factors among patients on renal replacement therapy at SPHMMC, Menelik II Referral Hospital and Zewditu memorial hospital Addis Ababa Ethiopia

Procedures: If you agree to take part in this study, you will be given the consent form to sign, and interviewed by health professional to assess whether you qualify to participate in the study or not. If you are fit for the study, the data collector will ask some questions which are important for the study like socio-demographic data, duration of hemodialysis, history of comorbidities, number of dialysis per week and 4mL of blood sample will be also collected for laboratory examination of platelet count PT and PTT determination.

Possible risks: The study has no health risk except minimum pain associated with blood with drawl procedure. This is just the result of the routine work which experienced by all then individuals that will involve in the research by experienced health practitioner.

Possible Benefit: Any incentive to you during participation in this study will not be given as compensation. But you will have the chance to know your general health status from the project without any payment because the cost will be covered by the project. In addition, your genuine answer have paramount importance to the outcome of the research and the findings of the study

will be used for better understanding of the prevalence of the thrombocytopenia among hemodialysis patients . So that it can be helpful for planning and intervention on the problem under the study.

Confidentiality: The information you provide for us will be confidential and only used for research purpose. There will be no information that will identify you in particular. The findings of the study will be general for the study area and will not reflect anything particular of individual persons. The questionnaire will be coded to exclude showing names. No reference will be made in oral or written reports that could link participants to the study.

Rights: To protect the participant from any risk and discomfort which may result due to the procedure of the study, we have written ethical clearance and cooperation letter obtained from Addis Ababa University College of Health Sciences, Department of Biochemistry.

Participation for this study is fully voluntary. You have the right to declare to participate or not in the study. If you decide to participate, you have the right to with draw from the study at any time. At the end, if you faced any problem related to the study or if you have any question about the study, you can contact me (the investigator) via phone: 0910122104/0926328294 or by using email address: seblea319@gmail.com.

Annex II English Version Consent form

I volunteer to participate in a research project conducted by Seblework Abeje from Addis Ababa University. I understand that the project is designed to gather information about hemodialysis-associated thrombocytopenia and associated factors among patients on renal replacement therapy at St. Paulo's general specialized hospital, Menelik II Referral Hospital and Zewditu memorial hospital Addis Ababa Ethiopia, I will be one of participants being selected for this research. I understand that I will not be paid for my participation. I may withdraw and discontinue participation from the study at any time without penalty. I understand that the researcher will not identify me by name in any reports using information obtained from this interview and that my confidentiality as a participant in this study will remain secure. Subsequent uses of records and data will be subject to standard data use policies which protect the anonymity of individuals and institutions.

I have been given a copy of this consent form and I have clearly understood the explanation provided to me. So, I hereby approve my consent with my signature to take part in the study.

_____	_____	_____
Participant's code number	Date	Signature
_____	_____	_____
Data Collector's name	Date	Signature

Thank you for participating in the study

Annex III English Version Questionnaire

Questionnaire on assessment of hemodialysis-associated thrombocytopenia and associated factors among ESRD patients in St. Paulo's specialized hospital Addis Ababa Ethiopia 2019

I. Socio demographic information

01. Identification number of respondents----------

101. Age _____

102. Sex 1. Male 2.female

103 Marital status 1.Single 2.Married 3.Divorced 4.widowed

104. Educational status 1) Illiterate 2) Read& write 3) primary school 4) secondary school 5) collage and above

105. Residence 1) urban 2) Rural

II). History of comorbidity and treatment

201. Duration of treatment …….

202. Duration of one session……

203. Number of dialysis per week…….

204. Types of member

205. Comorbidities 1) Diabetes mellitus 2) Hypertension 3) both 4) other

III) Laboratory Examination	before HD	after HD
301. Platelet count ____	……	……
302. PT_____	……	…….
303. PTT_____	……..	……

THANK YOU

Annex IV: አማርኛለተሳታፊዎችስለጥናቱመረጃ

ጤናይስጥልኝ! እኔ_____ እባላለሁ፡፡

በአሁኑሰአትበአዲስአበባዩኒቨርሲቲጤናሳይንስኮሌጅሕክምናት/ቤትበባዮኬሚስትሪት/ክፍልበማስተር ስድግሪየሚያጠናውሰብለወርቅአበጀለሚያደርገውየመመረቂያጥናትበመረጃሰብሳቢነትእየሰራሁእገኛ ለሁ፡፡እናምእርስዎበዚህየመመረቂያጥናትላይእዲሳተፉተመርጠዋል፡፡

እባክዎበዚህጥናትለመሳተፍከመስማማትዎበፊትከዚህቀጥሎየሚገኘዉንምንባብበጥሞናያንብቡናግ ልጽያልሆነልዎትንማንኛዉምሃሳብይጠይቁ፡፡

የጥናቱርዕስ፡-

የኩላሊትእጥበትክትትልበሚያደረጉታካሚዎችላይስለኘላትሌትማነስናተዛማጅጉዳዮችለማጥናትየሚ ደረግምርምርነው፡፡

የጥናቱተሳታፊለመሆንየሚጠበቅበዎት:

በዚህጥናትለመሳተፍየሚስማሙከሆነየደምናሙናእንደሚወሰድናለጥናቱእንዲሚዉልመስማማትይ ጠበቅብዎታል፡፡

ከተወሰደዉናሙናላይየሚገኙመረጃዎችከዚህሆስፒታልዉጭለሚገኙናለስራዉአግባብነትላላቸዉሰ ዎችቢነገርየማይቃወሙመሆኑንመስማማትይጠበቅብዎታል፡፡

ይሁንእንጅይህአይነቱመረጃየርስዎንማንነትየሚገልጡመረጃዎችንማለትምስም፤

አድራሻናየስልክቁጥርየመሳሰሉትንመረጃዎቸንአይጨምርም፡፡

ይልቁንምለዚህአገልግሎትብቻየሚዉልአርስዎንለማወቅየሚያስችልመለያቁጥርጥቅምላይእንዲዉል ይደረጋል፡፡

በተጨማሪምስለርስዎአጠቃላይየጤናሁኔታለሚቀርቡአንዳንድተጨማሪጥያቄዎችመልስመስጠት፡፡

በዚህጥናትመሳተፍሊያስከትላቻውየሚችሉቸግሮች:

ናሙናበሚሰበሰብበትወቅትምንምአይነትየከፋችግርአያጋጥምዎትም፡፡

ነገርግንደምሲወሰድመጠነኛየህመምስሜትሲያስከትልይችላል፡፡
ሆኖምግንናሙናዉንለመሰብሰብልምድያለዉባለሙያስለሚመደብናአስፈላጊዉየጥንቃቄእርምጃስለ
ሚወሰድየህመምስሜትአይኖርም፡፡በዚህጥናትመሳተፍሊገኙየሚችሉጥቅሞች:
ይህጥናትየማስተርስዲግሪመመረቂያእንደመሆኑመጠንበዚህጥናትበመካፈልምበገንዘብየሚያገኙት
ጥቅምባይኖርምከጥናቱበሚገኝውውጤትግንተጠቃሚነዎት፡፡
ከእርስዎየሚገኘዉመረጃየኩላሊትእጥበትክትትልበሚያደረጉታካሚዎችላይየሚከሰተዉንየኘላትሌት
ማነስችግርለመከላከልከፍተኛሚናስለሚኖረዉለምንጠየቅዎትጥያቄትክክለኛመረጃእንዲሰጡንበትህ
ትናእንጠይቃለን፡፡
የመረጃበሚስጥርአጠባበቅሁኔታ:
ስለራስዎየሰጡትማንኛዉምመረጃናከተወሰደዉናሙናላይየተገኘዉየላቦራቶሪዉጤትየሚዉለዉለጥ
ናቱአላማብቻነዉ፡፡
የጥናቱውጤትየተሳታፊዎችንአጠቃላይሁኔታእንጂየአንድንግለሰብምንምነገርአያንጸባርቅም፡፡
የተሳታፉዎችንስምላለማሳየትየራሳችንንመለያቁጥርየምንጠቀምይሆናል፡፡
ይህንማህደርሊያገኙየሚችሉትየተወሰኑየጥናቱተባባሪሰዎችብቻናቸው፡፡
ከዚያምበላይስለእርስዎያለውንማንኛውንምመረጃየተለየየይለፍቃልባለውየኮምፒውተርየመረጃማህደ
ርውስጥእንዲቀመጥይደረጋል፡፡
በዚህጥናትላለመሳተፍያለዎትመብት:
በዚህጥናትመሳተፍሙሉበሙሉበእርስዎፈቃደኝነትየተመሰረተበመሆኑበማንኛውምሰዓትናቦታየማ
ቋረጥሙሉመብትየተጠበቀከመሆኑምበላይእራስዎንከጥናቱበማግለልዎምክንያትየሚቀርብዎትምን
ምአይነትየሆስፒታልአገልግሎትአይኖርም፡፡
ከዚህምበተጨማሪጥናቱንበተመለከተማንኛውንምአይነትጥያቄየመጠየቅናገለጻየማግኘትመብትአለ
ዎት፡፡የላብራቶሪምርመራውጤቱንምበነጻማግኘትይችላሉ፡፡ለን፡፡
ጥያቄካልዎትወይምችግርካጋጠመዎት:
ይህንንጥናትበተመለከተወይምከዚህጥናትጋርበተዛመደመልኩችግርከገጠምዎትወይምጥያቄካለዎት
በስልክቁጥር፡ 0910122104/0926328294 ወይምበኢ-ሜይል:seblea319@gmail.com፤
የሚለውንአድራሻይጠቀሙ፡፡
Annex V: አማርኛየስምምነትማረጋገጫቅጽ
የዚህጥናትመሰረታዊዓላማእናሌሎችመረጃንበሚገባተገንዝቢያለሁ፡፡
ጥናቱበአዲስአበባበሚገኙየኩላሊትእጥበትታማሚዎችላይየኘላትሌትመጠንለመለካትእንደሆነበሚገባ

ተገንዝቢያለሁ፡፡ተሳትፎዬበፍቃደኝነትላይብቻየተመረኮዘእንደሆነምተረድቻለሁ፡፡
ማንኛዉምሰብዓዊምሆነህጋዊመብቴሳይነካከጥናቱራሴንማግለልእንደምችልምእንደሆነ፡፡
ስለጥናቱዝርዝርጉዳይበግልፅከተረዳሁትባሻገርበተጨማሪማብራሪያብፈልግመጠየቅእንደምችልምአ
ዉቂያለሁ፡፡
የጥናቱምባለቤትየዚህየጥናቱመረጃይፋየሚሆነውለእኔብቻእንደሆነእናስለሚወሰደውማንኛውምመረ
ጃዎችሆነየጥናትውጤትለማሰራጨትበስምሳይሆንበሚስጥር(ኮድ) እንደሆነምተረድቻለሁ፡፡
በመሆኑምበፈቃዴየዚህጥናትአካልእንድሆንስፈልግየምጠብቅብኝንሁሉለሚድረግበመወሰንመሆኑን
በፊርማዬአረጋግጣለሁ፡፡

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የተሳታፊየምስጥርቁጥርቀንፊርማ

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የመረጃሰብሳቢውስምቀንፊርማ

ስለፈቃደኝንዎትዎአመሰግናለሁ!

Annex VI አማርኛመጠይቅ

01. የመጠይቁተሳታፊዎችመለያቁጥር----------

ክፍል 1 ኢኮኖሚያዊናማህበራዊመረጃዎች			
ተ.ቁ	ጥያቄዎች	መልሶች	ኮድ
101	እድሜ	----------ዓመት	
102	ፃታ	1. ወንድ 2.ሲት	
103	የጋብቻሁኔታ	1. ያላገባ/ች 2. ያገባ/ች 3. የፈታ/ች 4. የሞተበት/ባት	
104	የትምህርትደረጃ	1. ማንበብእናመፃፍየማትችል 2. ማንበብናመፃፍብቻየምትችል 3. የመጀመሪያደረጃትምህርትየተማረች 4. የሁለተኛደረጃደረጃትምህርትየተማረች 5. ኮሌጅእናከዛበላይ	
105	የመኖሪያአድራሻ	1. ገጠር 2. ከተማ	
ክፍል 2 የህክምናናየተጋዳኝበሽታዎችታሪክ ----------			
201	ህክምናከጀመርሽ/ክ ስንትጊዜነዉ?	----------	
202	በሳምንትስንትጊዜየኩላሊት እጥበትያደርጋሉ	-	
203	ስንትሲዓትየኩላሊት እጥበትያደርጋሉ	-	
204	ተጋዳኝበሽታዎች	1.ስካር 2.የደምግፊት 4. ሌላ	

የመረጃሰብሳቢዉ/ዋስም _____

ፊርማ ----------

ቀን----------አመሰግናለሁ!!!!!