EVALUATION OF PROTHROMBIN TIME, INTERNATIONAL NORMALIZED RATIO AND ACTIVATED PARTIAL THROMBOPLASTIN TIME TESTS IN DIABETES MELLITUS PATIENTS AT MERU TEACHING AND REFERRAL HOSPITAL IN KENYA

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JOMO KENYATTA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY

2021

Evaluation of Prothrombin Time, International Normalized Ratio and Activated Partial Thromboplastin Time Tests In Diabetes Mellitus Patients At Meru Teaching And Referral Hospital In Kenya

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A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Medical Laboratory Sciences (Clinical Hematology and Blood Transfusion Sciences) of the Jomo Kenyatta University of Agriculture and Technology

2021

DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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This thesis has been submitted for examination with our approval as university supervisors

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DEDICATION

This research work is dedicated to my wife Esther, my children Faith and Francis, my brother Anthony and my mum Margaret for their moral support.

ACKNOWLEDGEMENT

I am extremely grateful to my supervisors Dr. Kimani and Dr. Kahato for their unwavering support and guidance since the conception of the study proposal leading to the completion of this research. Sincere gratitude to KNH/UoN ethical review committee for allowing me carryout this research as well as National Commission for Science, Technology and Innovation for giving me the permit to carry on with the research.

I salute the Chief Officer for health Meru County, the CEO Meru Teaching and Referral Hospital, laboratory manager and laboratory staff members for warmly allowing me carry on with this research. Thanks a lot. The Lord bless you.

I appreciate my research assistant Mr. Albanous; - you did a wonderful job, may the Lord be with you always. Catherine Makandi, your guidance in data analysis is highly appreciated. Thank you study subjects for your good will by participating in this research.

Above all, my sincere gratitude, appreciation and praises goes to the Mighty Lord and God for a wonderful health and excellent skills that He gave me during this wonderful work. Praise to you oh Lord forever and ever.

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ABBREVIATIONS AND ACRONYMS

APTT	Activated partial thromboplastin time
BMI	Body mass index
CVD	Cardiovascular disease
DM	Diabetes mellitus
FDPs	Fibrin degradation products
INR	International normalized ratio
ISI	International sensitivity index
MTRH	Meru Teaching and Referral Hospital
NCDs	Non communicable diseases
PAI-1	Plasminogen activator inhibitor type-1
PAP	Plasmin-antiplasmin complex
PT	Prothrombin time
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
TF	Tissue factor
TFP1	Tissue factor pathway inhibitor

tPA	Tissue plasminogen activator
VTE	Venous thrombotic embolism
vWF	Von Willebrand factor
WHO	World Health Organization

OPERATIONAL DEFINITIONS

Acute	An illness that occurs abruptly
Amputation	The action of surgically cutting off a limb
Activated Partial thromboplastin	is a test done to determine the integrity of the intrinsic pathway of the haemostatic mechanism
Atherogenesis	The process of forming atheromas, plaques in the inner lining of an artery
Atherogenic dyslipidaemia	This is increased values of triglycerides, decreased density lipoprotein and decreased levels of high density lipoprotein
Atherothrombotic	The formation of a blood clot within an artery resulting from atherosclerosis
Atherosclerosis	The thickening and hardening of arteries due to deposits of fats in their inner most lining
Autonomic neuropathy	These are symptoms occurring as a result of damaged nerves managing involuntary body functions
Cardiomyopathy	Diseases affecting the heart muscle
Cardiovascular	Denotes the heart and blood vessels
Cerebrovascular disease	Refers to a group of conditions that can lead to a cardiovascular event such as stroke
Cerebrovascular ischemia	A condition occurring as a result of insufficient supply of oxygen to the cerebrum.

Charcot joints	It is the deterioration of a weight bearing joint, as indicated by the destruction of the bone, resorption and deformity as a result of loss of sensation
Chronic	An illness or disease which has persisted for a long time
Diabetes mellitus	Disease that develops following the incapability of the body to manufacture insulin and /or incapability of the body to respond to the insulin hormone, leading to impaired breakdown of carbohydrates with an increase in blood sugars in blood circulation.
Dyslipidaemia	An increase of total or low-density lipoprotein cholesterol levels
Endocrine	Relating to glands which secrete hormones or other products directly into the blood
Fibrinolysis	This is the enzymatic breakdown of the fibrin in blood clots
Glycation	This is increased non-enzymatic deposition of glucose within structural proteins
Glycosylation	The enzymatic process of adding glycosyl groups to a protein to form a glycoprotein
Haemorrhage	This is blood loss through bleeding
Haemostasis	Mechanisms involved in preventing blood loss
Hyperaggregability	Increases ability of platelets to aggregate

Hypercoagulable state	This is an increased rate of forming clots in an abnormally short period than expected.
Hyperfibrinogenaemia	Elevated levels of fibrinogen in blood circulation
Hyperglycemia	An increase in sugar levels in blood and may be found in diabetic patients
Hyperinsulinaemia	Increased levels of insulin in the blood
Hyperosmolar syndrome	It is a complication found in the diabetic patients resulting from elevated levels of blood glucose
Hypofibrinolysis	An abnormally low rate of fibrinolysis
Incidence	It is the number of all new disease cases developing in a given time period
Insulin	This is a pancreas synthesized hormone regulating the level of glucose in the blood, allowing cells to use glucose for energy
International normalized ratio	This is a calculation based on prothrombin time test and is used to monitor patients under treatment with anticoagulants (warfarin)
In vivo	A process taking place in a living organism
Ketoacidosis	A complication associated with diabetes occurring due to increased acids (ketones) in blood.
Lipoprotein	It is a protein molecule composed of a protein part and a lipid part

Macrovascular complication	A disease affecting the large blood vessels as well as the medium sized vessels
Microvascular complication	A disease affecting the smaller blood veins especially the capillaries
Morbidity specific area	This is the rate at which a disease occurs in a
Mortality	This represents the number of deaths in a period of a given time
Nephropathy	Kidney disease
Peripheral neuropathy	A condition that results when damage to peripheral nerves occurs, and due to this, the hands and feet may become weak, numb and painful
Obesity	Being overweight, especially having a body mass index of 30 kg/m^2 and above
Peripheral vascular complication	A blood circulation disorder resulting to the narrowing of the blood vessels outside the heart and brain
Polydipsia	Excessive thirst
Polyphagia	Increased hunger
Polyuria	Increased urination

Prevalence	The total number of disease cases in a particular population at a given period of time expressed as a percentage of the population
Procoagulant state	Represent substances enhancing the conversion of prothrombin to thrombin
Prothrombin	A blood clotting factor known as factor II
Prothrombin time	A test done to determine the integrity of the extrinsic pathway of the haemostatic mechanism
Retinopathy	Disease affecting the eye retina due to blood veins being damaged in the back of the eye
Thromboembolism	Blood clot in motion inside a vessel which may lodge in another area from the site of formation
Thrombogenesis	The synthesis of a thrombus
Thrombolysis	The destruction or dissolution of a blood clot
Thrombomodulin	A glycoprotein found in endothelial cells that binds thrombin and participates in the activation of protein C as a cofactor
Thrombosis	Local coagulation of the blood in circulation leading to clot formation.
Thrombus	A blood clot formed in a blood vessel and remains anchored at the site of formation

ABSTRACT

Diabetes mellitus (DM) is one of the non-communicable diseases (NCDs) leading to disease burden in Kenya. Diabetics have an increased risk of developing complications such as thrombosis if proper care is not taken to those with the disease as a result of hyperglycemia. Many cases may not be detected as many health facilities do not screen for hyperglycemia. This study was done to evaluate the effect of hyperglycemia on coagulation mechanism since no study has been done on this. The study focused on evaluating the effects of hyperglycemia on prothrombin time (PT), international normalized ration (INR) and activated partial thromboplastin time (aPTT) in relation to sex, age and duration of illness in DM patients attending Meru Teaching and Referral Hospital. Ethical clearance was sought from Ethics and Research Committee of KNH/UoN ERC and research permit from National Commission for Science, Technology and Innovation (NACOSTI). A case control study, employing random systematic sampling method to recruit participants in the outpatient diabetic clinic in Meru Teaching and Referral Hospital was done from June 2019 to October 2019. 371 diabetic cases and 371 healthy controls aged 18-60 years and below were recruited upon meeting the inclusion criteria and upon consenting. Recruitment to the study was done with an aid of structured questionnaire. Coagulation parameters (PT, INR and aPTT) were analyzed directly with coagulation analyzer (Start4) while blood glucose parameters were analyzed at the point of contact with on call plus glucometer. The results were entered to MS-excel and uploaded to SPSS software package version 23 and computed statistically. Demographics attributes of study participants were expressed in percentage. Mean and standard deviation were used to express results. Linear regression was used to determine the relationship of coagulation parameters diabetic cases and those of controls. Comparison between coagulation parameters and duration of illness was done by use of analysis of variance (One way-ANOVA) while comparison of coagulation parameters according to sex was done by using independent-sample t test. Variation of coagulation parameters in diabetic participants in relation to age was done using one-way ANOVA, followed by Tukey Post HOC Test. Results revealed that (diabetic group) the male subjects were 152 (41%) and female subjects were 219 (59%) while in the healthy group, the males were 157 (42.3%) and females were 214 (57.7%). Prothrombin time was 12.726±1.307 seconds and 12.487±1.016 seconds in the diabetic subjects and normal group respectively (p=0.215). The International normalized ratio was 1.0142±0.137 and 0.9891±0.102 in diabetic participants and normal group respectively (p=0.221). There was no statistically significant difference (p=0.05) between the Prothrombin time and International normalized ration means of diabetic subjects and the normal group in relation to duration of illness, age and sex of the participants. The activated partial thromboplastin time (aPTT) was 30.656±3.74 seconds and 34.424±2.71 seconds in the diabetics and normal group respectively (p=0.445). Sex, and duration of illness did not have significant effect (p=0.564 and p=0.887 respectively) on the aPTT, but age had significant effect in different age groups in diabetic participants (p=0.005). In conclusion, the findings of this current study is that; PT, INR and aPTT were not significantly different in normal group as compared to

diabetes mellitus group. It is recommended that a study be done on both treated and untreated diabetics to determine if there is a significant difference in coagulation status between the two groups.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Diabetes, which is a long term metabolic disease condition, leads to an increased blood sugar levels (Awad *et al*, 2016). According to Singh (2013), Diabetes mellitus (DM), a common endocrine disease is associated with multiple etiologies, presents with chronic hyperglycemia with disturbances in the metabolism of the carbohydrates, fats, and proteins. The excess blood glucose brings about some of major symptoms associated with DM, such as polydipsia, polyuria and polyphagia (Dallatu, 2012). Diabetes was not a new disease to Indian physicians even before 1500BC (Shah & Mohan, 2015). Several ancient texts gave detailed descriptions of how it presents, leading to the classification of the disease as lean or hereditary, obese or food-induced diabetes, and this is similar to today's classification of diabetes as type 1 and type 2 (Shah & Mohan, 2015).

Diabetes is one of the oldest diseases known in human history, it was first reported in Egyptian manuscript approximately three centuries ago (Banoo and Nusrat, 2015). The prevalence of DM is drastically increasing worldwide and approaching epidemic proportions (Pahim & Megalamane, 2017). In 2013, according to Awad *et al,*. (2016), estimations indicated that over 382 million people all over the world suffered from diabetics. Ninety percent (90%) of the diabetics are Type 2 Diabetes mellitus while 10% of the cases are Type 1 Diabetes mellitus (Dallatu, 2012). The risk of atherosclerosis is increased in the diabetics; with coronary artery disease being the leading cause of death in these individuals (Erem *et al.*, 2004).

Persistent hyperglycemia in patients with DM, exposes red blood cells to elevated glucose levels, consequently, leading to glycation of several parameters such as haemoglobin, prothrombin, fibrinogen, as well as other proteins which take part in clotting mechanisms (Dallatu, 2012). The activation and function of the coagulation system is not complete due to glycation. Once the intrinsic and extrinsic clotting proteins

(coagulation proteins been coated with glucose) are glycated, the availability of these proteins is significantly decreased and it will affect the clotting capacity (Dallatu, 2012). Diabetic patients have disturbances of the haemostatic and fibrinolytic mechanisms, with the development of diabetic complications been associated with them (Onbafl, 1999). As a result of the disturbances haemostatic and fibrinolytic mechanisms, the incidence of cardiovascular events is increased (Onbafl, 1999). The major causes of morbidity and mortality in the diabetics result from thrombosis and related complications (Erem *et al.*, 2004; Onbafl, 1999).

1.2 Problem Statement

Diabetes mellitus is a disease condition burdening the health sectors across the globe (Fayeza *et.al*, 2015). According to Paula and Moura, (2010), as a result of increased cases of diabetics, the disease has mimicked a characteristic of an "epidemic" in recent years. The disease causes blindness, renal failure as well as lower limb amputation (MoPHS, 2010). As a result, diabetes prevalence is causing challenges globally as 1 adult in 11 has diabetes, a figure projected to increase to 1 in 10 adults come 2040 (Tankeu *et al*., 2017). According to WHO (2016), in 2014, 422 million adults were diabetic globally, in comparison to 108 million cases in 1980. Moreover, 1.5 million deaths occurred in 2015 worldwide as a result of diabetes with Africa recording 321,100 deaths, and so, diabetes is among the top major causes of non-communicable diseases (NCD) deaths (Mutyambizi *et al.*, (2018). Since 1980, diabetes prevalence has increased tremendously in the adults from 4.7% to 8.5%, hence reflecting an increased association with risk factors like overweight as well as obese (WHO, 2016). Kenya is estimated to have a 3.3% prevalence of diabetes which is predicted to increase to 4.5% by 2025 (MoPHS, 2010; Jones, 2013), despite the fact that around two thirds of diabetics may not be diagnosed (Jones, 2013). According to Madan *et al.,* (2010) and Awad, *et al.,* (2016), 80% of diabetic subjects die as a result of thrombosis, with 75% of the deaths resulting from cardiovascular events, the remainder resulting from cerebrovascular events as well as peripheral vascular complications. These complications associated with diabetics are responsible for most of its morbidity and mortality (Banoo and Nusrat, 2015).

Hypercoagulability is considered when patients have laboratory abnormalities linked to an increased thrombosis risk, with many of the diabetic subjects falling into this category *(*Madan *et al*., 2010).

1.3 Justification

The incidence of diabetes in Kenya has increased tremendously; but, awareness about this disease condition has not been well done across the country (El-busaidy *et al*., 2014). The undetected as well as the untreated diabetes results to human suffering and disability due to the complications associated with the disease, and also socioeconomic costs related to premature morbidity and mortality (MoPHS, 2010). The diabetics have atherothrombotic complications as well as venous thrombotic embolism (VTE), a phenomenon which has been recently observed (Lemkes *et al*., 2010). There is paucity of data in regard to diabetes in Africa (Motala *et al.*, 2003; Mutyambizi *et al.*, 2018) reason being that, hyperglycemia is not included in the routine tests done in many health facilities (Jones, 2013). Several studies have been done in relation to coagulation status in diabetic patients featuring the basic coagulation parameters with key interest in Prothrombin time (PT), International normalized ratio (INR), and activated partial thromboplastin time (aPTT). The findings of these studies have reported contradicting coagulation status in the diabetics; some revealed hypercoagulable state, others hypocoagulable state while others revealing normal coagulation status. These inconsistencies necessitated this study to be carried out. Therefore, Prothrombin time, International normalized ration, and Activated partial prothrombin time parameters were measured enabling the assessment of the risk of clotting complications in diabetic patients (Dallatu, 2012). Basic coagulation screening (PT, INR and aPTT) is also not routinely done on diabetic patients here in Kenya as it has not been included as a routine test for the diabetics. Several studies have been done in Kenya describing the prevalence of diabetes, none has compared diabetes and its relationship with basic coagulation tests (PT and APTT). Therefore, this study aimed at comparing basic coagulation tests (PT, INR and APTT) in DM to healthy controls to find out the impact of hyperglycemia on coagulation system.

1.4 Null Hypothesis

Hypercoagulable state is not observed in diabetic patients

1.5 Alternative Hypothesis

Hypercoagulable state is observed in diabetic patients

1.6 Research Questions

1. Is there any relationship between coagulation parameters (PT, INR and APTT) of Diabetes mellitus patients and healthy control?
2. Is there any relationship between coagulation parameters (PT, INR and APTT) of Diabetes mellitus patients and the duration of illness?
3. Is there any relationship between coagulation status of male and female Diabetes mellitus patients?
4. Is there any variation of coagulation status in Diabetes mellitus patients in relation to age?

1.7 Objectives

1.7.1 General Objective

To evaluate of prothrombin time (PT), international normalized ratio (INR) and activated partial thromboplastin time tests (aPTT) in Diabetes mellitus patients at Meru Teaching and Referral Hospital, Kenya

1.7.2 Specific Objectives

1 To determine the relationship of basic coagulation parameters (PT, INR and APTT) in Diabetes mellitus (DM) patients and Healthy controls.

2 To compare coagulation parameters of DM patients and the duration of illness.

3 To compare the coagulation parameters of male and female DM patients.

4 To evaluate the variation of coagulation parameters in DM patients in relation to age.

CHAPTER TWO

LITERATURE REVIEW

2.1 Background Information

Diabetes mellitus (DM), a common endocrine disease (Dallatu, 2012; Singh, 2013) presents with chronic hyperglycemia with impaired carbohydrates, fats, as well as protein metabolism (Dallatu, 2012; Singh, 2013). In addition, the increased glucose in blood results due to defective insulin secretion mechanisms, inability of insulin to perform its roles or both (Ephraim *et al.*, 2017). Diabetes mellitus is among the major causes of death today (Mariappan *et al.*, 2017) with recent studies indicating that, thrombotic complications results in 80% of deaths in diabetic subjects, with cardiovascular disease resulting to 75% to 80% of these deaths (Paula and Moura, 2010; Mariappan *et al.*, 2017), as well as cerebrovascular and peripheral vascular complications (Ankalayya *et al.*, (2016).

According to Kearney *et al.*, (2017), diabetes resulted to more than 1.5 million deaths worldwide in 2012. Disease burden in many African countries has gone up due to increasing cases of non-communicable diseases, some of which are; high blood pressure, stroke, coronary heart disease and diabetes, plus the existing challenges posed by tuberculosis, malaria and Human Immunodeficiency Virus (HIV) (Beran & Yudkin, 2006). The increased disease burden is a challenge to the healthcare systems of poor countries lacking adequate resources as they need to invest in systems, train healthcare workers who will be in a position of managing chronic diseases (Beran & Yudkin, 2006). Subjects suffering from diabetes, have Cardiovascular vascular disease (CVD) being the main cause of morbidity and mortality; despite having advances in therapy (Kearney *et al*., 2017).

Patients with Type 2 Diabetes mellitus (DM) have high chances of developing premature cerebral, coronary, and peripheral vascular disease, and which together take part as the leading cause of death in diabetic subjects (Kakouros *et al.*, (2011).

Moreover, insulin resistance (IR) manifests in a majority of individuals with Type 2 Diabetes mellitus (T2DM), and appears as a common precursor of both diabetes and macrovascular disease (Vinik *et al*., (2001). The risk of increased atherothrombosis is evident in the pre-diabetic stage subjects; as well, subjects with IR and normoglycemia are at risk of cardiovascular events, due to several number of risk factors (Alzahrani & Ajjan, 2010). The mechanisms leading to high chances of thrombosis risk in diabetes are complex, involving multiple pathways (Alzahrani & Ajjan, 2010). Diabetic subjects usually have premature atherosclerosis as well as an extensive vascular diseases, which predisposes them to plaque rupture, and the development of thrombus. In addition, these diabetic subjects have elevated thrombotic tendency resulting from increased reactivity of platelets, hyper-activation of pro-thrombotic coagulation factors, with fibrinolysis activity being decreased (Alzahrani & Ajjan, 2010).

Beran and Yudkin (2006), states that, in order to overcome the challenges of diabetes; the increasing burden of T2DM arising from urbanization as well as obesity should be stemmed, with provision of accessible care and appropriate medication to people diagnosed with the disease.

2.2 The Prevalence of Diabetes Mellitus

2.2.1 Global Prevalence

According to Pahim and Megalamane (2017), the prevalence of Diabetes mellitus (DM) is drastically increasing worldwide and approaching epidemic proportions. In 2000, the diabetic subjects globally were approximately 151 million; these estimates were projected to reach 221 million in 2010, hitting 324 million by 2025, of which, an approximate ninety percent (90%) of diabetes cases globally is characterized by insulin resistant, and or insufficient production of insulin (Paula & Moura, 2010). In 2008, 57 million deaths occurred worldwide, 36 million (63%) resulted from Non Communicable Diseases (El-busaidy *et al.*, 2014). According to Kearney *et al.*, (2017), 1.5 million deaths occurred globally, it was directly attributed to Diabetes mellitus in 2012. In the

same year, the total burden of deaths resulting from elevated blood sugars was estimated to be 3.7 million. The number is inclusive of 1.5 million diabetes deaths mentioned earlier, in addition to 2.2 million deaths resulting from CVDs, chronic kidney disease and tuberculosis associated with higher than normal blood glucose (WHO, 2016).

As a result of the rapid increased prevalence of diabetes globally, by 2030, that is by WHO prediction, the number of adults with diabetes would have increased globally to 370 million from 177 million in 2000 (Banoo and Nusrat, 2015; Santos *et al.*, 2015). According to Banoo and Nusrat (2015), it has been predicted that the developing countries have an increase in Diabetic mellitus prevalence in comparison to the developed countries, (69% versus 20%). The people aged 40-60 years are the most affected in developing countries, in comparison to those aged above 60 years in developed countries (Banoo and Nusrat, 2015). According to Chawla *et al.*,(2016) and Owiti *et al.*, (2017), in 2015, 415 million individuals globally had diabetes and predictions indicated this figure would shoot to 642 million cases in 2040 even if age specific prevalence remains constant (Bommer *et al.*, (2018).

In Brazil, by 2030, the number of subjects having diabetes will rise to 11.3 million from 4.5 million in 2000, making it one of the countries with highest burden of this disease burden worldwide (Santos *et al.*, 2015). In 2001, the prevalence of diabetes in Bangladesh was found to be 8.3%, comprising 15.2% urban dwellers and 8.3% rural residences (Fayeza *et al.*, 2015). In the United States of America, an approximation of 30.3 million (9.4%) people of all age groups were suffering from diabetes in 2015 (Centers for Disease Control, 2017). This figure included 30.2 million subjects of 18 years and above, 7.2 million (23.8%) of these subjects had no idea that they had diabetes (Centers for Disease Control, 2017). Moreover, there was an increased number of diabetic adults in relation to their age (Centers for Disease Control, 2017). In India, an estimated 69.2 million people have diabetes, this figure is projected to go up to 123.5 million by 2040 (Chawla *et al*,. 2016). In addition, an approximate of 193 million diabetic subjects worldwide are not diagnosed and hence are predisposed to develop complications of untreated chronic hyperglycemia (Chawla *et al*,. 2016). According to

Chen *et al.*, (2017), in China, the adults had an estimated 11.6% prevalence of diabetes, with 50.1% prediabetes prevalence. Therefore, taking the total number of Chinese population into consideration, there were an approximate figure of 113.9 million diabetics, and 493.4 million individuals with prediabetes, clearly pointing out that; diabetes poses a major challenge to the public health in China (Chen *et al*., 2017). An estimate of more than 17 million adults in Mexico have the metabolic syndrome, with only 3.5 million having been diagnosed with diabetes (Aburto-Mejiá *et al.*, 2017).

2.2.2 Prevalence in Africa

Diabetes mellitus at one time was considered a disease of the affluent and Africa was thought to be absolutely free from it; but now, it is among the major challenges facing many of the African health systems (Mutyambizi *et al.*, 2018). For sub-Saharan Africa, T2DM prevalence is projected to increase from 1.1% in 1995 to 1.3% in 2025, from an estimation of 3 million subjects to 8 million (Motala *et al.*, 2003). An estimate indicates that, the number of diabetic people in the African region will shoot upwards to 34.2 million in 2040, up from 14.2 million in 2015 (Mutyambizi *et al*., 2018). Diabetes prevalence in Ghana is approximately 3% of the country's general population (Ephraim *et al*., 2017).

2.2.3 Prevalence in Kenya

The non-communicable diseases (NCD's), forms a major source of morbidity and mortality in Kenya; and diabetes is one the NCDs (El-busaidy *et al*., 2014). Countries with low to middle income, Kenya among them, had approximately 29 million of such deaths (El-busaidy *et al*., 2014). In addition, NCD's, including diabetes, forms more than 50% of the hospital admissions, as well as 55% of hospital based deaths (El-busaidy *et al*., 2014). Kenya has a 3.6% diabetic prevalence, a figure projected to go up to 4.4% by 2035 if preventive measures are not put in place (Shannon & Haghparast-bidgoli, 2019). According to Owiti *et al.*, (2017), NCD's were responsible for 27% of all deaths in 2014, DM accounting for an approximate of 2%, even though this rate may be

an underestimate as a result of undiagnosed diabetic cases in the country. According to Shannon and Haghparast-bidgoli, (2019), in 2015, Kenya had more than 8,700 deaths diabetic related and almost all were aged below 60 years of age.

2.3 Pathogenesis of Diabetes mellitus

Type 1 Diabetes mellitus (T1DM) is triggered by autoimmune destruction of pancreatic beta cells by cluster of differentiation 4 ($CD4^+$) and cluster of differentiation 8 ($CD8^+$) T cells as well as by macrophages which infiltrates the islets (Baynest, 2015). Once the pancreatic beta cells are destroyed, deficiency of insulin secretion follows resulting to metabolic derangements associated with T1DM (Ozougwu *et al.,* 2013; Baynest, 2015). The pathogenesis of Type 2 Diabetes mellitus (T2DM) is associated with both impaired secretion of insulin by dysfunctional pancreatic beta cells and impaired insulin action through insulin resistance (IR) (Ozougwu *et al.,* 2013). Impaired glucose tolerance develop due to IR and hyperinsulinaemia (Baynest, 2015).

2.4 Complications Associated with Diabetes Mellitus

Diabetic patients are known for the associated risk of vascular events (Lemkes *et al.*, 2010). T2DM is one of mostly incriminated conditions for the onset of atherosclerosis, endothelial, vascular (Dayer *et al*., (2014), as well as thrombotic complications (Dayer *et al*., 2014; Stegenga *et al*., 2008). Furthermore, patients with hypercoagulation state have high risk of thrombosis, and so, uncontrolled T1DM patients can develop this condition and increase the risk of mortality due to abnormal clotting mechanism (Mariappan *et al*,. 2017). Eighty percent (80%) of diabetic subjects die, subject to thrombosis, with cardiovascular events leading to 75% of these deaths (Erem *et al*., 2004; Paula & Moura, 2010), and cerebrovascular events together with peripheral complications accounting for the remaining 25% (Erem *et al*., 2004). The thrombotic events in diabetics are accompanied by an increase in the procoagulant activity, accompanied by a decrease in fibrinolysis (Erem *et al*., 2004). According to Ephraim *et al.,* (2017), the mechanisms associated with increased thrombosis risk in diabetes are complex, involving several

pathways. Diabetic subjects usually have premature atherosclerosis as well as extensive vascular disease, and this predisposes them to plaque rupture as well as thrombus formation (Ephraim *et al*., 2017). In addition, Insulin resistance and T2DM have been associated with an increase in the risk of atherothrombotic complications (Sauls *et al*., (2007). According to Singh, (2013) and Dhule and Gawali (2014), there is a higher risk of developing coronary artery disease in subjects with T2DM, accounting for an approximate 60% of their mortality rate. In addition, there is an occurrence of endothelial dysfunction and elevated Von Willebrand factor (vWF) in T2DM (Erem *et al.*, 2004). Von Willebrand factor is synthesized by endothelial cells of T2DM individuals and it is a glycoprotein which is associated with vascular injury (Erem *et al*., 2004). Another important feature of T2DM is the concurrent existence of hyperglycemia and hyperinsulinaemia (Stegenga *et al.*, 2008). Moreover, thrombosis results in an approximate of 80% deaths in T2DM patients, with high mortality due to cardiovascular disease (CVD) in this patient population (Stegenga *et al*., 2006). T2DM subjects also suffer from metabolic disorders like atherogenic dyslipidaemia, hypertension, glucose intolerance and a prothrombotic state (Ephraim *et al*., 2017). T2DM often develops due to overweight and lack of exercise in addition (Mohan *et al.*, (2017) and develops as age progresses (Mariappan *et al,*. 2017).

The persistent hyperglycemia in diabetics causes long term destruction and dysfunction of various organs such as the eyes, kidneys, nerves, heart, including the blood vessels (Diabetes, 2009). Diabetic ketoacidosis and diabetes non ketotic coma are some of the acute complications occurring in DM (Diabetes, 2009; MoPHS, 2010). The risk of developing vascular complications is increased in subjects with diabetes, leading significantly, to a reduced life expectancy (Kearney *et al*., 2017). Many organ systems are usually affected by persistent complications of DM and hence being responsible for most of morbidity and mortality linked to the disease (Dhawale *et al.*, 2016). Therefore, the chronic complications associated with T2DM form a part of the principle cause of illness and death as well as a serious source of concern for those in charge of health sector in developed countries (Santos *et al.*, 2015). Microvascular and macrovascular

systems forms the basis of some of major complications arising from T2DM (Paula and Moura, 2010; Awad *et al*,. 2016).

2.4.1 Microvascular Complications

The history of diabetes is usually characterized by the variation as well as the severity of microvascular, and macrovascular complications (Sapkota *et al*., 2013; Pahim and Megalamane, 2017). Uncontrolled DM causes microangiopathy leading to diabetic nephropathy (Chaitanya & Kavuri, 2014) and kidney failure (Pahim & Megalamane, 2017). According to (Rask-madsen & King, 2014), there is increased glomerular perfusion and plasma filtration resulting from decreased resistant in both afferent and efferent arteriole; with albuminuria being a risk factor for diabetic nephropathy. Nephropathy, increases the risk for the appearance of other microvascular events like retinopathy as well as neuropathy (Fowler, 2008; Mohan *et al*., 2017; Pahim & Megalamane, 2017; Santos *et al*., 2015). They mostly result to permanent loss of eye sight, chronic kidney disease as well as non-traumatic leg amputations (Santos *et al*., 2015). Impaired vision is majorly caused by diabetic retinopathy in the developed countries with new cases of blindness in people aged between 20 – 64 years (Giusti *et al.*, 2000) resulting to 12,000 to 24,000 new cases of blindness yearly (Banoo and Nusrat, 2015). According to Dhawale *et al*,. (2016), nephropathy is indicated by the appearance of low, abnormal albumin levels in the urine referred to as "microalbuminuria". Complications associated with diabetes rise with the persistent increase in the prevalence of diabetes, and hence straining the available health-care resources (Beran & Yudkin, 2006). Studies have shown that, 16 – 55% of diabetic subjects had retinopathy , as well, the newly diagnosed subjects with T2DM forms 21 – 25% and presented with retinopathy (Beran & Yudkin, 2006). Therefore, blindness can be prevented only if retinopathy is diagnosed, and treated promptly (Dhawale *et al*,. 2016). In addition, diabetic neuropathy occurs due to the injury of small blood vessels supplying the nerves, as well as macrovascular conditions that may end up in diabetic neuropathy (Dhawale *et al*,. 2016).

2.4.2 Macrovascular Complications

Diabetes mellitus is associated with reduced life expectancy as well as an increased risk to develop macrovascular complications like the ischaemic cardiomyopathy, stroke and also peripheral vascular disease (Fowler, 2008; Mohan *et al*., 2017; Santos *et al*., 2015), and they are mostly linked to the disease and perhaps leading to death (Santos *et al.*, 2015). Moreover, the principal cause of morbidity as well as mortality in the diabetics is cardiovascular disease (CVD), with 80% of diabetes subjects dying due to cardiovascular complications (Alzahrani & Ajjan, 2010). The risk of developing atherothrombotic complications in people with diabetes is similar to those without diabetes but have an ischaemic heart disease history (Alzahrani & Ajjan, 2010). The macrovascular complications, are known as the major cause of morbidity in diabetic subjects, such as atherosclerosis, which are implicated in the circulatory disturbances presented by the diabetics (Chaitanya & Kavuri, 2014). Changes in platelet activity especially increased adhesiveness and aggregation abilities (Pahim & Megalamane, 2017), fibrinolytic aberration, coagulopathy and haemorrheological results to further aggravation of circulatory disturbances, in addition to changes in endothelial metabolism (Chaitanya & Kavuri, 2014). It is good to note that, when high risk patients are treated, chances of developing CVD is reduced in this group, this makes the risk be in line with individuals without diabetes (Paula & Moura, 2010).

2.4.3 Infections in Diabetes mellitus cases

Diabetic patients are prone to infections, and they die as a result of these infections more frequently as compared to non-diabetics (Stegenga *et al.*, 2008). The enhanced infection risk is associated with impaired immune system, especially the innate system (Stegenga *et al.*, 2008). Cytokines especially inflammatory cytokines, are the molecules orchestrating the first line of defense response, but their concentrations increase in the diabetics and may lead to enhanced insulin resistant (IR), as well as suppression of various functions of neutrophils involved in the destruction of the invading pathogens (Stegenga *et al.*, 2008). According to Shojaeian and Mehri-ghahfarrokhi, (2018), recent

studies have showed that viral infections can lead to development of diabetes include *enterovirus, rotavirus, herpes virus, cytomegalovirus, endogenous retrovirus and Ljungan* virus. According to Tavares *et al.*, 2012, T1DM develops when there is destruction of pancreatic beta cells. Likewise, viral infections as well as autoimmune disorders can take part in the destruction of beta cells resulting in the development of T1DM; and it is the *Enterovirus* which has been associated with much higher risk of T1DM development (Tavares *et al.*, 2012). According to Yaribeygi *et al.*, (2020), Coronavirus disease 19 (COVID-19) is an infectious disease caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). Diabetic people are prone to complications associated with COVID-19 with a higher risk of COVID-19 related mortality; as well as experiencing severe symptoms and complications compared to non-diabetics due to COVID-19 (Yaribeygi *et al.*, 2020). Some of the complications in diabetics associated with increased COVID-19 mortality are cardiovascular disease (CVD), heart failure and chronic kidney disease (Bae *et al*., 2021).

2.5 Classification of Diabetes mellitus

The major types of diabetes are: T1DM and T2DM (Jones, 2013).

2.5.1 Type 1 Diabetes Mellitus (T1DM)

In Type I Diabetes mellitus (T1DM), the body is incapable of producing insulin, with an approximate of 10% of all diabetic cases being type 1 (Awad *et al*,. 2016; Nnenna *et al.*, 2016). In T1DM, the pancreatic beta cells are destroyed by an autoimmune activity, this results in an absolute deficiency in insulin (Paula & Moura, 2010), and it can develop from childhood (Mariappan *et al*,. 2017). However, the disease has been shown to affect the adults as well (Shojaeian & Mehri-ghahfarrokhi, 2018). Subjects at increased risk of developing T1DM are identified by serological screening to detect pathologic prove of an autoimmune process taking place in the islets of the pancreas, as well as screening for genetic markers (Diabetes, 2009). T1DM subjects are totally dependent on insulin to regulate their blood glucose and in order to survive, T1DM is currently not preventable

(WHO, 2016). T1DM frequently affect the children, and represents 80 to 90% of diabetes in children and teenagers (Shojaeian & Mehri-ghahfarrokhi, 2018).

2.5.2 Type 2 Diabetes Mellitus (T2DM)

According to Banoo and Nusrat (2015), in 1936, the distinction between T1DM and T2DM was clearly made. In T2DM, the body produces insufficient amount of insulin for proper function, with an approximate of 90% of diabetic cases worldwide being Type 2 (Paula and Moura, 2010; Awad *et al,*. 2016). T2DM often ranges predominantly from lack of insulin to predominantly secretory abnormalities associated with insulin resistance (Punthakee *et al.,* 2018). This type of disease develops as age progresses (Mariappan *et al.,* 2017) but T2DM prevalence in paediatrics is increasing due to an increase in childhood obesity in many countries (Zheng *et al.,* 2017).

2.6 Symptoms of Diabetes Mellitus

Diabetes mellitus presents with the following commonest symptoms which include:- frequent urination, increased thirsty and hunger (Dallatu, 2012; Ephraim *et al.,* 2017). There is also an increase in weight, sometimes unusual weight loss, fatigue, wounds that take long to heal, sexual dysfunction of the males, numbness and tingling sensation in hands and the legs (Awad *et al,*. 2016). Blurred vision is sometimes a symptom associated with Diabetes mellitus (Diabetes, 2009). Moreover, in Type 1 Diabetes mellitus there is fatigue, frequent infections, as well as ketoacidosis in diabetics in case of severe dehydration especially in children and adolescents (Shojaeian & Mehri-ghahfarrokhi, 2018). In addition, the general symptoms presented by diabetics also include hypercoagulability and hypofibrinolysis (Paula & Moura, 2010).

2.7 Diagnosis of Diabetes mellitus

Screening patients for diabetes enables early detection of diabetes and pre-diabetes and this allows prompt intervention leading to reduction in future complications as management of diabetic patients is done without delays (Baynest, 2015). Diagnosis can

be done through random plasma test where fasting is not required and blood sugars of 200 mg/dl (11.1 mmol/L) and above indicates diabetes which has to be reconfirmed (Baynest, 2015). Fasting plasma glucose is another technique to diagnose diabetes. The patient fasts for eight hours before the test is done. Diabetes is confirmed if blood glucose more than 126 mg/dl (7 mmol/L) on two or more tests on different days is obtained (American Diabetes Association, 2015; Baynest, 2015) Glycated haemoglobin A fraction (HbA1C) is an indicator on the average glucose concentration serving as a good indicator for monitoring blood glucose control. A HbA1C value of ≥6.5 is considered diabetic (Baynest, 2015).

2.8 Treatment and management of Diabetes mellitus

Regular follow up of diabetic patients is vital with a health care provider as it helps in averting any of the long term diabetic complications such as nephropathy, retinopathy and neuropathy (Baynest, 2015). Management is aimed at preventing clinically significant glycosuria, water and electrolyte loss, infections as well as development of ketotic hyperosmolar coma. For Type 1 Diabetes mellitus, insulin therapy is indicated; same to Type 2 Diabetes mellitus patients whose hyperglycemia fails to respond to diet therapy only or in combination with oral hypoglycemic drugs (Baynest, 2015)

2.9 Haemostasis Mechanism (Cascade)

Haemostasis is a physiological mechanism which stops bleeding at an injured site as well as ensuring that the normal flow of blood in the circulation system is well maintained (Gale, 2011). The clotting of the blood involves transforming the liquid blood into a semi-solid gel, fibrinogen generates the fibrin polymers as well as fibrin monomers resulting to formation of a clot (Dallatu, 2012). The formation of impermeable platelet plug with fibrin deposition at the injured site lead to haemostasis (De Caterina *et al.,* 2013). The blood vessels have an endothelium which ensures the availability of an anticoagulant surface, and hence maintaining blood in its liquid state (Gale, 2011). According to Mackman *et al.,* (2007), the disruption of the endothelium

results in exposing platelets to collagen present in the vessel wall as well as factor VII/VIIa to tissue factor (TF) also known as factor III with some other proteins, such as vWF facilitating platelet binding onto the injured vessel wall. TF and FVIIa forms a complex, which is the extrinsic pathway suggested to be the central activator of the coagulation mechanism *in vivo* (Mackman *et al*,. 2007). When coagulation is activated, thrombin is formed from prothrombin and several clotting factors are involved which are: Factor V, VII, VIII, IX, X, XI and XII as well as activated platelets (Kluft & Jespersen, 2002). Once the activation of coagulation mechanism has been started, there is increased production of activator products from the platelets such as the β-thromboglobulin and platelet factor 4 (Kluft & Jespersen, 2002). In addition, there is prothrombin activation as well as an increase in thrombin inhibition. Upon the activation of the vascular wall, there is an increase in the synthesis of soluble intracellular adhesion molecule-1, soluble E-selectin, as well as vWF with decreased prostaglandin 1_2 (Kluft & Jespersen, 2002).

The process of haemostasis is usually activated in a very short period due to a tightly regulated mechanisms in case of an injury (Gale, 2011). The injured site forms the surface where the platelet –fibrin thrombus localizes hence preventing propagation into vascular lumen (De Caterina *et al*., 2013). Moreover, further thrombus propagation is limited when fibrin in thrombus activates its own dissolution through fibrinolysis by plasmin (De Caterina *et al.*, 2013). According to Gale, (2011), haemostasis has two major components, the primary as well as the secondary. In the primary stage, platelets aggregate leading to platelet plug formation as a result of platelet activation leading to adhesion to the injured site and onto each other sealing the injured site (Gale, 2011). In addition, more platelets are recruited as well as coagulation mechanism being amplified by the intrinsic pathway and hence enhancing thrombus propagation (Mackman *et al*,. 2007). The secondary stage involves the deposition of insoluble fibrin from the proteolytic coagulation process (Gale, 2011). The secondary haemostasis involves the intrinsic and extrinsic pathways which are vital in the clot formation and they converge

at the common pathway having been initiated by distinct processes leading to clot formation (Adonu *et al*., (2013).

In the blood, there are a dozen of coagulation factors, which are proteins in nature, and exist in an inactive state in the blood, but in the event of any kind of injury, they are activated and play their role of preventing blood loss (Dallatu, 2012). Fibrinogen can be transformed to fibrin in circulation as well as in the vasculature, then, factor II activated activates factor XIII leading to stabilization of the clot by cross-linking the fibrin formed (Kluft & Jespersen, 2002). It is good to note that, a thrombogenic surfaces provided by platelets enables the amplification of the coagulation process, with the rich-thrombus being stabilized by fibrin (Mackman *et al*,. 2007). According to Gale, (2011), the fibrinolysis pathway plays a significant role in haemostasis, with fibrinolysis activation leading to the generation of plasmin from its inactive form by the help of the plasminogen activator (t-PA), fibrinolysis is inhibited by PAI-1 (Kluft & Jespersen, 2002). The plasmin generated dissolves fibrin resulting in soluble fragments referred to as the fibrin degradation products or the D-dimers, and at the same time plasmin inhibitor inactivating plasmin giving rise to plasmin-antiplasmin complex (PAP), an inactive complex (Kluft & Jespersen, 2002). Moreover, as indicated by Gale (2011), there are several mechanisms that regulate and control these systems and hence help in ensuring that blood remains in its fluid form when there is no injury as well as forming a clot proportionally to the injury. Maintaining blood in the liquid form in the circulatory system as well as prevention of blood loss when there is an injury indicates clearly, a very delicate balance between tightly regulated players of haemostasis; the platelet function, coagulation as well as fibrinolysis (De Caterina *et al*., 2013). If there is any disturbance in this balance as a result of synthesis and deposition of insufficient amounts of fibrin at the injured site, haemostasis is impaired, resulting to bleeding, or thrombosis manifest if there is increased fibrin formation and deposition (De Caterina *et al*., 2013). According to Adonu *et al.*, (2013), diabetes disturbs the delicate balance existing in healthy flowing blood increasing the risk of developing thromboembolic conditions in the diabetics, and hence increasing morbidity to these subjects.

Coagulation is activated when TF comes into contact with plasma, and therefore binds coagulation factor VII/VIIa leading to the formation of complexes on cellular surfaces and hence triggering the coagulation mechanism (De Caterina *et al.*, 2013). In the extrinsic pathway, FX is activated by FVII/TF complex on phospholipid surface in the presence of calcium ions while in the intrinsic pathway, FX is activated by FIXa via formation of tenase complex with coagulation FVIII and calcium ions on phospholipid membranes (Dayer *et al.*, 2014)

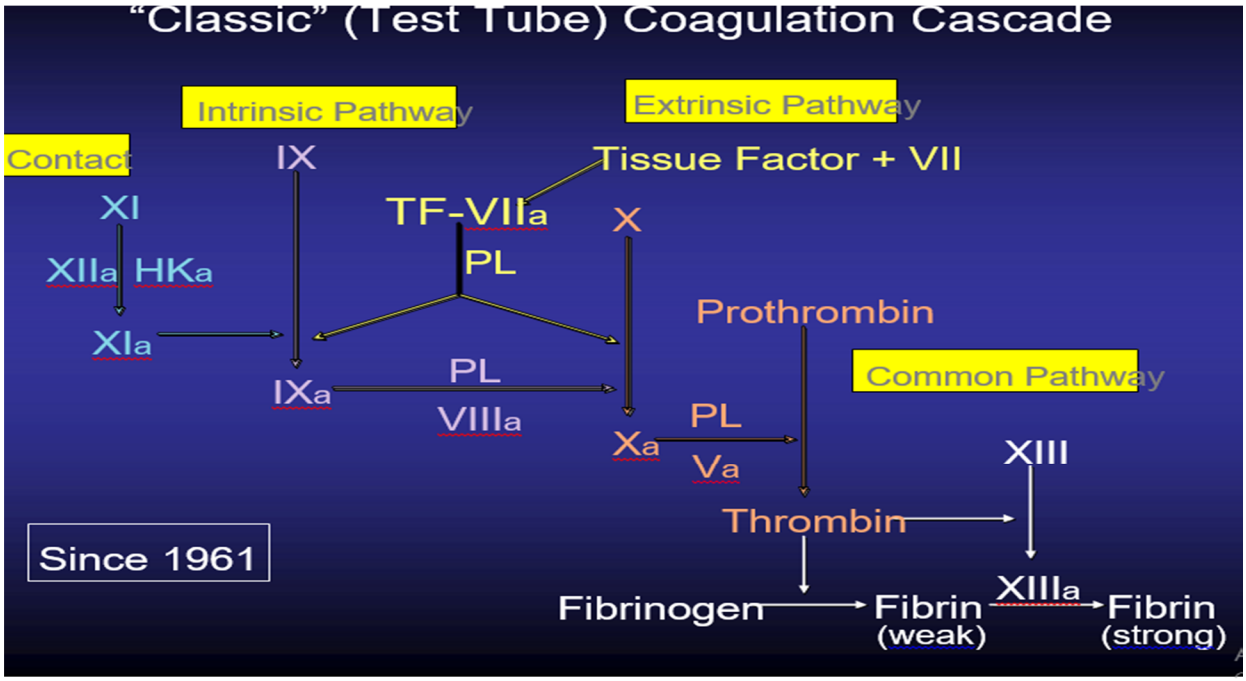

Figure 2.1: Classic Coagulation Cascade (Whitehill, 2008).

2.10 Hypercoagulability in Diabetes Mellitus

When coagulation parameters such as activated partial thromboplastin time (APTT) is shortened, it may be a reflection of increased coagulation state, which is then linked to an increase in thrombotic risk, as well as severe cardiovascular events (Sapkota *et al,*. 2013). As a result of accumulated circulating activated coagulation factors (e.g. VIII, IX and X) in plasma, this also leads to shortened APTT as a result of enhanced coagulation

activation *in vivo* (Sapkota *et al,*. 2013). According to Fayeza *et.al.,* (2015), hypercoagulability, a presentation in diabetics may increase the rate of atherosclerosis development, and also, it is a risk factor for the development of CVD. In addition (Fayeza *et.al,* 2015), coagulation disorders with reduced levels of natural coagulation inhibitors have been described in diabetic subjects. Some of the coagulation inhibitors include protein C, antithrombin III, as well as protein S. Clotting factors are also elevated, with an increased levels of PAI-1 contributing to hypercoagulability state in DM (Fayeza *et.al,* 2015). According to Onbafl, (1999), a suggestion has been put forward that, when coagulation inhibitors in the diabetics are glycosylated, their functions may be impaired. Tissue factor pathway is also a natural coagulation inhibitor. Hypercoagulability phenomenon as indicated by reduced Prothrombin tine (PT) and activated partial thromboplastin time (aPTT) in diabetic individuals, could lead to occlusive thrombi in a coronary artery (Fayeza *et.al,* 2015). International normalized ratio is a coagulation parameter derived from PT and they are directly related, that is; if PT is reduced, INR is reduced and vice versa. International normalized ratio is used in monitoring patients' treatment with oral anticoagulant and it is used to calculate the drug dosage (Mishra *et al,* 2017)

2.11 Hypercoagulability Risk factors in Diabetes Mellitus

Prothrombin time test as well as APTT test, are some of the haematological parameters giving a clue on the coagulation status of patients, and are used in evaluating the risk of clotting disorders in diabetic individuals (Dallatu, 2012). Some of hypercoagulability risk factors are as follows:-

2.11.1 Time of Exposure to Hyperglycemia

Mariappan *et al,*. (2017) showed that long period of exposure to hyperglycemic state leads to glycation of various proteins in the body. This exposure can affect the concentrations of various coagulation factors, as well as the other parameters involved in coagulation. Hyperglycemia may result to vessel damage which is a known risk factor

for an increased rate of atherosclerosis as well as vascular disease (Dhule & Gawali, 2014).

2.11.2 Increased Coagulation factors in DM

Some of clotting proteins factors such Factor XII, XI, IX, VII, VIII, vWF, Kallikrein and fibrinogen are raised in subjects with diabetes (Ankalayya *et al*., 2016; Erem *et al*., 2004; Madan *et al*., 2010). They also have increased platelet aggregation, endothelial cell dysfunction accompanied by an increased blood viscosity, and these factors contribute to some of the changes leading to increased thromboembolic incidence in the disease (Adonu *et al.*, 2013). In addition, antithrombin III is the natural inhibitor of factor X , its inhibition function is decreased by hyperglycemia, and this may lead to an increase in factor X (Onbafl, 1999). A similar study indicated that, high levels of fibrinogen, vWF as well as factor VII had a higher risk for atherosclerosis development, and hence could be looked at as risk factors for the evolution of cardiovascular complications (Adonu *et al.*, 2013). Moreover, the development of prothrombotic and hypercoagulable state is favoured by some changes in these coagulation proteins (Dallatu, 2012). This may lead to enhanced cardiovascular risk as the likelihood of experiencing an occlusive thrombus in a coronary or cerebral artery resulting to the formation of atherosclerotic lesion (Dallatu, 2012). In addition, in prothrombotic state, the levels of fibrinogen are increased, as well as PAI-1 levels with several abnormalities in platelet function (Ephraim *et al.*, 2017). According to Ankalayya *et al.*, (2016), in severe diabetes, there is decreased levels of anticoagulant protein C, with elevated coagulation activation markers and these are prothrombin activation fragment 1+2, and antithrombin complexes.

2.11.3 Fibrinolysis in Diabetes Mellitus

Clots in the body or in circulation are removed by the fibrinolytic system. This process is slow in diabetic individuals as a result of abnormal clot structure with increased resistant to degradation, with increased PAI-1, an inhibitor to tissue plasminogen

activator (Madan *et al*., 2010). There is still controversy as to whether fibrinolysis abnormalities contributes to the occurrence of diabetic microvascular and macrovascular events, since several studies conducted have showed variations in the activity of the fibrinolytic system, to be either increased, reduced or unchanged, but, most studies, have indicated increased coagulation due to hypofibrinolysis (Erem *et al.*, 2004). T2DM has an established hypofibrinolysis state which is defined by an increase in clot lysis time as well as increased levels of PAI-1 (Lemkes *et al*., 2010). Plasminogen activator (tPA) triggers the activation of fibrinolysis mechanism, whose decreased levels have been reported in the diabetic subjects (Erem *et al.*, 2004; Onbafl, 1999). More so, in diabetes, high levels of tissue plasminogen activator 1 (PAI-1) inhibitor has been reported (Onbafl, 1999) and is considered an independent factor for cardiovascular risk in T2DM (Erem *et al*., 2004). According to Stegenga *et al.,* (2006), the impairment of fibrinolysis as a result of elevated levels of PAI-1 is a constant finding in T2DM. It is good to note that, PAI-1, is principally involved in the inhibition of fibrinolysis (Aburto-Mejiá *et al.*, 2017). Higher levels of PAI-1 has been linked to an increased atherothrombosis risk due to hypofibrinolysis, suppression of vascular smooth muscle, cell migration and growth, predisposing to formation of atheroma plaques prone to rupture, by thin fibrous cap of collagen separating the lipid core from the arterial lumen (Aburto-Mejiá *et al*., 2017). IR, obesity, dyslipidaemia, and endothelial defects also induce PAI-1 overexpression and are linked with an augmented cardiovascular risk (Aburto-Mejiá *et al*., 2017).

2.11.4 Platelet abnormalities in Diabetes Mellitus

Platelets have a significant role as far as the integrity of normal haemostasis is concerned, atherosclerosis process and are also closely associated with cardiovascular events (Chen *et al.*, 2017). Individuals having DM have a higher risk to thrombotic activity as a result of platelet hyper-reactivity, as well as an increase in the activation of prothrombotic coagulation factors accompanied by decreased fibrinolysis (Ephraim *et al*., 2017), consequently increasing thrombosis risk (Kearney *et al.,* 2017). According to Chen *et al.,* (2017), researchers found the morphological changes of platelets and the increased platelet activity occurred in diabetic patients. DM is associated with increased

complications due to variety of abnormalities reported in diabetic platelets. These diabetic platelets in response to stimulating agents and spontaneous can exhibit aggregation phenomenon (Pahim & Megalamane, 2017). According to Dhule and Gawali, (2014), evidence associated with abnormality of platelet function in DM have been pointed out as alteration in the functions of the platelets, in addition to hyper-aggregation of platelets leading to accelerated atherogenesis. Platelet hyperaggregability and increased vWF levels in the diabetics which plays a vital role in platelet adhesion to sub endothelial cells, has been shown (Onbafl, 1999). Platelets in T2DM subjects attach on the vascular endothelium and have an increased tendency to aggregate than those of healthy subjects (Dhule & Gawali, 2014). The increased hyperactivity and baseline activation of platelets in DM patients come about as a result of factor combinations, including the effects brought about by insulin, hyperlipidemia, hyperglycemia, endothelial dysfunction, oxidative stress, as well as inflammatory state (Kakouros *et al*., 2011). Furthermore, raised parameters of β-thromboglobulin, platelet factor 4 released from α-granules and increased biosynthesis of thromboxane in platelets that is responsible for enhanced thrombotic tendency has been described in diabetic subjects (Onbafl, 1999). Thromboxane production is very high in patients with thrombotic complications, especially in the cardiovascular disease and in patients with poor glycemic control (Chen *et al.*, 2017).

2.11.5 Increased Fibrinogen levels in DM

Fibrinogen in the plasma is an important component of coagulation process, playing a very vital role in determining the viscosity of the blood, the flow of blood in circulation (Dhawale *et al,*. 2016), blood rheology, thrombogenesis, and platelet aggregation (Sapkota *et al,*. 2013). Epidemiological studies have suggested that, increased fibrinogen parameters have been associated with a high risk of disorders of the cardiovascular system inclusive of ischaemic heart disease and stroke as well as (Dhawale *et al,*. 2016). Moreover, reports have shown that in diabetic individuals, the risk of CVD is enhanced by elevated levels of fibrinogen (Dhawale *et al,*. 2016). Increased levels of fibrinogen, a risk factor among others in the development of

macrovascular disease, occurs through its different modes of action such as enlarged fibrin clots, elevated blood viscosity, increased tissue deposition, activation of atherosclerosis as well as vascular thickening (Dhawale *et al*,. 2016). Insulin leads to an acute and an increased production of fibrinogen in diabetic individuals and not the healthy ones (Dhawale *et al*,. 2016). In addition, studies have shown a statistical significant correlation between the levels of both fibrinogen and insulin (Sapkota *et al*,. 2013). According to Sapkota *et al*,. 2013, free radicals have been found to activate thrombin in diabetic individuals and hence, an oxidative stress, could act as a link between the state of diabetes and hyperfibrinogenemia. In addition, raised levels of fibrinogen in untreated Non-Insulin Dependent Diabetes mellitus (NIDDM) patients is associated with vascular damage induction (Dallatu, 2012). This gives a suggestion of hyperfibrinogenemia, which will result in hypercoagulable state demonstrated by shortened APTT, and consequently could as well be a risk marker for CVD as results to an increased thrombin generation and hence higher chances of occurrence of a thrombotic complication (Awad *et al*,. 2016).

2.11.6 Endothelial dysfunction in Diabetes Mellitus

The vascular endothelium, has a very vital role to play as far as the regulation of local haemostatic process is concerned (Erem *et al.*, 2004). There is endothelial dysfunction in diabetic individuals (Awad *et al*,. 2016). According to Chaitanya and Kavuri, (2014), thrombotic myocardial infarction in diabetics, may come up due to ruptured atherosclerotic plaques, exposure to procoagulant proteins which activate clotting of blood, or occur as a result of exposure of blood to damaged endothelium. Endothelium cell surface hosts the major defense mechanisms which prevent unwanted coagulation, whereby, procoagulant factors are produced by the endothelium including vWF, as well as anticoagulant factors like thrombomodulin and heparan sulfate (Erem *et al.*, 2004). The vascular endothelium, which prevents thrombosis, is defective in diabetes and it plays the role of enhancing activation of platelets as well as clotting factors in diabetic individuals (Ankalayya *et al*., 2016; Madan *et al*., 2010). Furthermore, the abnormalities linked to the endothelial lining participates in the elevation of coagulation factors and

platelet activation in diabetics (Ankalayya *et al*., 2016). A procoagulant state is observed in diabetic patients and this may be a contributing factor to the risk of catastrophic cardiovascular complications. The hypercoagulable state could result from the lack of equilibrium between plasma haemostatic factors and the surface of the endothelium (Erem *et al*., 2004).

2.11.7 Hyperglycemia in Diabetes mellitus (DM)

Diabetes is characterized by hyperglycemia, which is accompanied by decreased insulin in the blood, dyslipidaemia, hypertension and obesity (Lemkes *et al*., 2010). Therefore, the effect of diabetes on the coagulation mechanism cannot be easily associated with either one of these factors (Lemkes *et al*., 2010). Cardiovascular disease (CVD) development due hypercoagulability in diabetes subjects has been demonstrated by an increased concentrations of prothrombin fragment 1+2, which is linked to the presence of confirmed CVD in diabetic patients in contrast to non CVD patients (Lemkes *et al.*, 2010). The state of chronic hyperglycemia has been linked to long-term damage as well as non-performance of various organ systems especially the nerves, eyes, kidneys, and the heart (Chawla *et al*,. 2016). According to Nnenna *et al.*, (2016), the consequence of increased glucose levels in diabetics is impaired carbohydrate, fat, and protein metabolism.

2.11.8 Obesity

According to Leon, (2015), obesity, a common co-morbidity of DM, especially T2DM has been associated with a high rates of CV morbidity and mortality. In a study done by Mwambungu, (2014), T2DM hypercoagulability status was highly associated with obesity, and so, the obese T2DM subjects were more at risk of developing hypercoagulability compared to the non-obese T2DM patients. Therefore, obesity becomes a risk factor as far as the development of atherosclerosis is concerned (Mwambungu, 2014). IR syndrome, together with obesity are highly associated with atherosclerosis and may lead to enhanced inflammatory process in the stages of

atherosclerosis. In addition, individuals with metabolic disorders have abnormal fibrinolysis markers with an increased fibrinolytic dysfunction in people with diabetes as well as those with abdominal obesity (Awad *et al,*. 2016). There is enhanced thrombotic tendency in the obese subjects as a result of up regulation of TF, an altered expression of proteins involved in the clotting process as well as atherosclerosis (Chaitanya & Kavuri, 2014). The overweight and obese are encouraged to embrace weight loss as a treatment remedy, leading to the improvement of their CV risk profile, as well reduce the risk of CVD as stated by Leon, (2015). Leon, (2015), advises the overweight and obese diabetics to embrace a 5% weight loss in a span of 4 years with studies demonstrating attainability of this goal in pre-diabetic as well as diabetic patients. According to Rechenberg *et al.,* 2017, it is important to note that, the overweight and obese youths have an increased T1DM incidence of 3 – 4% annual rate and this is a significant public health concern.

2.12 Insulin Resistance in Type 2 Diabetes mellitus (T2DM)

The decrease in sensitivity of target tissues to insulin, a phenomena referred to as insulin resistance (IR) leads to the development of T2DM (Banoo and Nusrat, 2015). Some of the factors contributing to IR are failure to exercise, genetics, obesity, as well as advancing age (Vinik *et al*., 2001). According to Vinik *et al.*, (2001), patients with IR have some metabolic disturbances such as atherogenic dyslipidaemia, glucose intolerance, hypertension, as well as a prothrombotic state. People with the metabolic disorders present with certain patterns of coagulation factors that enhance thrombosis or slow down thrombolysis (Vinik *et al*., 2001). There is increased levels of fibrinogen, abnormalities affecting the function of platelets as well as elevated PAI-1 in thrombotic state (Vinik *et al*., 2001).

2.13 Prothrombin Time and Activated Partial Thromboplastin Time

The reduction of PT and APTT parameters form the risk factors associated with thromboembolic cardiovascular disease in T2DM (Fayeza *et al,* 2015). The

hypercoagulability state as shown by reduced time taken to form a clot in PT and APTT in diabetic patients and may result to occlusive thrombosis of a coronary artery (Fayeza *et al.,* 2015). Studies have reported contradicting coagulation profile in diabetes patients; some reporting shortened PT and APTT while others reported normal PT and APTT values (Ephraim *et al.*, 2017). The screening of PT, APTT and fibrinogen parameters are the commonest tests performed in individuals with suspected coagulopathy disorders (Dallatu, 2012). There are insufficient studies conducted in this region to explore the effects of DM on coagulation status.

2.13.1 Activated Partial Thromboplastin Time

Thromboplastin, also known as thrombokinase is made up of tissue factor and phospholipids; it is found in plasma and enables the coagulation of blood by converting prothrombin to thrombin. Activated Partial thromboplastin time is a suitable test for uncovering deficiencies in clotting factors, in addition to replacement therapy monitoring in subjects at risk of haemorrhage; with a recent report indicating a correlation between shortened APTT parameter and thrombosis risk (Sauls *et al*., 2007). In addition, elevated levels of factor VIII or IX can contribute to shortened APTT, and thrombosis as well as been linked to elevated levels of prothrombin (Sauls *et al*., 2007). According to Sapkota *et al.*, (2013), the status of thromboembolic risk in the diabetics can be assessed by APTT testing. Moreover, APTT is a useful screening test for detecting the abnormalities associated with the intrinsic and the common pathways, in addition to monitoring the effects of circulating heparin as an anticoagulant. It measures the activities of all the proteins involved, both in the intrinsic and the common pathways (Dallatu, 2012; Dhule & Gawali, 2014). Therefore, hypercoagulable state could be illustrated by shortened clotting times (Dhule & Gawali, 2014).

2.13.2 Prothrombin Time

Prothrombin time is one of the coagulation tests done specifically to detect the abnormalities associated with the extrinsic and the common pathways of the coagulation

process (Dallatu, 2012; Dhule & Gawali, 2014). PT is the laboratory test of choice which monitors the integrity of extrinsic pathway of coagulation as well as the common pathway. TF is necessary for the initiation of extrinsic coagulation pathway through factor VII activation, with raised glucose and hyperinsulinaemia in T2DM inducing a marked increase in TF activity (Dayer *et al.*, 2014)

2.13.3 International Normalized Ratio (INR)

International normalized ratio is a hematological parameter used to standardize prothrombin time test during treatment with oral anticoagulants especially warfarin (Mishra *et al*, 2017). According to Depond, (1983), it normalizes the coagulation time by correcting the differences in reagent responsiveness ensuring standardization of oral anticoagulant treatment. It is calculated form Prothrombin time and expressed numerically without units.

2.14 Principles of the Methods

2.14.1 Glucose Estimation

The On Call Plus glucometer uses test strips into which, a chemical reagent system incorporated. The test strips together with the glucometer, measure glucose levels in whole blood. An appropriate size of blood drop is applied at the end tip of the test strip and the blood being absorbed in the reaction well, the ideal reaction area. The reaction leads to the formation of an electric current which is detected by the glucometer. The glucose concentration is inversely proportional to the electrical current formed, with results being displayed on the meter's screen. The glucometer is calibrated to display plasma equivalent results. The reference range for fasting blood sugars (FBS) in diabetic patients under treatment is 4.4 – 7.2 mmol/L (Targets, 2017). FBS within the reference

range were normoglycemic which indicated good glucose control while FBS above 7.2 mmol/L were hyperglycemic indicating poor glucose control.

2.14.2 Coagulation

2.14.2.1 Prothrombin Time Principle

Prothrombin time measures the duration taken by plasma to coagulate when thromboplastin and calcium are added. When plasma is recalcification in the existence of thromboplastin, factor X activated (Xa) is generated, leading to the production of thrombin culminating with an insoluble fibrin clot. The test measures the competency of the extrinsic and the competency of the factors taking part in the common pathway. The reference range is 10 – 14 seconds (Nicholas *et al.*, 2019). Any result below 10 seconds indicated hypercoagulable state and above 14 seconds indicated hypocoagulable state. The INR reference range used was 0.8 – 1.2. The INR values were calculated directly by the machine upon calibrating the machine with a normal control. Formular for calculating INR is as follows:-

$$\text{INR} = \left(\frac{\text{PT of test sample}}{\text{PT of control sample}}\right)^{\text{ISI}}$$

2.14.2.2 Activated Partial Thromboplastin Principle

This technique leads to plasma forming a clot when calcium is added to the plasma in the presence of a platelet substitute and Kaolin which activates factor XII. The procedure minimizes test variability by standardizing the contact activation and by optimizing the concentration of platelet-like phospholipids. The APTT checks the competency of the intrinsic coagulation pathway. The reference range is 30 – 40 seconds (Nicholas *et al.*, 2019). Any result below 30 seconds indicated hypercoagulable state and above 40 seconds indicated hypocoagulable state.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Site

This study was conducted in Meru Teaching and Referral Hospital in the outpatient Diabetic clinic. It is a public Facility located in Township sub-location, Municipality location, Miriga Mieru East division, North Imenti in Meru County. It has a bed capacity of 350, and has 10 cots. The hospital has an outpatient capacity of 18,000 patients monthly, with the diabetic clinic receiving more than 50 patients on weekly basis, which is Fridays, the day set for diabetic visits. The Hospital serves residents of Meru sub - counties, and neighboring Counties such as Isiolo, Marsabit, Tharaka Nithi and Samburu.

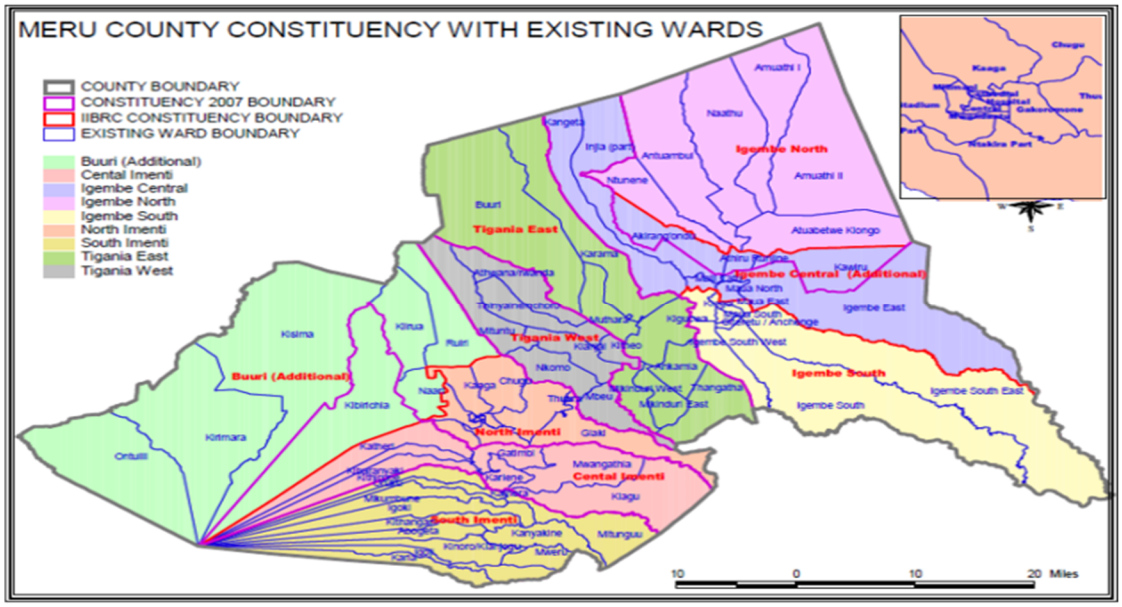

Figure 3.1: Meru County Map

Source: Kenya National Bureau Statistics (2013)

3.2 Study Design

The study design was case control study in the diabetic clinic in Meru Teaching and Referral Hospital. This was done between June 2019 and October 2019. Random systematic sampling method was used to recruit study participants; this ensured all the study subjects were given a chance to participate in the study. Both qualitative and quantitative research methodologies were employed which enhanced comprehensive analysis of the research problem. The mixed methodology was informed by the research problem enabling the researcher collect and organize data; hence making informed inferences. The study employed a case control study to collect data from 371 diabetic participants and 371 healthy controls where the relationship of coagulation parameters (PT, INR, and aPTT) between the diabetics and healthy subjects was determined. Informed consent form was signed by study participants and data entry form was prepared. PT, aPTT and glucose estimation procedures were done appropriately. Structured questionnaires and screening check list were also included.

3.3 Study Population

The study population comprised of DM patients in the outpatient diabetic clinic in Meru Teaching and Referral Hospital, both males and females upon giving consent.

3.4 Inclusion Criteria

Diabetic subjects and healthy subjects of both sex aged 18 - 60 years upon giving consent. This is the age with stable coagulation mechanism. A screening checklist was used which helped recruiting the appropriate study participant for the study. Upon meeting the criteria, a questionnaire was administered to get demographic and other required information from study participants.

3.5 Exclusion Criteria

Diabetic patients and healthy subjects of both sex aged above 60 years. Below 18 years, there is variation of coagulation mechanism. Above sixty years (60), the integrity of the vascular system may be compromised and procoagulant factors may be released into the circulation affecting coagulation. Cancer patients release procoagulant factors in circulation which activate coagulation while the pregnant women have elevated coagulation factors such as fibrinogen and this will affect the parameters under test. Patients under anticoagulant medications were not recruited since they had a coagulation disorder which they were receiving medication for as well as those with known history of coagulation disorders. Those patients with liver disease (e.g. liver cirrhosis, viral hepatitis infection etc.) were excluded since coagulation factors are synthesized in the liver and so coagulation factor production could be slowed or inhibited by the liver disease. Hypertensive and those having cardiovascular disease were excluded.

3.6 Study Variables

The dependent variables were Prothrombin Time (PT), International Normalized Ration (INR), and Activated Partial Thromboplastin Time (aPTT) while the independent variables were age, sex, fasting blood sugars (FBS) and duration of illness.

3.7 Assumption of the Study

An assumption was made that the study participants gave the right information as per the questionnaire.

3.8 Sample Size Determination

Sample size calculations were done using step by step sample size determination by Bartlett *et al.*, (2001) method: -

$$n \frac{Z^2 P(1-P)}{d^2}$$

Whereby,

n= required sample size

Z = a statistic for the level of confidence, 0.25=1.96

P = the expected prevalence or proportion.

According to a study carried out by Mwambungu (2014) in Zambia, there was a prevalence of 59.2% hypercoagulable status in T2DM patients. Therefore **P** = 59.2%, P = 0.592, and

d = precision = 5%, d = 0.05).

$\boldsymbol{n} = \frac{(1.96)20.592(1-0.592)}{0.05^2} = 370.66 \approx 371.$ Therefore, sample size was 371.

3.9 Sampling Method

Random systematic sampling method was used in the recruitment of study participants of 18 - 60 years attending Meru Teaching and Referral Hospital upon filling consent forms.

3.10 Data Collection

The collection of data was done by use of a structured questionnaire. Participant's history including demographic data was obtained directly from the participants. Information obtained included age, sex, duration of illness (defined as the day when someone was diagnosed with Diabetes disease), and medication especially anticoagulant therapy.

3.11 Specimen Collection and Processing

Venous blood was collected using a syringe and a needle from veins at the antecubital area (4.5 mls) in 3.2% trisodium citrate (0.5 mls). This translates to nine parts of blood + one part of anticoagulant for estimation of PT and APTT. The blood was well mixed immediately with anticoagulant to prevent clotting. It was centrifuged at 3500 rotations per minute for 10 minutes to get platelet free plasma. The plasma was then separated immediately using a plastic Pasteur pipette and analyzed using coagulation analyzer (Brand name - *Start 4*). For blood sugar estimation, glucose oxidase method was used (enzymatic) with a glucometer (brand name - ON CALL PLUS). One drop of capillary blood was used for blood glucose analysis.

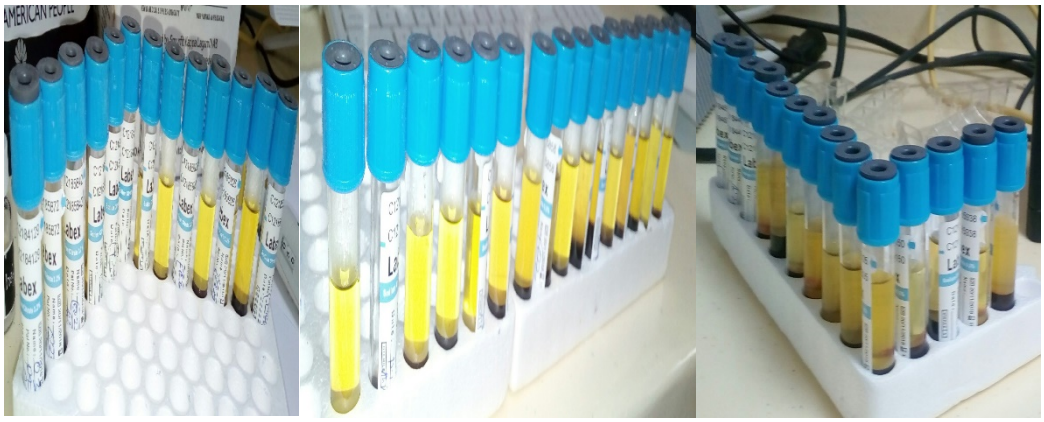

Figure 3.2: Samples for coagulation testing in 3.2% Sodium citrate tubes

Source: Field Data

3.12 Data Management

All study data was keyed into Micro soft-Excel and analyzed with statistical package for social sciences (SPSS) version 23. The data collected was both quantitative and qualitative and was analyzed by descriptive and inferential statistical methods. Inferential statistics helped in making conclusions of the study findings while descriptive statistics enabled summarization of data into measures of central tendency and measures

of variability. Comparison of haemostatic parameters (PT, INR and APTT) between male and females was done by use of independent sample t- test parameters, a method suitable to make comparison between two independent groups. Linear regression was used to determine the relationship between coagulation parameters (PT, INR, and APTT) of diabetic patients and the healthy controls. The analysis of variance (one-way ANOVA test) was used to compare coagulation parameters and the duration of illness since illness was categorized into four groups. ANOVA was also used to evaluate the variation of coagulation parameters in diabetic participants in relation to age, followed by Tukey's post hoc test. The age was categorized into four age groups (18-30, 31-40, 41-50, and 51-60 years) hence making this statistical method suitable for this objective. The alpha level was set at 5% ($p<0.05$). The data was presented with tables and graphs.

3.13 Data categorization

The data collected was grouped according age groups (18-30, 31-40, 41-50 and 51-60), gender (male and female), and duration of illness

3.14 Ethical Consideration

Permission to conduct the research was obtained from the Department of Post graduate school, through the Department of Medical Laboratory Sciences of Jomo Kenyatta University of Agriculture and Technology (JKUAT). Ethical clearance was sought from the Ethics and Research Committee of KNH/UoN ERC (appendix VIII), this was then forwarded to the NACOSTI (appendix IX) to obtain a research permit. It was then presented to the Chief Officer for Health Meru county and then to the Chief Executive Officer of Meru Teaching and Referral Hospital and management team in order for the researcher to be allowed to collect the desired data. Informed consent was sought from all study subjects.

3.15 Quality Management

Internal quality control (IQC) was adhered to throughout all the stages of testing process. Two levels of control reference materials were used for IQC namely; normal control plasma, and abnormal control plasma (pathological high). Controls were treatment in a similar manner as the test samples.

3.16 Pre-analytical Phase

Only fasting patients and healthy subjects participated in the study. Proper patient identification, collection of samples in the right collection tubes, the appropriate blood volumes, and labelling of collected samples was ensured. All samples were given a unique identification number which was traceable throughout the testing process.

3.17 Analytical Phase

Quality control material for both normal plasma and abnormal plasma was run alongside the samples.

3.18 Post Analytical Phase

All the results were checked for completeness and accuracy.

CHAPTER FOUR

RESULTS

4.1 Introduction

This chapter presents the results of data analysis, discussions of the outcome in relation to the objectives of the study as well as the hypotheses. The independent samples t test was used in the analysis of the data as it qualified for parametric analysis. Other data analysis methods used were analysis of variance and linear regression due to the quantitative nature of the data.

4.2 Demographic attributes of the study participants

Table 4.1: Demographic attributes of the study participants (diabetics)

	V		N	%
Diabetic Group	Gender	Male	152	41
		Female	219	59
	Age Group in Years	18 – 30	28	7.5
		31 – 40	78	21
		41 – 50	123	33.2
		51 - 60	142	38.3

V, variable; N, sample size; %, Percentage

Table 4.1 shows a cross tabulation of independent variables of the study participants. A total of 371 diabetic subjects took part in the study. Male subjects were 152 (41%) while female participants were 219 (59%). The age group of the participants ranged from 18-60 years. The age of the participants were categorized into four groups; 18 - 30 years having 28 (7.5%) participants, 31 - 40 years with 78 (21%) participants, 41 - 50 had 123 (33.2%) participants, while 51 - 60 years had 142 (38.3%) study subjects. Their mean

age was 45.99 (SD = 9.501). The age range was 42 (60 – 18) and 48.29 as the median age. The duration of illness had a mean of 4.14 years, with a standard deviation of 2.773 while the range was 9 years.

4.3 Determination of the relationship of coagulation parameters of diabetics and normal controls

Table 4.2: Coagulation status of diabetic participants: *Linear regression***; p-value = <0.05**

Variables	Subjects	N	M	SD	R	R Square	F-value	*p*-value
PT	Diabetic	371	12.726	1.307	0.065	0.04	1.542	0.215
	Normal group	371	12.487	1.016				
INR	Diabetic	371	1.0142	0.137	0.064	0.04	1.500	0.221
	Normal group	371	0.9891	0.102				
APTT	Diabetic	371	30.656	3.74	0.04	0.002	0.586	0.445
	Normal group	371	34.424	2.71				

PT, prothrombin time; INR, international normalized ratio; APTT, activated partial prothrombin time; N, sample size; M, mean; SD, standard deviation.

A linear regression was done to determine the relationship of coagulation status in diabetic patients and the normal group. Table 4.2 shows the means of the two sample groups and their SD, the R and R^2 values, and the ANOVA. The R value of 0.065

indicates a very low degree of correlation between PT of the diabetic patients and normal group. The R =0.065=6.5% indicates weak relationship. The mean PT for the diabetics was (12.726±1.3070 seconds) while the PT of normal group was (12.487±1.0157 seconds) but there was no statistically significant difference F = 1.542; p = 0.215.

The table shows low degree of correlation (R=0.064) between patient's INR and normal group INR. An R of 6.4% signifies very weak relationship. The mean INR for the diabetics was (1.0142±0.13735) while the normal group was (0.9891±0.10222), there was no statistically significant difference (F = 1.500; p = 0.221).

The table further shows a low degree of correlation (R=0.040) between patient's APTT and normal group APTT. This shows a weak relationship indicating that only 4% of the dependent variable has been explained by the independent variable. The mean APTT for the diabetics (30.656±3.7374) was lower than that of normal group (34.424±2.711) but having no statistically significant difference (F = 0.586; p = 0.445).

4.4 Comparison between coagulation parameters and duration of illness

Table 4.3: Comparison between coagulation parameters and duration of illness: One-way ANOVA Test, p-value = < 0.05

V	DYs	N	M	SD	df	F-value	*p*-value
PT	0 – 3	214	12.747	0.991	BG – 3	1.288	0.278
	4 – 6	72	12.875	2.019			
	7 – 9	60	12.655	1.236	WG -		
	>10	25	12.296	1.243	367		
INR	0 – 3	214	1.0153	0.994		1.122	0.340
	4 – 6	72	1.0301	0.224			
	7 – 9	60	1.0083	0.125			
	>10	25	0.9728	0.119			
APTT	0 – 3	214	30.592	3.648		0.213	0.887
	4 – 6	72	30.944	3.827			
	7 – 9	60	30.662	3.676			
	>10	25	30.364	4.503			

PT, prothrombin time; INR, international normalized ratio; APTT, activated partial prothrombin time; DYs, duration in years; N, sample size; M, mean; SD, standard deviation; V, Variables; BG, Between groups; WG, Within groups; df, degrees of freedom.

Table 4.3 shows the INR means between the duration of illness groups as revealed by one-way ANOVA ($F(3,367) = 1.122$, $p = 0.340$) revealed no statistically significant differences between the group means.

The PT means between the groups as revealed by the one-way ANOVA ($F(3,367)= 1.288$, $p = 0.278$) showed no statistically significant difference between the groups. The APTT between the groups as revealed by one-way ANOVA[$F(3,367) = 0.213$, $p = 0.887$] revealed no statistically significant differences between the different durations of illness.

4.5 Comparison of coagulation parameters according to sex

Table 4.4: Comparison of haemostatic parameters of males and females: Independent-sample t-test parameters, P-value = < 0.05

Variable	Sex	RR	N	M	SD	t-value	P-value
PT	Male	10 – 12	152	12.8	1.09	0.591	0.55
	Female		219	12.7	1.44		
INR	Male	0.90 –	152	1.02	0.11	0.418	0.676
	Female	1.2	219	1.01	0.15		
APTT	Male	30 - 40	152	30.5	3.48	-0.577	
	Female		219	30.8	3.91		0.564

PT, prothrombin time; INR, international normalized ratio; APTT, activated partial prothrombin time; RR, reference range; N, sample size; M, mean; SD, standard deviation.

An independent-sample t-test was done to compare the coagulation parameters of diabetic participants according to sex. Table 4.4 shows the mean of male PT (12.8±1.09) was higher than the mean for female PT (12.7±1.44) but the difference was not significant statistically (t-value = 0.591; p-value = 0.55).

The mean INR for males (1.02±0.11) was higher than the mean for female INR (1.01±0.15) with no statistical significant (t-value = 0.418; p-value = 0.676).

The mean APTT for males (30.5±3.48) was lower than mean APTT for females (30.8±3.91) but the difference was not statistically significant (t-value = -0.577; p-value = 0.564).

4.6 Variation of coagulation parameters in diabetic participants in relation to age

Table 4.5: Variation of coagulation parameters in diabetic patients in relation to age: One-way ANOVA Test, p-value = < 0.05

V	AGY	RR	N	M	SD	df	F	P-Value
INR	18 – 30		28	1.02	0.101	BG = 3	0.224	0.880
	31- 40		78	1.01	1.00	WG =		
	41 – 50	0.8 – 1.2	123	1.02	0.96	367		
	51 – 60		142	1.01	0.185			
PT	18 – 30		28	12.8	0.99		0.281	0.839
	31- 40		78	12.6	1.00			
	41 – 50	10 – 14	123	12.8	0.97			
	51 – 60	Seconds	142	12.7	1.71			
APTT	18 – 30		28	29.4	2.98		4.395	0.005
	31 - 40	30 – 40	78	31.6	3.81			
	41 – 50	Seconds	123	31.0	3.57			
	51 – 60		142	30.1	3.84			

PT, prothrombin time; INR, international normalized ratio; APTT, activated partial prothrombin time; RR, reference range; N, sample size; M, mean; SD, standard deviation; AGY, Age group in years; V, Variables; BG, Between groups; WG, Within groups; df, degrees of freedom.

Table 4.5 shows that, the INR means between the four age groups as revealed by one-way ANOVA ($F(3,367) = 0.224$, $p = 0.880$) were not statistically significant between the

group means. The PT means between the groups ($F(3,367)= 0.281$, $p = 0.839$) indicated no statistically significant difference between the groups.

The APTT between the groups [$F(3,367) = 4.395$, $p = 0.005$] revealed a statistically significant difference between the group means. Tukey post hoc test was done to determine which specific group differed.

Table 4.6: Post HOC Test to determine specific (APTT) age group difference

V	AGY	AGY	M	*P*-value
		31 – 40	-2.1847	0.037
APTT	18 - 30	41 – 50	-1.6049	0.162
		51 - 60	-0.6455	0.832
		18 – 30	2.1847	0.037
	31 - 40	41 – 50	0.5798	0.698
		51 - 60	1.5392	0.017
		18 – 30	1.6049	0.162
	41 - 50	31 – 40	-0.5798	0.698
		51 - 60	0.9594	0.151
		18 – 30	0.6455	0.832
	51 - 60	31 – 40	-1.5392	0.017
		41 - 50	-0.9594	0.151

APTT, activated partial prothrombin time; AGY, Age group in years; V, Variables; M, mean.

Table 4.6 revealed the categories of age groups which differed. There was a statistically significant reduction of APTT in age group 18 - 30 (29.4±2.98 seconds, $p = 0.037$), and 51- 60 (30.1±3.81 seconds, $p = 0.017$) compared to the age group 31- 40 (31.6±3.81 seconds). There was no statistical significance in APTT variation between age group 18 - 30 and 41- 50 ($p = 0.162$), 18-30 and 51- 60 ($p = 0.832$), 31- 40 and 41- 50 ($p = 0.698$), and lastly 41- 50 and 51- 60 ($p = 0.151$).

CHAPTER FIVE

DISCUSSION AND CONCLUSIONS

5.1 Discussion

The goal of the study was to evaluate PT, INR and APTT in diabetic patients attending Meru Teaching and Referral Hospital (MTRH), to compare coagulation parameters according to gender, duration of illness, variation of coagulation in diabetics according to age, and to determine the relationship between coagulation parameters of diabetic patients (coagulation status) and the normal control. Three hundred and seventy one (371) diabetic patients and 371 normal group were studied. The majority of diabetic cases were females 219 (59%) while the male cases were 152 (41%). The APTT of the cases (diabetics) was similar to that of the normal group. PT of diabetic subjects was also similar to that of normal group. International normalized ratio is a parameter calculated from the PT, which was insignificantly elevated in the diabetic subjects in comparison to normal group. This results differs from Mwambungu (2014) in Zambia, who reported significantly shortened APTT and insignificantly shortened PT in diabetic subjects than non-diabetic control participants. This clearly indicated incompetent intrinsic pathway and therefore hypercoagulable state in subjects who participated in the study.

The PT in this study was normal indicating a competent extrinsic coagulation pathway, showing adequacy of coagulation factors involved in this pathway. Mwambungu's (2014), results were consistent with Ephraim *et al.,* (2017) and Awad *et al*., (2016) who reported significantly shortened APTT, but their PT findings differed which was significantly shortened in Ephraim *et al.*, (2017) while in Awad *et al.*, (2016) report, PT and INR were consistent with the findings of current study with no statistically significant difference in the PT and INR of diabetic participants and the healthy participants. The variation in APTT and PT results observed among different studies could be associated with the sample size used. For example, Ephraim *et al* had a small

sample size of 100 both diabetic subjects and controls. This may have contributed to the variations observed between this current study and the previous studies. In Mwambungu's study, 213 T2DM cases participated in the study, which was also less than the sample size used in this study; with 19 participants being more than 60 years old. The variation in study variables especially age could have led to the differences in APTT, PT and INR in this current study as compared to others. With advance age, coagulation factors increase for example fibrinogen, factors VI, VIII, IX, XII, and von Willebrand among others (Mari *et al.*, 2008). According to Mari *et al.*, (2008), impaired fibrinolysis mechanism also manifest with ageing. Agarwal *et al.*, (2018) study revealed shortened APTT and PT and hence being inconsistent with findings of this study, the reason could be attributed to age of the participants as they were aged 35 – 70 years while participants in this current study were younger aged 18 – 60 years. In addition, the sample size was smaller, 60 diabetics and 30 controls as compared to this study could have resulted to the disparities between this studies. According to a study done by Chaitanya and Kavuri, (2014), their study revealed prolonged PT and APTT in diabetic patients, which differed from the findings of this study. This implies that the extrinsic and intrinsic pathways were affected and hence the diabetic patients could suffer from excessive bleeding in case of injuries. According to Ogedegbe (2002), the APTT and PT prolongation may be associated with factor deficiency or due to circulating anticoagulants. Their study also included hypertensive diabetic subjects which may have resulted to the differences as this current study only dealt with diabetic patients without complications; this is because during the recruitment of the subjects, only diabetic patients not suffering from other illness were recruited. The INR findings of this study differed from Ephraim *et al.*, (2017), who reported significantly decreased INR parameters in diabetics than in non-diabetics. A study done by Dallatu (2012) concurred with the findings of this current study which revealed that there was no statistically significant difference in treated diabetic participants and the control subjects. Madan *et al.*, (2010), found no statistical significance in APTT and PT of both diabetics and non-diabetic controls just as the findings of this study. Normal PT and APTT could be as a

result of treatment of diabetics in this study with antidiabetic drugs enabling them have a normal coagulation status.

Coagulation parameters were correlated with the duration of diabetes illness. In the present study, no statistical significant was detected between coagulation parameters and duration of diabetes illness. This means that the duration of illness did not affect the coagulation status of the individual. The findings are in harmony with Awad *et al.,* (2016) who found no statistically significant different between duration of illness and Diabetes mellitus. As well, Sapkota *et al.,* (2013), findings revealed insignificant association between duration of diabetes and APTT in the diabetic subjects. However, the findings of this current study were inconsistent with observations of other studies as Mwambungu (2014), reported the likelihood of hypercoagulable state of diabetic participants who had the illness for more than 10 years in comparison to those who had the illness for a duration of less than 5 years.

This study did a comparative study of the coagulation parameters of diabetic patients according to gender. Female participants were the majority as compared to men. The means of coagulation parameters analyzed (PT, INR and APTT) were within the expected reference ranges hence normal. This current study revealed no statistically significance difference between the PT of males and that of females. The INR findings had no significant difference between sexes. Both mean PT and mean INR of males were similar to those of females. The mean APTT was also similar for both male and female participants. This current study reveals that APTT findings do not correlate with Mwambungu (2014), findings who observed statistically significant low mean APTT in females than in males. The PT was consistent with Mwambungu (2014) findings, which revealed similar mean PT in males than in females.

Variation of coagulation parameters in diabetic participants in relation to age was studied. The participants were divided into four (4) age groups. The study observed that there was no significantly difference in PT and INR means of the different age groups. The APTT means of different age groups revealed an overall statistically significant

difference. Tukey's post hoc test was done to reveal which specific group differed and it revealed the age group means differing significantly from other group means.

The tukey's post hoc test revealed that there was significant different in the means of 18 – 30 and 31 – 40 age groups which were significantly reduced compared to 31 – 40 age group. This indicated reduced APTT in 18-30 and 51-60 age groups. A study done by Mwambungu (2014) revealed that diabetic subjects 51 years of age and over were at risk of being hypercoagulable as compared to those aged below 51 years, this was in line with the observations of this study as the diabetic subjects in the age group 51 – 60 years had their APTT hypercoagulable. According to Mwambungu, (2014), changes occurring to the vascular system as the age increase could be associated with hypercoagulation status. According to Favaloro *et al*., (2014), coagulation disorders are known to increase with aging such a venous thromboembolism resulting from haemostasis changes which reflects a heightened procoagulant status compared to young age. This indicates defects in the intrinsic as well as in the common pathways of the coagulation cascade (Dhule & Gawali, 2014). There was no significantly difference in the means of other age groups (31 – 40 and 41 – 50, 41 – 50 and 18 – 30, 41 – 50 and 51 – 60, 51 – 60 and 18 – 30).

5.2 Study Limitations

The most expected limitation was language barrier as the native community are Meru who speak Kimeru and some never understood English or Kiswahili. Local interpreters were incorporated to help in overcoming this challenge.

5.3 Conclusions

1. This current study revealed that the coagulation parameters (PT, INR and aPTT) of diabetics were similar to those of the control group and were not statistically significantly affected by the diabetic state of the study subjects. This indicated that the extrinsic and common pathways of coagulation cascade were intact and very competent in the treated diabetic subjects.

2. The study further revealed that the coagulation parameters of the diabetics were not affected by the diabetic status of the study subjects indicating normal coagulation profile.
3. Gender did not have significant effect on coagulation and therefore coagulation was normal for males and females in the study.
4. Age did not have a significant effect on INR and PT, but there was variation in aPTT indicating significant difference in age group means and this revealed hypercoagulable state in this age groups (18-30 and 51-60).

5.4 Recommendations

1. This study was conducted on diabetic patients under treatment. Therefore, there is a need to conduct a similar study on both treated and untreated (newly diagnosed) diabetics to determine if there is significant difference in the coagulation status between the two groups.
2. Assessment of other parameters associated with coagulation screen to be researched on to see whether they are affected in diabetics (e.g. thrombin time, factor assays, platelet functional assay and morphological examination of platelets
3. Risk factors need to be assessed in future (e.g. smoking, obesity among others)

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APPENDICES

Appendix I: Informed Consent Form

Topic: Evaluation of prothrombin time and activated partial thromboplastin time tests in diabetes mellitus patients at Meru Teaching and Referral Hospital

Consent Explanation: My name is **Stephen Wambua Mutua** (Cellphone; 0723388875) a Masters student in the Department of Medical Laboratory Sciences, Jomo Kenyatta University of Agriculture and Technology (JKUAT). PO Box 20732 Nairobi, Kenya; Phone: +254-6-752711. My supervisors are: **Dr. Kimani S.N** (Cell phone: 0721972652) and **Dr. Michael Kahato** (Cell phone: 0722921248). I am conducting a study to evaluate the prothrombin time and activated partial thromboplastin time tests in Diabetes mellitus patients. The information in this form will help you make an informed decision whether or not to take part in this study. Kindly read through keenly and be encouraged freely to ask any question about the study. I will read it out to those who are not able to read.

Description: Previous studies in other countries have indicated conflicting information in the relationship between DM and hypercoagulability status.

Purpose: This study is interested in finding the relationship between DM and hypercoagulation status.

Benefits: There will be no tokens to the participants. However, the final findings will ultimately help the participants know their coagulation status. Depending on findings, the physicians or clinicians in diabetic clinic will decide whether more investigations will be required on patients whose basic coagulation will be prolonged so that the right treatment and management can be given in time to prevent complications associated with coagulation disorders in T2DM.

Risks: The procedure of sample collection has no side effects but might cause a slight discomfort such as haematoma, which will be taken care of professionally if it occurs. The samples will only be used for the intended purpose of this study and any personal information collected going to be handled highly confidential.

Procedure: Once you accept to take part in this study, blood sample will be collected for analysis.

Voluntarism: Enrolment to the study is at free will.

Subject's rights: As a voluntary study participant, you have the right to withdraw your consent or discontinue participating at any time without penalty. Your personal privacy will be maintained in all published and written data emanating from the study.

In case you have questions about your rights as a study participant and or are dissatisfied at any point with any aspect of this study, you are free to contact - anonymously, if you wish – KNH/UoN ERC (Chairperson of the Scientific Steering Committee, PO Box 20732 Nairobi, Kenya; Phone: 02-7263000 Ext 44102.

I, the study participant have read this form or had it read to me in a language that I comprehend. I have clearly discussed the Information with study staff. My questions

have been answered professionally. My decision whether or not to take part in the study is voluntary. If I decide to join the study I may withdraw at any point.

By signing this form I do not give up any rights that I have as a study participant.

_____	_____	_____
Participant Name	Participant Signature/ Thumbprint	Date
_____	_____	_____
Study Staff Conducting Study	Staff Signature	Date

Appendix II: Data Entry Form

S/NO / Lab: No	AGE in Years	SEX	FBS in mmol/L	PT	INR	APTT in seconds
1.						
2.						
3.						
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Appendix III: Prothrombin Time Procedure

- The coagulometer (Start 4) was switched on 30 minutes before analysis to warm up.
- After warming up, from the main menu. Test parameters were selected appropriately by pressing the (3) key and confirmed with enter.
- PT test was selected by pressing the (1) key and confirmed with enter.
- Patient ID number was entered. Enter key was pressed after every ID entry. Lastly, enter key was pressed twice the when last number was entered.
- Cuvette strips were placed in the incubation area for pre-warming at 37^0c for 3 minutes.
- Metallic balls were dispensed to each cuvette.
- 50µL of plasma was dispensed in the cuvettes (control or test plasma).
- The timer was started by pressing the timer key.
- When the analyzer started to beep, the cuvettes were transferred to the test column area.
- The Finnpippette was primed once with the start reagent (Thromboplastin reagent with 0.025M Calcium chloride)
- The Finnpippete was activated by pressing the pipette key
- 100µL of the pre-warmed start reagent at 37^0c was dispensed into each cuvette.

Results

- Final results were displayed in seconds and INR

- Patients results were reported in relation to the normal control sample
- Reference range for the normal control is 12 – 14 seconds and INR of 0.8 – 1.2

Appendix IV: Activated Partial Thromboplastin Time Procedure

- The coagulometer (Start 4) was switched on 30 minutes before analysis to warm up.
- After warming up, from the main menu. Test parameters were selected by pressing the (3) key and confirmed with enter.
- APTT test was selected by pressing the (2) key and confirmed with enter.
- Patient ID number was entered. Enter key was pressed after every ID entry. Lastly, enter key was pressed twice the when last number was entered.
- Cuvette strips were placed in the incubation area for pre-warming at 37^0c for 3 minutes.
- Metallic balls were dispensed to each cuvette.
- 50µL of plasma was dispensed in the cuvettes (control or test plasma).
- 50µL of APTT reagent was dispensed in each cuvette.
- Timing started when the timer key was pressed.
- When the analyzer started to beep, the cuvettes were transferred to the test column area.
- The Finnpippette was primed once with Calcium chloride (0.025M).
- The Finnpippete was activated by pressing the pipette key
- 50µL of the pre-warmed Calcium chloride at 37^0c was dispensed into each cuvette.

Results

- Final results were recorded in seconds
- Patients results were reported in relation to the normal control sample
- Reference range for the normal control is 30 – 40 seconds.

Appendix V: Glucose Estimation Procedure

- Before analyzing patient's samples, the glucometer was coded.
- After coding, the test samples were then analyzed.
- Test strip was inserted into the strip port.
- The blinking test strip and blood drop icon indicated that the test strip was inserted correctly.
- The drop of blood was touched with the tip of the test strip. The meter beeped to indicate the sample was sufficient and measurement started.
- The meter counted from 9 to 1 and then displayed the results. The meter also beeped to indicate that the measurement was complete.
- Results were recorded, the test strip was removed and discarded in a safety box.

Results

- The meter accurately measures blood glucose concentrations between 1.1 to 33.3 mmol/L. "HI" and "LO" indicates results outside this range.
- Reference ranges blood glucose for healthy individuals and diabetics under treatment
    - Fasting – 3.9 – 5.6 mmol/L – Healthy individuals
    - Fasting (4.4 – 7.2) – Diabetics under treatment

Appendix VI: Questionnaires

Please respond to all the questions in the questionnaire appropriately and honestly. The information given will be treated highly confident and will only be used for research purpose only. This will be explained to the participants who do not understand English in a language they comprehend. (Parent or guardian to respond or fill the questionnaire on behalf of those aged below 18 years).

1. Age in years: ☐
2. Gender: Female ☐ Male ☐
3. For how long have you had diabetes? 0-3 years ☐ 4-6 years ☐ 7 ☐ years >10 ☐ years
4. Is there any history of diabetes in your family? Yes: ☐ No: ☐

Appendix VII: Screening Checklist for Recruitment Procedure

Are you sick today? (other kind of sickness excluding diabetes)				
Are you on any medication at the moment? (For other diseases or conditions excluding diabetes)				
Are you aged above 60 years?				
Could you be suffering from any form of Cancer?				
Have you ever been told that you have diabetes (high blood sugar in your blood)?				
Do you suffer from any coagulation disorder? (e.g. Bleeding disorder?)				
Do you have a liver disease (hepatitis, liver cirrhosis) or jaundice?				
Female	Are you pregnant?			
	Are you on any medication at the moment?			

Appendix VIII: Kenyatta National Hospital –University of Nairobi Ethics and Research Committee

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Ref: KNH-ERC/A/55

20th February, 2019

APPROVED 20 FEB 2019

Stephen Wambua Mutua
Reg.No.HSB331-3250/2016
School of Biomedical Sciences
College of Health Sciences (CoHES)
J.K.U.A.T

Dear Stephen

RESEARCH PROPOSAL – EVALUATION OF PROTHROMBIN TIME AND ACTIVATED PARTIAL THROMBOPLASTIN TIME TESTS IN DIABETES MELLITUS PATIENTS AT MERU TEACHING AND REFERRAL HOSPITAL (P815/12/2018)

This is to inform you that the KNH- UoN Ethics & Research Committee (KNH- UoN ERC) has reviewed and **approved** your above research proposal. The approval period is 20th February 2019 – 19th February 2020.

This approval is subject to compliance with the following requirements:

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

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

Protect to discover

Yours sincerely,

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

c.c. The Principal, College of Health Sciences, UoN
The Director, CS, KNH
The Chairperson, KNH- UoN ERC
The Assistant Director, Health Information, KNH
Supervisors: Dr. Kimani S.N(Kenya Methodist University), Dr. Michael Kahato(JKUAT)

Appendix IX: Research Permit by National Commission for Science, Technology And Innovation

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **134259**

Date of Issue: **23/August/2019**

RESEARCH LICENSE

This is to Certify that Mr.. Stephen Mutua of Jomo Kenyatta University of Agriculture and Technology, has been licensed to conduct research in Meru on the topic: EVALUATION OF PROTHROMBIN TIME AND ACTIVATED PARTIAL THROMBOPLASTIN TIME TESTS IN DIABETES MELLITUS PATIENTS AT MERU TEACHING AND REFERRAL HOSPITAL for the period ending : 23/August/2020.

License No: **NACOSTI/P/19/424**

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Appendix X: Research Authorization County Government of Meru – Department Of Health

COUNTY GOVERNMENT OF MERU

DEPARTMENT OF HEALTH

County Government
Headquarters
P.O. Box 120-60200
MERU

Email: merucountyhealth@gmail.com
When replying please quote

Ref: CGM/COH/HR/1/3/Vol.4 (175) **29th May, 2019**

STEPHEN WAMBUA
Reg. HSB331-3250/2016

RE: RESEARCH AUTHORIZATION

This is to inform you that your request to carry out a research on **"Evaluation of prothrombin time and activated partial thromboplastin time tests in diabetes mellitus patients at Meru Teaching and Referral Hospital,"** has been approved.

Kindly share the research results with us.

Thank you.

Dr. Kariana Kimonye
CHIEF OFFICER OF HEALTH

Cc: Chief Executive Officer
Meru Teaching & Referral Hospital

Appendix XI: Research Authorization Letter from JKUAT Post Graduate School

JOMO KENYATTA UNIVERSITY
OF
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P.O. BOX 62000
NAIROBI – 00200
KENYA
Email: director@bps.jkuat.ac.ke
TEL: 254-67-5870000/1-5

REF: JKU/2/11/HSB331-3250/2016 **17TH FEBRUARY, 2020**

MUTUA STEPHEN WAMBUA
C/o SOBMS
JKUAT

Dear Mr. Wambua,

RE: APPROVAL OF RESEARCH PROPOSAL AND APPOINTMENT OF SUPERVISORS

Kindly note that your MSc. research proposal entitled: **"EVALUATION OF PROTHROMBIN AND ACTIVATED PARTIAL THROMBOPLASTIN TIME TESTS IN DIABETES MELLITUS PATIENTS AT MERU TEACHING REFERRAL HOSPITAL."** has been approved. The following are your approved supervisors:-

1. Dr. Kimani S. N. -KEMU
2. Dr. Michael Kahato – MLS, JKUAT

Yours sincerely,

PROF. MATHEW KINYANJUI
DIRECTOR, BOARD OF POSTGRADUATE STUDIES
Copy to: Dean, SOBMS

/cm

Appendix XII: Journal of Medical Science and Clinical Research Publication

JMSCR Vol||08||Issue||01||Page 618-624||January 2020

http://jmscr.igmpublication.org/home/
ISSN (e)-2347-176x ISSN (p) 2455-0450
crossref DOI: https://dx.doi.org/10.18535/jmscr/v8i1.97

IGM Publication Journal Of Medical Science And Clinical Research
An Official Publication Of IGM Publication

Evaluation of Prothrombin time and Activated Partial Thromboplastin Time Tests in Diabetes Mellitus Patients at Meru Teaching and Referral Hospital

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Abstract
Introduction: *Diabetes mellitus is a chronic and a progressive disease. The risk of at herothrombotic complication is increased in diabetics. Research has shown a diverse of Diabetes mellitus related abnormalities especially in haemostasis and thrombosis. The main objective of this study was to investigate the Prothrombin time and Activated partial thromboplastin time in Diabetes mellitus patients.*
Methodology: *Descriptive analytical cross-sectional study was employed to recruit 371 participants. Venous blood was collected (4.5 mls) in 3.2% trisodium citrate (0.5 mls) for prothrombin time and activated partial thromboplastin time testing. It was analyzed using coagulation analyzer (Start 4). Capillary blood was used for glucose estimation by glucose oxidase method.*
Results: *There was low degree of correlation between prothrombin time, international normalized ratio and activated partial thromboplastin time of diabetics as compared to the controls (R=4%, p=0.215, R=4%, P=0.221 and R=4%, P=0.445 respectively). There was statistical significant in activated partial thromboplastin time in relation to age (p=0.005) with no statistical significant in prothrombin time (0.839) and international normalized ratio (p=0.880).*
Conclusion: *Prothrombin time and international normalized ratio were not significantly affected by the diabetic state of the study subjects. Activated partial thromboplastin time had a significant difference in age group means revealing hypercoagulable state. Preventive measures should be undertaken in these age groups to ensure complications associated with hypercoagulable state do not occur.*
Keywords: *Prothrombin time, Activated partial thromboplastin time, International normalized ratio, hypercoagulable.*

Introduction

Diabetes, which is a long term disease condition, leads to an increased blood sugar levels[(1)]. According to Singh[(2)], it is a common endocrine disease is associated with multiple etiologies, presents with chronic hyperglycemia with disturbances in the metabolism of the carbohydrates, fats, and proteins. The excess blood glucose brings about some of major symptoms associated with Diabetes mellitus, such as polydipsia, polyuria and polyphagia[(3).] Diabetes was not a new disease to

Indian physicians even before 1500BC[4]. Diabetes is among the oldest diseases known in human history, it was first reported in Egyptian manuscript approximately three centuries ago[5]. The prevalence of Diabetes mellitus is drastically increasing worldwide and approaching epidemic proportions[6]. In 2013, according to Awad et al,.[1], estimations indicated that over 382 million people worldwide suffered from diabetes. Therefore, about 90% of the diabetic cases are Type 2 Diabetes mellitus while 10% of the cases are Type 1 Diabetes mellitus and so, diabetes is a serious health problem [3]. The risk of atherosclerosis is very high in the diabetics with coronary artery disease being the major cause of death in these individuals [7].

Persistent hyperglycemia in patients with Diabetes mellitus, leads to red cells being exposed to elevated glucose levels, consequently, leading to glycation of several parameters such as haemoglobin, prothrombin, fibrinogen, as well as other proteins involved in clotting mechanisms [3]. Once the intrinsic and extrinsic clotting factors are glycated, the availability of these factors is significantly decreased and it will affect the clotting capacity [3]. Diabetic patients have disturbances of the haemostatic and fibrinolytic mechanisms, with the development of diabetic complications being associated with them, as well as the incidence of cardiovascular events being increased[8]. The main causes of morbidity and mortality in the diabetics result from thrombosis and related complications[7,8]. Several studies have been done in Kenya describing the prevalence of diabetes, none has compared diabetes and its relationship with basic coagulation tests. Therefore, this study aims to compare basic coagulation tests (Prothrombin time and Activated partial thromboplastin time) in Diabetes mellitus.

Methodology

The study design used was descriptive analytical cross-sectional study in June 2019 to October 2019. The study population comprised of Diabetic patients in the outpatient diabetic clinic and both males and females were recruited. Study participants were aged below 60 years meeting the inclusion criteria. The sampling technique used was random systematic method to recruit study participants upon consenting and filling consent forms. Those aged below 18 years, consent was sought from their parents or guardians. Ethical clearance was obtained from the Kenyatta National Hospital-University of Nairobi Ethics and Research Committee (KNH/UoN ERC).

Venous blood was collected (4.5 mls) in 3.2% trisodium citrate (0.5 mls) for prothrombin time and activated partial prothrombin time. One drop of capillary blood was used for blood glucose analysis by glucose oxidase method.

Study data was keyed into MS-EXCEL and analyzed with SPSS version 23. Comparison of haemostatic parameters (Prothrombin time, International normalized ratio, and Activated partial thromboplastin time) between male and females was done by use of independent sample t- test parameters. Linear regression was used to determine the relationship between coagulation parameters (Prothrombin time, International normalized ratio, and Activated partial thromboplastin time) and glucose parameters in diabetic patients. ANOVA was used to evaluate the variation of coagulation parameters in diabetic participants in relation to age. The alpha level was set at 5% ($p<0.05$). The data was presented using tables and graphs.

Results

Figure 1 shows a cross tabulation of independent variables of the study participants. A total of 371 diabetic subjects participated in the study. Male subjects were 152 (41%) while female participants were 219 (59%). The age group of the participants ranged from 18-60 years. The age of the participants were grouped into four groups; 18-30 years having 28 (7.5%) participants, 31-40 years with 78 (21%) participants, 41-50 had 123 (33.2%) participants, while 51-60 years had 142 (38.3%) study subjects. Their mean age was 45.99 with a SD of 9.501. The age range was 42 and 48.29 was the median age.

Figure 1 Demographic Attributes of study Participants

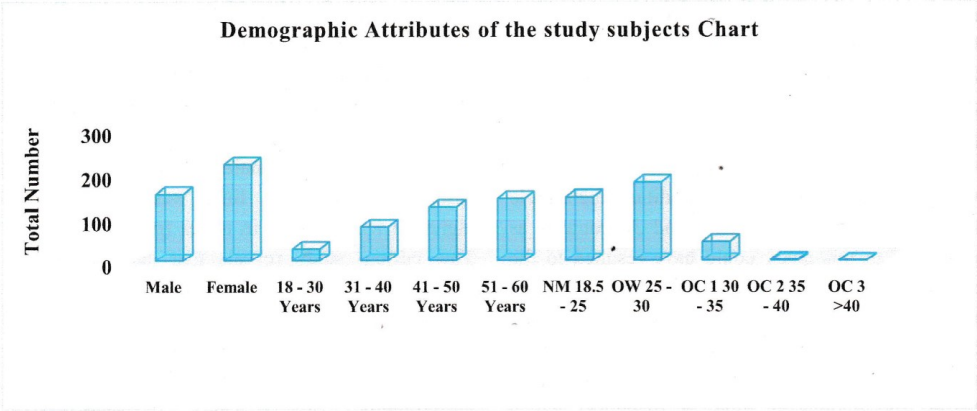

Table 1 Coagulation Status of Diabetic participants: ***Linear regression***; p-value = <0.05

Variables	Subjects	Sample	Mean	SD	R	R Square	F-value	*p*-value
PT	Patient PT	371	12.726	1.307	0.065	0.04	1.542	0.215
	PT Control	371	12.487	1.016				
INR	INR for Patient	371	1.0142	0.137	0.064	0.04	1.500	0.221
	INR Control	371	0.9891	0.102				
APTT	Patient APTT	371	30.656	3.74	0.04	0.002	0.586	0.445
	APTT Control	371	34.424	2.71				

A linear regression was conducted to determine the relationship of coagulation status in diabetic patients and control samples. **Table 1** shows the means of the two sample groups and their standard deviation, the R value and the p value. The R value of 0.065 indicates a very low degree of correlation between prothrombin time of the diabetic patients and prothrombin time of control samples. The R =0.065=6.5% indicates weak relationship. The mean prothrombin time for the diabetics (12.726±1.3070 seconds) was higher than the prothrombin time control (12.487±1.0157 seconds) but the difference was not statistically significant F-value = 1.542; p-value = 0.215.

The table shows R=0.064 indicating low degree of correlation between patient's international normalized ratio and control INR. R = 0.064=6.4% signifying very weak relationship. It also reveals the mean and the standard deviation for international normalized ratio. The mean international normalized ratio for the diabetics (1.0142±0.13735) was higher than the international normalized ratio of control samples (0.9891±0.10222) but the difference was not statistically significant F-value = 1.500; p-value = 0.221.

The table further reveals the following: R=0.040 indicating low degree of correlation between patient's activated partial thromboplastin time and control activated thromboplastin time. This is 4% signifying very weak relationship indicating that only 4% of the dependent variable has been explained by the independent variable. The mean activated partial thromboplastin time for the diabetics (30.656±3.7374) was lower than that of the activated partial thromboplastin time of controls (34.424±2.711) but the difference was not statistically significant F-value = 0.586; p-value = 0.445.

An independent-sample t-test was conducted to compare the coagulation parameters of diabetic participants according to sex. **Table 2** shows the mean of male prothrombin time (12.8±1.09) was higher than then mean for female prothrombin time (12.7±1.44) but the difference was not statistically significant as the p-value was greater than the threshold value of 0.05 t-value = 0.591; p-value = 0.55.

The mean international normalized ratio for males (1.02±0.11) was higher than the mean for female international normalized ratio (1.01) with no statistical significance t-value = 0.418; p-value = 0.676.The mean activated partial thromboplastin time for males (30.5±3.48) was lower than mean activated partial thromboplastin time for females (30.8±3.91) but the difference was not statistically significance t-value = -0.577; p-value = 0.564.

Table 2 Comparison of haemostatic parameters of males and females: Independent-sample t-test parameters, *P*-value = < 0.05

Variable	Sex	Reference range	Total number	Mean	Standard deviation	t-value	P-value
Prothrombin time	Male	10 – 12 Seconds	152	12.8	1.09	0.591	0.55
	Female		219	12.7	1.44		
International normalized ratio	Male	0.90 – 1.2	152	1.02	0.11	0.418	0.676
	Female		219	1.01	0.15		
Activated partial thromboplastin time	Male	30 - 40 Seconds	152	30.5	3.48	-0.577	0.564
	Female		219	30.8	3.91		

Table 3 Variation of coagulation parameters in diabetic patients in relation to age: One-way ANOVA Test, *p*-value = < 0.05

Variable	Age group	Reference range	Total number	Mean	Standard deviation	df	F	P-Value
International normalized ratio	18 – 30	0.8 – 1.2	28	1.02	0.101	Between groups = 3 Within groups = 367	0.224	0.880
	31- 40		78	1.01	1.00			
	41 – 50		123	1.02	0.96			
	51 – 60		142	1.01	0.185			
Prothrombin time	18 – 30	10 – 14 Seconds	28	12.8	0.99		0.281	0.839
	31- 40		78	12.6	1.00			
	41 – 50		123	12.8	0.97			
	51 – 60		142	12.7	1.71			
Activated partial thromboplastin time	18 – 30	30 – 40 Seconds	28	29.4	2.98		4.395	0.005
	31- 40		78	31.6	3.81			
	41 – 50		123	31.0	3.57			
	51 – 60		142	30.1	3.84			

Table 3 shows that, at *p*-value < 0.05, the international normalized ratio means between the four age groups as revealed by one-way ANOVA F(3,367) = 0.224, $p = 0.880$.Therefore, there was no statistically significant differences between the group means. The prothrombin time means between the groups as revealed by the one-way ANOVA F (3,367) = 0.281, $p = 0.839$ indicating no statistically significant difference between the groups as presented in table 3.The activated partial thromboplastin time between the groups as revealed by one-way ANOVA F (3,367) = 4.395, $p = 0.005$. The *p*-value 0.005 is below the significance *p*-value 0.05, revealing a statistically significant difference between the group means. Tukey post hoc test was done to determine which specific group differed.

Table 4 Post HOC Test to determine which specific (APTT) age group differed as seen in **Table 3**: Tukey

Variable	Age group	Age group	Mean	*P*-value
APTT	18 - 30	31 – 40	-2.1847	0.037
		41 – 50	-1.6049	0.162
		51 - 60	-0.6455	0.832
	31 - 40	18 – 30	2.1847	0.037
		41 – 50	0.5798	0.698
		51 - 60	1.5392	0.017
	41 - 50	18 – 30	1.6049	0.162
		31 – 40	-0.5798	0.698
		51 - 60	0.9594	0.151
	51 - 60	18 – 30	0.6455	0.832
		31 – 40	-1.5392	0.017
		41 - 50	-0.9594	0.151

Table 4 Reveals there was a statistically significant difference between activated partial thromboplastin timein age groups by one-way ANOVA F(3, 267) = 4.395, *p*-value = 0.005. There was a statistically significant difference in activated partial thromboplastin time variation between age groups 18-30 and 31-40 (*p*-value = 0.037), and 31-40 and 51-60 (*p*-value = 0.017).There was no statistical significance in activated partial thromboplastin time variation between age group 18-30 and 41-50 (*p*-value = 0.162), 18-30 and 51-60 (*p*-value = 0.832), 31-40 and 41-50 (*p*-value = 0.698), and lastly 41-50 and 51-60 (*p*-value = 0.151.

Discussions

The objective of the study was to evaluate prothrombin time and activated partial thromboplastin time in diabetic patients. Three hundred and seventy one (371) diabetic patients and 371 normal control samples were studied. The majority of diabetic participants were females 219 (59%) while the male participants were 152 (41%). The activated partial prothrombin time of the participants was insignificantly shorter than that of the normal control samples while prothrombin time of diabetic subjects was insignificantly prolonged than that of normal control samples. International normalized ratio was insignificantly increased in the diabetic subjects as compared to normal control samples. This results differs from Mwambungu's[9] who reported significantly reduced activated partial thromboplastin time and insignificantly shortened prothrombin time in diabetic subjects than non-diabetic control participants. This clearly indicated incompetent intrinsic pathway and therefore hypercoagulable state in participants who took part in that study. The prothrombin time in that study was normal indicating a competent extrinsic coagulation pathway, showing adequacy of coagulation factors involved in this pathway. Mwambungu's[9] results were consistent with Ephraim *et al.*, [10] and Awad *et al.*,[1] who reported significantly shortened activated partial thromboplastin time, but their prothrombin time findings differed which was significantly shortened in Ephraim *et al.*,[10] while in Awad *et al.*,[1] report, prothrombin time and international normalized ratio were consistent with the findings of this study with no statistically significant different in the prothrombin time and international normalized ratio of diabetic participants and the healthy participants. The variation in activated partial thromboplastin time and prothrombin time results observed among different researchers could be associated with the sample size used. For example, Ephraim *et al.*, [10] had a small sample size of 100 both diabetic subjects and controls. This may have contributed to the variations observed between this current study and the previous studies. In Mwambungu's[9]study, 213 type 2 diabetic participants participated in the study, which was also less than the sample size used in this study with 19 participants being more than 60 years old. The variation in study variables especially age could have led to the differences in activated partial thromboplastin time, prothrombin time and international normalized ratio in this study

as compared to others. A study done by Agarwal *et al.*,[11] revealed shortened activated partial thromboplastin time and prothrombin time and hence being inconsistent with findings of this study, the reason could be attributed to age of the participants as participants were aged 35 – 70 years while participants in this current study were younger aged 18 – 60 years. In addition, the sample size was smaller, 60 diabetics and 30 controls as compared to this study could have resulted to the disparities between this studies, and the race (Indian race vs African race) as well. According to a study done by Chaitanya and Kavuri[12], their study revealed prolonged prothrombin time and activated partial thromboplastin time in diabetics, which differed from the findings of this study. This implies that the extrinsic and intrinsic pathways were affected and hence the diabetic patients could suffer from excessive bleeding in case of injuries. According to Ogedegbe[13] the activated partial thromboplastin time and prothrombin time prolongation may be associated with factor deficiency or due to circulating anticoagulants. The international normalized ratio findings of this study differed fromEphraim *et al.*, [10]who reported significantly decreased international normalized ratio in diabetics than in non-diabetics. A study done by Dallatu[3] was in agreement with the findings of this current study which revealed that there was no statistically significant difference in treated diabetic participants and the control subjects. Madan *et al.*,[14], found no statistical significance in activated partial thromboplastin time and prothrombin time of both diabetics and non-diabetics just as the findings of this study. Normal prothrombin time and activated partial thromboplastin time could be associated with treatment of diabetics in this study with antidiabetic drugs enabling them have a normal coagulation status.

Female participants were the majority (59%) as compared to men (41%). The prothrombin time, international normalized ratio and activated partial thromboplastin time were within the reference range hence normal. There was no statistically significance difference between the prothrombin time (p-value = 0.55) of males and that of females. The international normalized ratio findings (p-value = 0.676) had no significant difference between sex. Both mean prothrombin time and mean international normalized ratio in males were insignificantly higher than that of females while mean activated partial thromboplastin time was insignificantly higher in females than in males (p-value = 0.564). This current study reveals that activated partial thromboplastin time findings do not correlate with Mwambungu's[9] findings who observed statistically significant low mean activated partial thromboplastin time in females than in males. The prothrombin time was consistent with Mwambungu's[9] findings, which revealed insignificant higher mean prothrombin time in males than in females.

Variation of coagulation parameters in diabetic participants in relation to age was studied. The participants were divided into four (4) age groups. The study observed that there was no significant difference in prothrombin time and international normalized ratio means of the different age groups with a p-value of 0.839 and 0.880 respectively. The activated partial thromboplastin time means of different age groups revealed an overall statistically significant difference with a p-value of 0.005, which was below the set alpha level ($p = <0.05$). Post hoc test was done to determine which specific group differed.

The post hoc test (Tukey) revealed that there was significant different in the means of 18 – 30 and 31 – 40 age groups with a mean difference of 2.18 and a p-value of 0.037. The group means difference of 31 – 40 and 51 – 60 age groups was 1.54 with a p-value of 0.017 which was significant. A study done by Mwambungu[9]revealed that diabetic subjects aged 51 years and above were at risk of being hypercoagulable as compared to those below 51 years of age, this was in line with the findings of this study as the diabetic subjects in the age group 51 – 60 years had their activated thromboplastin time statistically significant indicating hypercoagulable state. According to Mwambungu[9],

changes occurring to the vascular system as the age increase could be associated with hypercoagulation status. This indicates abnormalities in the intrinsic and common pathways of the coagulation cascade [15]. There was no significant difference in the means of groups 31 – 40 and 41 – 50, 41 – 50 and 18 – 30, 41 – 50 and 51 – 60, 51 – 60 and 18 – 30 ($p>0.05$).

Conclusions

This study revealed that the extrinsic pathway of coagulation was intact in diabetics with hypercoagulable state in different age groups indicating incompetence of the intrinsic coagulation pathway. Preventive measures should be undertaken in these age groups to ensure complications associated with hypercoagulable state do not occur.

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