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Evaluation of Fibrinogen Level among Patients with Diabetes Mellitus Type2

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قال تعالى:

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(وَقُل رَّبِّ زِدۡنِي عِلۡمٗا)

صدق الله العظيم

سورة طه (١١٤)

DEDICATION

To the memory of my mother

For her advices which remain as a candle of lighting my way.

To my father

With deep appreciation for his love and encouragement.

To my lovely husband

For his advices, help and support.

To my dear sisters and brothers

To my faithful friends who I can't live without them.

Acknowledgement

Praise to Allah who gave me the health and strength to conduct this study. I would like to thank very much my supervisorDr. Elshazali Widaa Ali for his support encouragement and supervision.Also thank everyone who supported and advised me to finish this research.Finally, I would like to thank diabetic patients who subjected to this study for their cooperation.

Abstract

Diabetes mellitus is a syndrome characteriezed by chronic hyperglycemia and relative insulin deficiency, resistance, or both. Its complication result in major health costs. These include macrovascular disease, leading to coronary artery disease, peripheral vascular disease and stroke; microvascular damage causing diabetic retinopathy and nephropathy, and contributing to diabetic neuropathy. Haemostatic factors especially hyperfibrinogenemia is implicated as a source of these complications. This study aimed to estimate the fibrinogen level and its association with glycemic control in type 2 diabetes mellitus patients.

A total of 70 type2 diabetic patients were recruited for this cross-sectional study. Two blood samples were collected from each participant; one for fibrinogen estimation in tri- sodium citrate anticoagulant container (1.8 ml venous blood) and the other for Hb A1c estimation (2.5 ml) in EDTA container. Fibrinogen level was measured by Clauss method and Hb A1c was measured using Clover*A1c system which is a fully automated boronate affinity assay for the determination of the percentage of hemoglobin A1c in whole blood.

Patient’s data were collected using structured interview questionnaire; age of study population was ranged from 30-82 years. 54.3% were females and 46.7% were males. The duration of disease among patients was ranged from 5-30 years. Data were analyzed using statistical package for social sciences (SPSS), version 14.

Mean fibrinogen level was slightly higher than normal (Mean±SD=469±91.3 mg/dl); 60% of patients were found to have hyperfibrinogenemia and 40% with normal fibrinogen level.

There was no statistically significant difference in mean fibrinogen level in patients with good and those with bad control (*P. value*=0.76).

No statistically significant correlation was found between fibrinogen level and Hb A1c (r =0.1, *P.value* = 0.30), and age (r=0.3, *P.value*=0.3); while there was positivestatistically significant correlation between fibrinogen level and duration of DM (r = 0.2, *p. value* = 0.03).

No statistically significant associationbetween fibrinogen level and complications (*P.value*=0.38).

The results showed that, there was a statistically significant higher mean fibrinogen level in femalescompared tomales (Mean±SD= 490±83.3 and442±94.7 mg/dl respectively,*P. value*=0.02).

In conclusion, although, there was a significant correlation between duration of DM and fibrinogen level but no association was found between glycemic control and fibrinogen level.

المستخلص

مرض السكري هو متلازمة تتميز بارتفاع مزمن في سكر الدم ونقص جزئي في الانسولين، مقاومة الانسولين، او الاثنين معا. مرض السكري غالبا لايشفى بشكل كامل، وعلى الرغم من ذلك فمرضى السكري يستطيعون عيش حياة طبيعية. مضاعفاته قد تتسبب في اضرار صحية بالغة. هذه المضاعفات تتضمن: امراض الاوعية الدموية الكبيرة (والتي تزيد من نسبة حدوث امراض الشريان التاجي، امراض الاوعية الدموية الطرفية، السكته الدماغيه) و تلف الاوعية الدموية الصغيرة (قد تسبب اعتلال الشبكية، اعتلال الكلية، والاعتلال العصبي). عوامل التخثر المختلفة خاصة ارتفاع مستوى الفبرينوجين في الدم هو مصدر هذه المضاعفات.

هدفت هذه الدراسة لقياس مستوى الفبرينوجين في الدم وسط مرضى السكري ومعرفة علاقته بالسيطرة على نسبة السكر في الدم.

شملت الدراسة 70 مريض مصاب بالنوع الثاني من مرض السكري ، اخذت عينة دم وفحص فيها مستوى الفبرينوجين ومعدل السكر التراكمي.

معلومات المريض جمعت بواسطة استبيان من خلال معاينة وحللت بواسطة الحزمة الاحصائية للعلوم الاجتماعية (14).

وجد في هذه الدراسة ان متوسط مستوى الفبرينوجين في مرضى السكري مرتفع قليلا من المعدل الطبيعي (المتوسط±الانحراف المعياري)= 469±91 مل/دسل، 40% منهم لديهم مستوى طبيعي من الفبرينوجين بينما 60% لديهم ارتفاع في مستوى الفبرينوجين.

لاتوجد علاقة بين متوسط مستوى الفبرينوجين : والسيطره على نسبة السكر في الدم (القيمة الإحتمالية= 0.30) ، والمضاعفات (القيمة الإحتمالية=0.38)، و عمر المريض (القيمة الإحتمالية= 0.3) بينما توجد علاقة ذات دلالة معنوية بين متوسط مستوى الفبرينوجين والمدة الزمنية للمرض (القيمة الإحتمالية= 0.03).

اظهرت النتائج ارتفاع في متوسط مستوى الفبرينوجين عند الاناث عندما قورن مع الذكور (المتوسط±الانحراف المعياري = 490±83.3، 442±94.7 مل/دسل على التوالي ،القيمة= 0.02**).**

List of Contents

Title	Page
Dedication	I
Acknowledgement	II
Abstract	III
Arabic abstract	IV
List of contents	VI
List of tables	XII
List of figures	XIII
List of abbreviations	XIV
Chapter One : Introduction and Literature Review	
1.1 Hemostasis	1
1.1.1 Platelets	1
1.1.1.1 Platelet structure	1
1.1.1.2 Major platelet granules	2

1.1.1.3 Platelet receptors	3
1.1.2 Primary hemostasis	3
1.1.2.1 Mechanism of primary hemostasis	4
1.1.2.1.1 Platelet adhesion and activation	4
1.1.2.1.2 Platelet aggregation	4
1.1.3 Secondary hemostasis	5
1.1.3.1 Coagulation factors	5
1.1.3.2 Coagulation cascade	6
1.1.3.2.1 Intrinsic pathway	6
1.1.3.2.2 Extrinsic pathway	6
1.1.3.2.3 Common pathway	6
1.1.4 Fibrinolysis	7
1.1.4.1 Plasmin and fibrin split products (FDPs)	7
1.1.5 Classification of hemostatic disorders	7

1.1.5.1 Acquired disorders	7
1.1.5.2 Inherited disorders	7
1.1.6 Laboratory evaluation	8
1.1.7 Fibrinogen	8
1.1.8 Hypercoagulable states (thrombophilia)	9
1.1.8.1 Inherited hypercoagulable states	10
1.1.8.1.1 Antithrombin deficiency	10
1.1.8.1.2 Protein C deficiency	11
1.1.8.1.3 Activated protein C resistance (factor V Leiden mutation)	11
1.1.8.1.4 Prothrombin G20210A mutation	12
1.1.8.1.5 Protein S deficiency	12
1.1.8.1.6 Hyperhomocysteinemia	13
1.1.8.1.7 Miscellaneous	13
1.1.8.2 Screening for inherited thrombophilias	13
1.1.8.3 Acquired hypercoagulable states	14

1.2 Diabetes mellitus	14
1.2.1 An outline of glucose metabolism	15
1.2.2 Glucose utilization	16
1.2.3 Type 1 Diabetes mellitus	16
1.2.4 Type 2 Diabetes mellitus	17
1.2.5 Diagnosis and investigation of diabetes	18
1.2.6 Compliacations of Diabetes mellitus	19
1.2.6.1 Macrovascular complications	19
1.2.6.1.1 Diabetic cardiomyopathy	19
1.2.6.2 Microvascular complications	20
1.2.6.2.1 Diabetic retinopathy	20
1.2.6.2.2 Diabetic nephropathy	21
1.2.6.2.3 Diabetic neuropathy	22
1.2.7 Hemoglobin A1c	23
1.3 Previous studies	25

Rationale	27
Objectives	28
Chapter Two: Materials and Methods	
2.1.1 Study design	29
2.1.2 Study area	29
2.1.3 Study duration	29
2.1.4 Study population	29
2.1.5 Inclusion criteria	29
2.1.6 Exclusion criteria	29
2.1.7 Ethical considerations	30
2.1.8 Data collection	30
2.1.9 Sample collection	30
2.1.10 preparation of platelet poor plasma	30
2.2.1 Fibrinogen estimation	30

2.2.4 Estimation of Hb A1c	32
2.3 Data analysis	33
Chapter Three: Results	34
Chapter Four: Discussion, Conclusion and Recommendations	
4.1 Discussion	39
4.2 Conclusion	40
4.3 Recommendations	41
References	42
Appendixes	47

List of Tables

Table 1.1	Coagulation factors nomenclature	5
Table 2.1	Dilutions of fibrinogen calibrator stock solution with owren's buffer	31
Table 3.1	Comparison of mean fibrinogen level in patients with good and those with bad control	36
Table 3.2	Comparison of mean fibrinogen level in patients with complications and those without complications	37
Table 3.3	Comparison of mean fibrinogen level according to gender	38

List of Figures

Figure 3.1	Gender distribution in study population	34
Figure 3.2	Distribution of study population according to glycemic control.	35
Figure 3.3	Frequency of complications among study subjects.	35
Figure 3.4	Correlation between HbA1c and fibrinogen level.	36
Figure 3.5	Correlation between fibrinogen level and age.	37
Figure 3.6	Correlation between duration of DM and fibrinogen level.	38

Abbreviations

Abbreviation	Full text
A	Adenine
ADA	American Diabetes Association
Arg-Gly-Asp	Argenine Glysine Aspartate
APA	Antiphospholipid antibodies
APC	Activated Protien C
APCR	Activated Protien C Resistance
APTT	Activated Partial Thromboplastine Time
AT	Anti thrombin
BMI	Body Mass Index
CD	Cluster of Designation
CHD	Coronary Heart Disease
CVD	Cardiovascular Disease
DCCT	Diabetes Control and Complications Trial
DIC	Disseminated Intravascular Coagulation
DM	Diabetes Mellitus
EPCR	Endothelial Cell Protein C Receptor
FDP	Fibrin Degradation Products
G	Guanine

GAD	Glutamic Acid Decarboxylase
GD	Gestational Diabetes
GP	Glycoprotein
HB A1c	Hemoglobin A1c
HLA	Human Leukocyte Antigen
HMWK	High Molecular Weight Kinenogen
HPLC	High Performance Liquid Chromatography
ICA	Islet Cell Antibody
IDDM	Insulin dependent diabetes mellitus
LAMP	Lysosome Associated Membrane Protein
MHC	Major Histocompatibility Complex
MODY	Maturity Onset Diabetes of the Young
mRNA	messenger Ribonucleic Acid
MTHFR	Methylene tetrahydrofolate Reductase
NIDDM	Non-Insulin Dependent DiabetesMellitus
OD	Optical Density
PL	Phospholipid
PPP	Platelet Poor Plasma
PT	Prothrombin Time
PTT	Partial Thromboplastine Time
RPM	Round Per Minute

SD	Standard Deviation
SPSS	Statistical Package for Social Sciences
TAFI	Thrombin Activatable Fibrinolysis Inhibitor
T1DM	Type 1Diabetes Mellitus
VEGF	Vascular Endothelial Growth Factor
vWF	von Willebrand Factor

Chapter One

Chapter One
Introduction and Literature Review

1.1 Hemostasis

Human hemostatic system provides a natural balance between procoagulant and anticoagulant forces. The procoagulant force includes platelet adhesion and aggregation and fibrin clot formation; anticoagulant forces include natural inhibitors of coagulation and fibrinolysis. Under normal circumstances, hemostasis is regulated to promote blood flow: however, it is also prepared to clot blood rapidly to arrest blood flow and prevent exsanguinations. After bleeding is successfully halted, thesystem remodels the damaged vessel to restore normal blood flow. The major components of hemostatic system, which function is concert, are platelets and other formed element of blood, such as monocyte and red cells, plasma proteins (the coagulation and fibrinolytic factors and inhibitors) and the vessel wall itself (Longo, 2010).

1.1.1 Platelets

Platelets are discoid-shaped anucleate cells approximately 2 to 3 _m in diameter. The normal platelet number is approximately 150,000 to 350,000/_L (150 to 350 _109/L). Platelets contain actin filaments, losing, and other contractile proteins, which help them, retain their shape and allow platelet plugs to contract. (Kern, 2002).

1.1.1.1Platelet structure

Electron microscopy reveals a fuzzy coat (glycocalix) extending 14 to 20 nm from the platelet surface, which is thought to be composed of membrane glycoproteins, glycolipids, mucopolysaccharides, and adsorbed plasma proteins. Platelets move in an electric field as if they have a net negative surface charge. Platelet composed of plasma membrane which is a trilaminar unit composed of a bilayer of

phospholipids in which cholesterol, glycolipids, and glycoproteins are embedded, cytoskeletal elements, membrane skeleton which is a planar network of thin, elongated spectrin tetramers interconnected by the ends of actin filaments is present immediately below the plasma membrane and the membranes of the open canalicular system, microtubules which present below the plasma membrane probably plays an important role in platelet formation from megakaryocytes and contributes to the platelet's discoid shape, microfilaments in which the platelet is rich in actin, a protein that can polymerize into microfilamentous bundles. In resting platelets, microfilaments are not prominent. However, when platelets change shape, the filopodia they form contain bundles of microfilaments made up of actin and associated proteins and platelet organelles that includePeroxisomes, mitochondria, lysosomes and dense Bodies (Lichtman *et al*., 2007).

1.1.1.2 Major platelet granules

1.1.1.2.1 α-granules which contain the following

Coagulant proteins (fibrinogen, factor V), platelet specific proteins (platelet factor 4, -β thromboglobulin), mitogenic and angiogenic factors (platelet -derived growth factor), transforming growth factor β, vascular endothelial growth factor), adhesive glycoproteins and a-granule membrane -specific proteins (thrombospondin, von Willebrand factor (vWF), multimerin, P-selectin) (Colman *et al.,* 2000).

1.1.1.2.2 Dense granules

Adenosine diphosphate, adenosin triphosphate, calcium, serotonin. In addition, dense granules contain guanosin triphosphate/guanosine diphosohate and high concentrations of pyrophosphate, phosphate and magnesium. A number of additional proteins are present, some released and some retained in the platelet cytosol. Dense bodies, numbering approximately five per platelet, are

exceptionally electron dense and easily appreciated by electron microscopy due to their distinctive "bull's eye" appearance (Colman *et al.,* 2000).

1.1.1.2.3 Lysosomal granules

Lysosomes are small, acidified vesicles, approximately 200nm in diameter, that are identifiable only with specific cytochemical stains for acid phosphatase and arylsulfatase or through immunocytochemistry for Cathepsin D and Lysosome associated membrane protein (LAMP-1/LAMP-2, these are expressed on the plasma membrane after activation). Lysosomal constituents are released more slowly an incompletely. They may have a greater role in lysis of thrombi than in the immediate hemostatic response (Greer *et al.*, 2003).

1.1.1.3 Platelet receptors

Platelets have a variety of surface glycoprotein, some of which act as receptors for vWF, fibrinogen, or other adhesive proteins. Many platelet receptors consist of complexes of two or more glycoproteins. The most important platelet receptors are GP Ib-IX/V (previously designated GP Ib- IX): the platelet receptor for vWF. Designated CD42 in cluster designation (CD) system, GPIIb-IIIa: The platelet receptor for fibrinogen, which also acts as a receptor for vWF, fibronectin and other adhesive proteins. Designated CD41/CD61 in the CD system. GPIIb-IIIa exists on the resting platelet on a -low affinity or inactive form (Kern, 2002).

1.1.2 Primary hemostasis

Primary hemostasis primarily involves platelets and vWF and results in the formation of a platelet plug. If the endothelial injury is small, this may be adequate to stop bleeding. However, if the injury is greater, participation by the coagulation cascade is required (Kern, 2002).

1.1.2.1 Mechanism of primary hemostasis

1.1.2.1.1 Platelet adhesion and activation

Following blood vessel injury, platelets adhere to the exposed sub endothelial connective tissues. Sub endothelial microfibrils bind the larger multimers of vWF which bind to platelet membrane Ib complex.Under the influence of shear stress platelet more along the surface of vessels until the platelet surface GPIa /IIa engages collagen and halts translocation. Following adhesion, platelets become more spherical and extrude long pseudo pods which enhance interaction between adjacent platelet. Platelets activation is then achieved by GP IIb /IIIa binding fibrinogen to produce platelet aggregation. The GP Ia /IIa receptor complex also forms a secondary binding site with vWF further promoting adhesion (Hoffbrand *et al.,* 2000).

1.1.2.1.2 Platelet aggregation

Platelet aggregation is a complex phenomenon that is the end result of a series of adhesion- and activation -related processes. Well-known essential components of this process include an agonist, calcium and the adhesive proteins fibrinogen and vWF. Divalent cations,such as calcium and magnesium, are required for platelet aggregation in trace amounts, and these alter the specificity of the integrin aIIbβ 3 for its ligands. Fibrinogen and vWF play dominant roles in platelet aggregation through binding to aIIbβ 3 and also by the ability of the former to generate polymerized fibrin as support for the platelet in the thrombus.Arg -Gly -Asp, which acts as a bridge between adjacent platelet allowing aggregation toproceed. In addition more aIIbβ 3 translocates to the platelet surface membrane from the degranulation a-granule pool where an additional receptor is stored. These changes facilitate irreversible binding to fibrinogen. An important role for tyrosine kinase -and phosphatase-associated phosphorylation-dephosphorylation in integrin

activation exists as assessed by the blockage of fibrinogen binding and platelet aggregation by enzyme inhibitors (Greer *et al.,* 2003).

1.1.3 Secondary hemostasis

1.1.3.1 Coagulation factors

Defined as plasma proteins in the coagulation system that circulate as inactive zymogens or cofactors, when activated by tissue damage ,they form complexes that ultimately produce thrombin, an enzyme that cleaves fibrinogen to fibrinclot and stops the bleeding (Kern, 2002).

Table(1.1): Coagulation factors nomenclature.

Factor	**Nomenclature**
Factor I	Fibrinogen
Factor II	Prothrombin
Factor III	Thromboplastin
Factor IV	Calcium
Factor V	Proaccelerin
Factor VII	Proconvertin
Factor VIII	anti-hemophilic factor
Factor IX	Christmas factor
Factor X	Stuart-prower factor
Factor XI	Plasma thromboplastin antecedent
Factor XII	Hageman factor
Factor XIII	Fibrin-stabilizing factor
PK	Pre kallikarein
HMWK	High Molecular weight kinenogen

1.1.3.2 Coagulation cascade

Coagulation cascade include intrinsic pathway, extrinsic pathway and common pathway.

1.1.3.2.1 Intrinsic pathway

Surface bound factor XII becomes susceptible to limited cleavage by kallikarein and also ultimately to auto activation by factor XIIa itself. The same activation occurs for HMWK which interact with both factor pre kallikarein . HMWK is also cofactor for the activation of factor XI by factor XIIa. Factor IXa is formed by proteolytic activation of factor IX by factor XIa. Factor IXa forms a complex with factor VIIIa,calcium and PL to complete the factor X activator, termed (tenase). Asimillar complex is assembled by factor Xa, Va, calcium and PL to form the prothrombin activator which convert factor I tired Ia (fibrinogen to fibrin) (Firkin *et al.*, 1996).

1.1.3.2.2 Extrinsic pathway

Extrinsic coagulation pathway is activated by the exposure to tissue thromboplastin(factor III) which normally extrinsic to blood. Factor VII bind to factor III and becomes activated. A long with ionized calcium, factor VII is a potent activator of the common pathway (factor X) (Liffick, 1997).

1.1.3.2.3 Common pathway

The common coagulation pathway is utilized by both intrinsic and extrinsic pathway to accomplish the common goal of the converting fibrinogen to fibrin. Factor X in association with cofactor factor V on the PL surface and calcium converts prothrombin to thrombin, which then converts fibrinogen to fibrin (Liffick, 1997).

1.1.4 Fibrinolysis

An important as the formation of a clot at a site of injury is its removal when its usefulness is over. The clot is removed by enzymatic digestion, a process referred to as fibrinolysis (Bernard *et al.*, 1994).

1.1.4.1 Plasmin and fibrin split products (FDPs)

Fibrinolysis is accomplished by Plasmin which circulating precursors. Plasminogen is converted to Plasmin by two activators: tissue Plasminogen activator and urokinase. When digesting a clot, Plasmin cuts the covalently linked fibrin polymers. The fragments of polymer cut away by Plasmin are released from the clot as FDPs (Bernard *et al.*, 1994).

1.1.5 Classification of hemostatic disorders

Hemostatic disorders can be classified as either hereditary or acquired, or according to the mechanism of defects.

1.1.5.1 Acquired disorders

The thrombocytopenias, liver diseases, vitamin K deficiency, renal failure, haematological disorders (acute leukemias, myelodysplasias, monoclonal gammopathies, essential thrombocythemia), acquired antibodies against coagulation factors, disseminated intravascular coagulation, drugs and vascular disorders (Lichtman *et al.*, 2003).

1.1.5.2 Inherited disorders

Deficiencies of coagulation factors (hemophilia A, hemophilia B, defeciencies of factor II, V, VII, X, XI and XIII, and von willebrand disease), platelet disorders(Glanzmann thrombosthenia, Bernard-Soulier syndrome, platelet granules disorders), fibrinolytic disorders, vascular (hemorrhagic telangictasas) and connective tissue disorders (Lichtman *et al.*, 2003).

1.1.6 Laboratory evaluation

The initial laboratory study of the bleeding patient should be guided by the information obtained from the clinical evaluation. It is generally agreed that the most essential information usually can be obtained from the three tests summarized in which in view of their availability, simplicity, and low cost , are well suited to serve as primary screening tests. The platelet count provides the most reliable and reproducible test of primary hemostasis. The APTT measures all of the coagulation factors involved in the intrinsic and common pathways and generally is accepted as the best single screening test for disorders of blood coagulation. When supplemented with the PT, which assesses the extrinsic as well as the common pathways, the abnormality usually can be localized to one of the three pathways and the factors involved therein and the results of these three tests thus provide a presumptive diagnosis, which can then be clarified further by the confirmatory method. The bleeding time test has been omitted from this evaluation because of its no specificity in the general clinicalsitting (Hoffbrand *et al.,*2005).

1.1.7 Fibrinogen

The high prevalence of classic cardiac risk factors in patients with type 2 diabetes mellitus does not explain the increased cardiovascular related morbidity and mortality in these patients. Fibrinogen may have a role in this excess risk. The prevalence of type 2 diabetes mellitus is expected to rise more rapidly in future because of increasing obesity and reduced activity levels (Alvin *et al* ,2001).

In the past decade, the potential role of hemostatic factors, particularly fibrinogen, in atheroscleros=is and its complications has generated considerable attention. Studies have shown that formation of an occlusive thrombus, on a damaged atherosclerotic lesion is the most common precipitating factor of acute myocardial

infarction. Evidence also suggests that fibrinogen has a role, both in the early stages of plaque formation and late complications of cardiovascular disease (Bruno *et al.,* 1996).

The excess cardiovascular morbidity and mortality among diabetics have not been fully explained by major risk factors such as hypertension, cigarette smoking and hypercholesterolemia. Increased attention is being paid to, disordered hemostatic mechanism in pathogenesis of both large vessel and small vessel disease in diabetes (Fuller *et al.*, 1979).

Impaired glucose tolerance exerts an influence by enhancing thrombogenic factors such as, fibrinogen in the diabetics (Kannel *et al.,* 1987).

Fibrinogen, itself is determined by several modifiable and non-modifiable determinants like age, sex, smoking, body mass index (BMI), hypertension, alcoholism, glycemic control, lipid profile and urine albumin excretion rate (Anjula *et al.,* 2001).

1.1.8 Hypercoagulable states (thrombophilia)

Thrombophilia is the technical term for hypercoagulable states. Virchow originally defined the conditions that predispose to thrombosis as abnormalities in the blood vessel wall, abnormalities in the blood, and abnormalities of blood flow (stasis). His definition remains valid today.Thrombophilia can be either inherited or acquired. Suggestions of an inherited thrombophilia include thrombosis without any predisposing condition (ie, no surgery, injury, prolonged inactivity), thrombosis at a young age (less than about 40 to 45), thrombosis in unusual sites (upper extremities, mesenteric vessels, hepatic or portal veins, cerebral veins), and a family history of thrombosis.We now know that many individuals with

thromboemboli who appear to have an obvious predisposing factor for thrombosis (ie, recent surgery) also have an inherited thrombophilia(Kern, 2002).

1.1.8.1 Inherited hypercoagulable states

Inherited thrombophilic states can be due to a deficiency of a natural anticoagulant, such as antithrombin or protein C; a mutation in a clotting factor, making it resistant to inhibition (factor V Leiden); or resistance to fibrinolysis. Most of the inherited deficiencies of natural anticoagulants (AT, proteins C and S) are inherited in an autosomal dominant fashion.Infants who are homozygous for a deficiency of one of these factors die shortly after childbirth due to overwhelming systemic thrombosis,with necrosis of skin and other tissues (neonatal purpura fulminans).Resistance to activated protein C (factor V Leiden) is inherited as an autosomal trait with variable penetrance in heterozygotes (Kern, 2002).

1.1.8.1.1 Antithrombin deficiency

Antithrombin (previously called antithrombin III) is synthesizedin the liver. It is a glycoprotein member of the familyof serine protease inhibitors. Its inhibitory effect is not confinedto thrombin; antithrombin also inhibits the activatedclotting factors IXa, Xa, XIa, XIIa and tissue factor-boundFVIIa. Free enzymes are preferentially inhibited, those thatare part of the prothrombinase or tenasecomplexes being lessaccessible for inhibition. The rate of complex formationbetween antithrombin and activated clotting factors is markedlyaccelerated by heparin and by proteoglycans on the vascularendothelium (Hoffbrand *et al.,* 2005).

1.1.8.1.2 Protein C deficiency

Protein C is a member of the family of vitamin K-dependentglycoproteins. It is synthesized in the liver and, prior to activationby thrombin to activated protein C (APC), it circulates as atwo-chain zymogen. The activation process is enhanced approximately1000-fold when thrombin is bound to thrombomodulinon the endothelial surface of blood vessels. This binding blocksthe ability of thrombin to catalyse fibrin formation, factor XIIIactivation, platelet activation and feedback activation of coagulationcofactors. In some blood vessels, protein C activation is further augmented by the binding of protein C to a transmembraneprotein, endothelial cell protein C receptor (EPCR). OnceAPC is generated, it binds to protein S on the surface of activatedcells and this complex then inactivates FVa and FVIIIa.Activated protein C reduces platelet prothrombinase activityby degrading platelet-bound FVa at the receptor for FXa. It alsoplays a major role in the modulation of leukocyte function andreduction of the inflammatory response in septicaemia (Hoffbrand *et al.,* 2005).

1.1.8.1.3 Activated protein C resistance (factor V Leidenmutation)

The term *APCR* is defined as an abnormally reduced anticoagulant response of a subject's plasma to APC based on *in vitro* testing. A "normal" range for response to APC is established for the various coagulation or other related assay conditions used to assess response to APC.Theoretically, any genetic abnormality of a protein C pathway component that interferes with the expression of APC activity can cause APCR, as could acquired abnormalities such as antibodies against protein C pathway components. Although the causes of many cases of acquired APCR are unknown, the majority (>90%) of hereditary APCR subjects have the same genetic abnormality, factor V Leiden with a G1691A alteration causing Arg506Gln

substitution. This gene alteration arose in a single Caucasian founder approximately 21,000 to 34,000 years ago (Lichtman *et al*., 2007).

1.1.8.1.4 Prothrombin G20210A mutation

Replacement of G by A at nt 20210 in the 3'-untranslated region of the prothrombin gene augments translation and stability of prothrombin mRNA.This process results in elevated synthesis and secretion of prothrombin by the liver. The elevated level of plasma prothrombin with a mean of 132 percent of normal in heterozygotes may contribute directly to increased thrombotic risk by causing increased thrombin generation or decreased fibrinolytic activity because of enhanced activation of TAFI. Another basis for prothrombotic action might derive from the ability of prothrombin to inhibit APC's inactivation of factor Va (Lichtman *et al*., 2007).

1.1.8.1.5 Protein S deficiency

Protein S, another vitamin K-dependent glycoprotein is producedby the liver, endothelial cells, megakaryocytes and thetesticular Leydig cells. Approximately 60% circulates bound to the -chain of C4b-binding protein and is inactive. The remaining40%, designated free protein S, is uncomplexed and is theactive moiety. The bioavailability of protein S is closely linkedto the concentration of C4b-binding protein, which acts asan important regulator of the APC–protein S inhibitory pathway.Free protein S increases the affinity of APC for negatively
charged phospholipid surfaces on platelets or the endothelium,enhancing complex formation of APC with FVa and FVIIIa. Inaddition, protein S has an independent anticoagulant effect onthe free form of FIXa–FVIIIa–phospholipid complex (tenase)and the FVa–FXa–phospholipid complex (prothrombinase) (Hoffbrand *et al.,* 2005).

1.1.8.1.6 Hyperhomocysteinemia

A plasma homocysteine level above the normal range defines hyperhomocysteinemia. Severe hyperhomocysteinemia (plasma levels >100 mol/L), also identifiable as homocystinuria, occurs in approximately one in 200,000 to 300,000 individuals in the general population and is transmitted as an autosomal recessive trait. The most common causes for homocysteinuria are mutations in cystathionine -synthase. Rarely, other mutations in 5,10-MTHFR, or methionine synthase give rise to homocystinuria. Such severe abnormalities are associated with neurologic abnormalities, mental retardation, ectopialentis, premature cardiovascular disease, stroke, venous thrombosis, and arterial thrombosis (Lichtman *et al*., 2007).

1.1.8.1.7 Miscellaneous

A variety of other inherited thrombophilic states have been described, including abnormal fibrinogen molecules (dysfibrinogenemia),deficiency of heparin cofactor II, defects in the fibrinolytic system, and others. All of these are quite rare (Kern, 2002).

1.1.8.2 Screening for inherited thrombophilias

Screening for an inherited thrombophilia should be considered in patients with thrombosis and a family history of thrombosis, thrombosis in unusual sites, thrombosis at an early age (less than ~40 to 45 years), or thrombosis without an obvious predisposing cause.The testing protocol would depend on the ethnic background of the patient, the particular thrombotic manifestation, and whether a specific inherited thrombophilia has been identified in relatives. As a general rule, tests should include an assay for APC resistance, AT and protein C and S

levels,and possibly an assay for the prothrombin G20210A mutation.Acquired thrombophilic conditions, such as a lupus anticoagulant, should also be considered.Some people have advocated that all women who are going to be put on oral contraceptives or post-menopausal estrogen replacement should be screened for inherited thrombophilia. The cost of this per episode of thrombosis prevented would probably be excessive, and it is not currently standard practice to screen for thrombophilia before prescribing oral contraceptives or estrogens. However, if the woman has a family history that suggests the presence of an inherited thrombophilia,it might be prudent to consider such screening (Kern, 2002).

1.1.8.3 Acquired hypercoagulable states

In many cases the hypercoagulable state conditions will act in synergy with an inherited defect to cause clinical thrombosisthe acquired conditions can be classified as iatrogenic, secondary to another disease and physiological .Iatrogenic include : major surgery, estrogen treatment, protease inhibitors, heparin- induced thrombocytopenia, secondary to another disease: cancer, myeloproliferative disorders with thrombocytosis, paroxysmal nocturnal hemoglobinuria , trauma, stroke, congestive heart failure, obesity, immobility due to infection, cardiolipin antibodies, nephrotic syndrome,Physiological:age≥40, puerperium, pregnancy, seasonal variation, long travels(Lugassy *et al.*, 2001).

1.2 Diabetes mellitus

The pancreas is both an endocrine gland that produces hormones insulin, glucagon and somatostatin, and an exocrine gland that produces digestive enzymes. The peptide hormones aresecreted from cells located in the islets of Langerhans (beta cells produce insulin,alpha cells produce glucagon and cell produce somatostatin). These hormones playable important role in regulating the metabolic activities of

the body,particularly the hemostsis of blood glucose. Hyperinsulinemia (due for example to insulinoma) can cause severe hypoglycemia. Relative or absolute lack of insulin, such as in diabetes mellitus , can cause serious hyperglycemia, if this condition is left untreated, retinopathy, nephrophathy, neuropathy and cardiovascular complications may result (Clark *et al.*,2012).

Diabetes mellitus may be categorized into several types but the two major types are type 1 and type 2. On the basis of etiology, the term type 1 and type 2 were widely used to describe IDDM and NIDDM, respectively. Gestational Diabetes (GD) mellitus refers to the onset or initial recognition of glucose intolerance during pregnancy, usually in the second or third trimester. It occurs in about 4% of all pregnancies. Patients with GD have a 30% to 50% chance of developing DM, usually type 2 DM. Other types include genetic defects of the pancreatic ß cell or in insulin action pathways (insulin receptor mutations or post-receptor defects) as well as disease of the exocrine pancreas (e.g., Pancreatitis, pancreatic reaction, or cystic fibrosis) are less common causes of DM. Endocrinopathies producing insulin counterregulatory hormones excess (e.g., Cushing's syndrome, acromegaly) may result in DM.According to the ADA recommendation changes in 1997, the fasting glucose concentration should be used in routine screening for diabetes as well as epidemiological studies; the threshold for fasting glucose was changed from 7.8mmol/L (140 mg/dl) to 7.0 mmol/L (126 mg/dl); however the 2-h glucose criterion remains as = 11.1 mmol/L (200 mg/dL) (Bastaki, 2005).

1.2.1 An outline of glucose metabolism

Blood glucose levels are closely regulated in health and rarely stray outside the range of 3.5-8.0 mmol/l (63-144 mg/dl), despite the varying demands of food, fasting and exercise. The principal organ of glucose hemostasis is the liver, which absorbs and stores glucose (as glucagon) in the post absorptive state and releases it

into the circulation between meals to match the rate of glucose utilization by peripheral tissues (Kumar and Clarck,2002).

1.2.2 Glucose utilization

The brain is the major consumer of glucose. Itsrequirement is 1mg/kg body weight per minute. Glucose up take by brain is obligatory and is not dependant on insulin, and the glucose used is oxidized to carbon dioxide and water. Other tissues such as muscle and fat are facultative glucose consumers (Kumar andClarck,2002).

The American diabetes association(ADA) recognizes four clinical classification of diabetes: type 1 diabetes (formerly, insulin- dependent diabetes mellitus), type 2 diabetes mellitus (formerly non-insulin dependent diabetes), gestational diabetes and diabetes due to other cause (for example, genetic defects or medications) (Clark *etal.,*2012).

1.2.3 Type 1 Diabetes mellitus

Type 1 most commonly afflicts individuals in puberty or early adulthood, but some latent forms can occur later in life. The disease is characterized by an absolute deficiency of insulin caused by massive beta cell necrosis. Loss of beta cell function is usually ascribed to autoimmune mediated processes directed against beta cell, and it may be triggered by an invasion of viruses or the action of chemical toxins. As a result of destruction of these cells , the pancreas fails to respond to glucose and the type 1 diabetic shows classic symptoms of insulin deficiency (polydipsia, polyphagia, polyuria and weight loss). Types 1 diabetic require exogenous insulin to avoid catabolic state that results from and is characterized by hyperglycemia and life threatening ketoacidosis (Clark *etal.*, 2012).

Several features characterize type 1 diabetes mellitus as an autoimmune disease: Presence of immuno-competent and accessory cells in infiltrated pancreatic islets, association of susceptibility to disease with the class II (immune response) genes of the major histocompatibility complex (MHC; human leukocyte antigens HLA) and presence of islet cell specific auto antibodies.Approximately 85% of patients have circulating islet cell antibodies, and the majorities also have detectable anti-insulin antibodies before receiving insulin therapy. Most islet cell antibodies are directed against glutamic acid decarboxylase (GAD) within pancreatic B cells. The autoimmune destruction of pancreatic β-cells, leads to a deficiency of insulin secretion which results in the metabolic derangements associated with T1DM. In addition to the loss of insulin secretion, the function of pancreatic α-cells is also abnormal and there is excessive secretion of glucagons in T1DM patients. Normally, hyperglycemia leads to reduced glucagons secretion, however, in patients with T1DM, glucagons secretion is not suppressed by hyperglycemia. The resultant inappropriately elevated glucagons levels exacerbate the metabolic defects due to insulin defi-ciency (Bastaki, 2005).

1.2.4 Type 2 Diabetes mellitus

There is no evidence of immune involvement in pathogenesis of types2 diabetes . Although insulin can bind normally to its receptor on the surface of cells in type 2 diabetic, unknown genetic abnormalities attenuate insulinsignaling within the cell, producing (insulin resistance). Furthermore, patients with type 2 diabetes cannot secrete enough insulin to overcome this burden of insulin resistance (Kumar and Clarck,2002).

In situations where resistance to insulin predominates, the mass of β-cells undergoes a transformation capable of increasing the insulin supply and compensating for the excessive and anomalous demand. In absolute terms, the

plasma insulin concentration (both fasting and meal stimulated) usually is increased, although "relative" to the severity of insulin resistance, the plasma insulin concentration is insufficient to maintain normal glucose homeostasis. Keeping in mind the intimate relationship between the secretion of insulin and the sensitivity of hormone action in the complicated control of glucose homeostasis, it is practically impossible to separate the contribution of each to the etiopathogenesis of DM2. Insulin resistance and hyperinsulinemia eventually lead to impaired glucose tolerance. Except for maturity onset diabetes of the young (MODY),the mode of inheritance for type 2 diabetes mellitus is unclear. MODY, inherited as an autosomal dominant trait, may result from mutations in glucokinase gene on chromosome 7p. MODY is defined as hyperglycemia diagnosed before the age of twenty-five years and treatable for over five years without insulin in cases where islet cell antibodies (ICA) are negative (Bastaki, 2005).

1.2.5 Diagnosis and investigation of diabetes

The diagnosis is usually simple. Blood glucose is so closely controlled by the body that even small devitions become important. In symptomatic patient, a single elevated plasma glucose more than or equal 11mmol/l, measured by a reliable method, Indicates diabetes (Clark *etal.,*2012).

In asymptomatic or mildly symptomatic patients the diagnosis is made on:

Two fasting venous plasma glucose levels above 7mmol/l (126mg/dl) or two random values more than or equal 11.1mmol/l(200mg/dl) in venous plasma. A glucose tolerance test is only needed for borderline cases.Other investigations:Other routine investigations include screening the urine for protein, a full blood count, urea and electrolytes, liver biochemistry and random lipids.Hypertension is present in one -third of European patients with type 2 and in 50/ of African and Caribbean patients (Kumar and Clarck,2002).

1.2.6Compliacations of Diabetes mellitus

The long-term vascular complications of diabetes include retinopathy, nephropathy, neuropathy and macrovascular disease. The outcomes are visual impairment and blindness due to diabetic retinopathy, renal failure and hypertension due to diabetic nephropathy, pain, paraesthesiae, muscle weakness and autonomic dysfunction due to diabetic neuropathy, cardiac disease, peripheral vascular disease and stroke due to macrovascular disease .Clinically evident diabetes-related vascular complications should be rare in childhood and adolescence. However, early functional and structural abnormalities may be present a few years after the onset of the disease. Childhood and adolescence is a period during which intensive education and treatment may prevent or delay the onset and progression of complications (Donaghue *et al*., 2009).

1.2.6.1 Macrovascular complications

Macrovascular complications include fatal and non - fatal coronary heart disease (CHD) events, stroke and peripheral arterial disease. Cardiovascular disease (CVD) accounts for most (> 75%) of the premature mortality and shortened life expectancy among patients with diabetes (Bilous and Donnelly, 2010).

1.2.6.1.1 Diabetic cardiomyopathy

There are two main types of cardiomyopathy: primary cardiomyopathy, where the cardiac function is aggravated by a defect in the heart itself, and secondary cardiomyopathy, where cardiac performance is affected because of a systemic syndrome. Cardiomyopathy leads to heart failure, which can be either diastolic heart failure, with preserved ejection fraction, or systolic heart failure, with reduced ejection fraction. Diabetes can lead to heart failure, not only by

augmenting the impact of classical cardiovascular risk factors (e.g. accelerating the appearance and progression of coronary artery disease through macroangiopathy), but also via a direct deleterious effect on the myocardium. This condition is known as diabetic cardiomyopathy, defined as the presence of myocardial involvement in patients with diabetes, characterized by dilatation and hypertrophy of the left ventricle, with the concomitant appearance of diastolic and/or systolic dysfunction, and its presence is independent of the coexistence of ischemic or hypertensive or valvular heart disease. Myocardial fibrosis and myocyte hypertrophy are the most frequently proposed mechanisms to explain cardiac changes in diabetic cardiomyopathy. Several studies have shown that diabetes causes defects in cellular calcium transport, defects in myocardial contractile proteins and an increase in collagen formation which result in anatomic and physiological changes in the myocardium (Trachans *et al*, 2014).

1.2.6.2 Microvascular complications

1.2.6.2.1 Diabetic retinopathy

Diabetic retinopathy may be the most common microvascular complication of diabetes. It is responsible for ~ 10,000 new cases of blindness every year in the United States alone (Fowler, 2008).

Approximately 25% of Type 1 patients have some retinopathy after five years. These numbers increase to almost 60% after 10 years and greater than 80% after 15 years. In Type 2 patients older than age 30 with a known duration of diabetes of less than five years, 40% of patients taking insulin and 24% of those not taking insulin are found to have retinopathy. After 10 years, the numbers increase to 53% and 84%, respectively. Proliferative diabetic retinopathy is found in approximately

2% of type 2 patients who have diabetes for less than five years, and 25% who have had diabetes for 25 years or more (Ferrucci *and* Yeh., 2016).

Aldose reductase may participate in the development of diabetes complications. It is the initial enzyme in the intracellular polyol pathway. This pathway involves the conversion of glucose into glucose alcohol (sorbitol). High glucose levels increase the flux of sugar molecules through the polyol pathway, which causes sorbitol accumulation in cells. Osmotic stress from sorbitol accumulation has been postulated as an underlying mechanism in the development of diabetic microvascular complications, including diabetic retinopathy. Oxidative stress may also play an important role in cellular injury from hyperglycemia. High glucose levels can stimulate free radical production and reactive oxygen species formation. Growth factors, including vascular endothelial growth factor (VEGF), growth hormone, and transforming growth factor β, have also been postulated to play important roles in the development of diabetic retinopathy. VEGF production is increased in diabetic retinopathy, possibly in response to hypoxia. In animal models, suppressing VEGF production is associated with less progression of retinopathy (Fowler, 2008).

1.2.6.2.2 Diabetic nephropathy

Diabetic nephropathy is the leading cause of end stage renal disease worldwide and is associated with increased cardiovascular risk. The earliest clinical manifestation is of microalbuminuria. Tight blood glucose and blood pressure control reduce the risk of microalbuminuria. Once microalbuminuria is present, the rate of progression to end stage renal disease and of cardiovascular disease can be delayed by aggressive management of blood pressure, glucose, and lipids. Inhibition of the renin-angiotensin system is important to reduce intraglomerular pressure but other

classes of antihypertensive agent may also be needed to gain adequate control of systemic blood pressure. Such measures can at least half the rate of progression of nephropathy and cardiovascular disease (Marshall, 2004).

The classical definition of diabetic nephropathy is of a progressive rise in urine albumin excretion, coupled with increasing blood pressure, leading to declining glomerular filtration and eventually end stage renal failure. Patients generally have diabetic retinopathy. Recently, greater appreciation of the close links between nephropathy and cardiovascular disease have lead to the inclusion of premature cardiovascular disease, cardiovascular risk increasing in parallel with albuminuria. Diabetes causes unique changes in kidney structure. Classic glomerulosclerosis is characterized by increased glomerular basement membrane width, diffuse mesangial sclerosis, hyalinosis, microaneurysm, and hyaline arteriosclerosis Tubular and interstitial changes are also present. Areas of extreme mesangial expansion called Kimmelstiel-Wilson nodules or nodular mesangial expansion are observed in 40– 50% of patients developing proteinuria . Micro- and macroalbuminuric patients with type 2 diabetes have more structural heterogeneity than patients with type 1 diabetes (Gross *et al*., 2005).

1.2.6.2.3 Diabetic neuropathy

The diabetic neuropathies are heterogeneous, affecting different parts of the nervous system that present with diverse clinical manifestations. They may be focal or diffuse. Most common among the neuropathies are chronic sensorimotor distal symmetric polyneuropathy and the autonomic neuropathies. distal symmetric polyneuropathy is a diagnosis of exclusion (Boulton *et al*., 2005).

Causative factors include persistent hyperglycemia, microvascular insufficiency, oxidative and nitrosative stress, defective neurotropism, and autoimmune-mediated nerve destruction (Vinik *et al*., 2013).

The precise nature of injury to the peripheral nerves from hyperglycemia is not known but likely is related to mechanisms such as polyol accumulation, injury from advanced glycosylated end products, and oxidative stress. Peripheral neuropathy in diabetes may manifest in several different forms, including sensory, focal/multifocal, and autonomic neuropathies. More than 80% of amputations occur after foot ulceration or injury, which can result from diabetic neuropathy (Fowler, 2008).

1.2.7 Hemoglobin A1c

Glycated hemoglobin (hemoglobin A1c, HbA1c, A1C, or Hb1c; sometimes also referred to as being Hb1c or HGBA1C) is a form of hemoglobin that is measured primarily to identify the three-month average plasma glucose concentration. The test is limited to a three-month average because the lifespan of a red blood cell is four months (120 days). However, since RBCs do not all undergo lysis at the same time, HbA1C is taken as a limited measure of 3 months. It is formed in a non-enzymatic glycation pathway by hemoglobin's exposure to plasma glucose. HbA1c is a measure of the beta-N-1-deoxy fructosyl component of hemoglobin (Miedema, 2005).

The origin of the naming derives from Hemoglobin type A being separated on cation exchange chromatography. The first fraction to separate, probably considered to be pure Hemoglobin A, was designated HbA0, the following fractions were designated HbA1a, HbA1b, and HbA1c, respective of their order of elution. There have subsequently been many more sub fractions as separation

techniques have improved .Normal levels of glucose produce a normal amount of glycated hemoglobin. As the average amount of plasma glucose increases, the fraction of glycated hemoglobin increases in a predictable way. This serves as a marker for average blood glucose levels over the previous three months before the measurement as this is the lifespan of red blood cells. In diabetes mellitus, higher amounts of glycated hemoglobin, indicating poorer control of blood glucose levels, have been associated with cardiovascular disease, nephropathy, neuropathy, and retinopathy(Peterson *et al.,* 1998).

1.2.7.1 Measuring HbA1c

A number of techniques are used to measure hemoglobin A1c.Laboratories use:High-performance liquid chromatography (HPLC): The HbA1c result is calculated as a ratio to total hemoglobin by using a chromatogram, immunoassay, enzymatic, capillary electrophoresis and boronate affinity chromatograph.Higher levels of HbA1c are found in people with persistently elevated blood sugar, as in diabetes mellitus. While diabetic patient treatment goals vary, many include a target range of HbA1c values. A diabetic person with good glucose control has a HbA1c level that is close to or within the reference range. The International Diabetes Federation and the American College of Endocrinology recommend HbA1c values below 48 mmol/mol (6.5 DCCT %), while the American Diabetes Association recommends HbA1c be below 53 mmol/mol (7.0 DCCT %) for most patients. Recent results from large trials suggest that a target below 53 mmol/mol (7 DCCT %) for older adults with type 2 diabetes may be excessive: Below 53 mmol/mol (7 DCCT %) the health benefits of reduced A1C become smaller, and the intensive glycemic control required to reach this level leads to an increased rate of dangerous hypoglycemic episodes (Lehman and Krumholz.,2009).

1.2.7.2 Indications and use

Glycated hemoglobin testing is recommended for both checking the blood sugar control in people who might be pre-diabetic and monitoring blood sugar control in patients with more elevated levels, termed diabetes mellitus. There is a significant proportion of people who are unaware of their elevated HbA1c level before they have blood lab work. For a single blood sample, it provides far more revealing information on glycemic behavior than a fasting blood sugar value. However, fasting blood sugar tests are crucial in making treatment decisions. Diagnosis of diabetes during pregnancy continues to require fasting and glucose tolerance measurements for gestational diabetes, and not the glycated hemoglobin (Walid

etal., 2009).

1.3 Previous studies

Many previous studies were reported in correlation between fibrinogen level and HbA1c in diabetic patients.

A study by Jain *et al* (2001) included 50 diabetic subjects and 10 age, sex and body mass index matched controls estimated fibrinogen levels and Hb A1c, higher plasma fibrinogen level were found in diabetics as compared with control group which statistically significant (Jain *et al*., 2001).

Bembde (2011) recruited 100 type 2 diabetic subjects and 100 age and sex matched controls and finrinogen level were estimated, the result showedhigher plasma fibrinogen level in diabetics as compared with control group which statistically significant (Bembde., 2011).

A cross- sectional study of a population- based cohort enrolled total 1574 patients with NIDDM who represented 81% of the initial cohort of 1967, fibrinogen level

and HbA1c were estimated and reported that diabetic patients had high prevalence of hyperfibrinogenemia (Bruno *et al.,* 1996).

Plasma fibrinogen and HbA1c were measured in 116 ambulatory typeI and type II diabetics with or without clinical evidence of macro-or microvascular complications, fibrinogen levels were elevated disproportionately in patients with type II diabetes and vascular complications (Ganda *et al.,* 1992).

Soliman *et al* (2005), in Egypt conducted a study in fibrinogen level and Hb A1c in 48 control person(24 male, 24 female), 96 diabetic patients (48 male, 48 female) , the result showed significant elevation in fibrinogen levels and Hb A1c in diabetic patients in comparison with control group (Soliman., 2005)

Pacilli *et al* (2013) , examined fibrinogen level and Hb A1c in 375 coronary artery disease (CAD)-negative and 320 CAD- positive diabetic patients , the result showed statistically significant association between fibrinogen level and cardiovascular disease in diabetic patients (Pacilli *et al*., 2013).

Rationale

The prevalence of type2 DM in Sudan has been estimated to be 4% (Hala *et al*., 2013).

Serious complications can occur in diabetic patients as a result of chronic hyperglycemia such as nephropathy, retinopathy and vascular complications which considered as a major cause of mortality and morbidity among diabetics. Macrovascular complications tend to affect the heart (coronary artery disease), the central nervous system (cerebrovascular disease) and lower limbs (peripheral vascular disease).

Many haemostatic abnormalities reported to be associated with vascular complications such as: increased levels of endothelial markers (t-PA antigen, vWF Ag), clotting factors (fibrinogen, factor VII, factor VIII and factor IX) and shortened APTT. Identification of hyperfibrinogenemia as a cause of vascular complications in diabetic patients will have a positive impact on patient's management improvement.

Objectives

General objective

To evaluate fibrinogen level among patients with type 2 diabetes mellitus.

Specific objectives

- To estimate fibrinogen level and HbA1c in diabetic patients.
- To compare fibrinogen level in patients with good and bad glycemic control
- To correlate fibrinogen level with development of complications in diabetic patients.
- To investigate the correlation between duration of disease and fibrinogen level in diabetic patients

Chapter Two

Chapter Two

Materials and Methods

2.1 Materials

2.1.1 Study design

This study is a descriptive cross-sectional study.

2.1.2 Study area

The study was conducted at professor. Mahdi Mohammed Ali clinic for diabetes and endocrine glands; Khartoum, Sudan.

2.1.3 Study duration

The study was conducted in the period from March - May 2017.

2.1.4Study population

Patients with diabetes mellitus type 2.

2.1.5 Inclusion criteria

Diabetic patients (type2) with duration of disease 5 years or more.

2.1.6 Exclusion criteria

Diabetic patients with known coagulation disorders and those using anticoagulant therapy were excluded.

2.1.7 Ethical considerations

The consent of the selected individuals to be enrolled in the study was obtained after being informed with all detailed objectives of the study and its health benefit in future.

2.1.8 Data collection

Patients' data (age, sex, duration of disease, complications) were collected using structured interview questionnaire.

2.1.9 Sample collection

Two samples were collected; one for fibrinogen estimation in tri-sodium citrate vacotainer tube 1.8 ml venous blood (Blood: anticoagulant ratio 1:9) and the other one for Hb A1c estimation(2.5ml venous blood in EDTA).

2.1.10 Preparation of platelet poor plasma

Platelet poor plasma was prepared for estimation of fibrinogen level, blood samples were centrifuged, after well mixing, for 15 minutes at 3000 rpm.

2.2 Methods

2.2.1 Fibrinogen estimation

2.2.1.1 Principle

Fibrinogen level was measured by Clauss method. The principle based on the clotting of diluted sample with a strong thrombin solution; the plasma diluted to give a low level of any inhibitors (e.g. FDP and heparin). A strong thrombin solution is used so that, the clotting time over a wide range is independent of the thrombin concentration.

2.2.1.2 Procedure

- Each of thethrombin reagent and fibrinogen calibrator vials (technoclone) were reconstituted exactly with one ml of distilled water; left at RT for 5minutes and then gently swirled till the solution attained homogensity.
- Serial dilutions of fibrinogen calibrator were prepared as demonstrated in table (2.1)

Table (2.1): Dilutions of fibrinogen calibrator stock solution with owren's buffer.

Test tube NO.	I	II	III	IV
Owren's buffer	160	180	190	195microlitre
Fibrinogen calibrator	40	20	10	5microlitre
Dilution	1:5	1:10	1:20	1:40
Fibrinogen concentration	500	349	210	176 mg/dl

Thrombin time was measured for each of the dilutions using automated coagulometer (stago) and the clotting time of each dilution was blotted against its corresponding concentration (dilution 1:10 were represented as 100% concentration) on log-log paper and the points were joined together to create linear line.

2.2.2Thrombin time

First of all cuvette was placed corresponding to the determinations that were done on the thermostat , a magnetic stirrer was inserted in every cuvette and waited for the instrument to reach 37ºC; after that, 200 microliter of diluted plasma were added into the cuvette. When the thermostatisation time is finished, the cuvette was placed on the reading well. The chronometer was remained inactive for some seconds and then it was showed 000:0. At this moment, 100 microlitre of the thrombin reagent was added with a disposable tip pipette, the liquid was left to get down with one blow and all the reaction was started at the same time.

When the reagent and plasma were in contact an O. D. variation was produced, that automatically activated the digital chronometer and the magnetic mixer. When the clot was starts to formed, an optical density variation was produced and stopped the chronometer and the mixer. The clotting time appeared on the display.

2.2.3 Determination of fibrinogen level

Fibrinogen level was determined from the table prepared by manufacturer.

2.2.4 Estimation of HbA1c

2.2.4.1 Principle

HbA1c was measured using Clover*A1c system which is a fully automated boronate affinity assay for the determination of the percentage of hemoglobin A1c in whole blood.

The test cartridge was composed of a cartridge and reagent pack containing the reagents necessary for the determination of hemoglobin A1c, with a sample collecting area for blood sample collection. The reagent pack was pre -filled with

reagent solution and rinsing solution. The reagent solution contains agents that lyse erythrocytes and bind hemoglobin specially, as well as boronate resin that binds cis-diols of glycated hemoglobin. The total hemoglobin was photometrically measured by the diffused reflectance of the optical sensor composed of both a LED (light emitting diode) and a PD (photo diode).

Then, assembled cartridge was rotated and rinsing solution washed out non-glycated hemoglobin from the blood sample, enabling photomectical measurement of glycated hemoglobin.

2.2.4.2 Procedure

When the power was connected, the display shows "warming up" until the device be ready for test. Then, the lid was opened and the pouch open was tornin the side with serrated edge. Gently the cartridge was inserted into the cartridge compartment; the cartridge barcode was held facing left. The reagent pack was mixed gently 5-6 times then the blood sample was applied in the sampling area by carefully touching the blood drop. After that, the reagent pack was inserted into the cartridge compartment of the analyzer and the lid was closed. Finally, the test result was displayed in(%)within 5 minutes.

2.3 Data analysis

Data was analyzed using statistical package for social sciences (SPSS) computer, version 14. Qualitative variables presented as frequency and percentage; quantitative variables presented as mean± SD; Means of quantitative variables was compared by independent 2-sample test; Pearson correlation was used to test the correlation between quantitative variables.

Chapter Three

Chapter Three

Results

This study was conducted on 70 patients with type 2 diabetes mellitus in Khartoum, Sudan, in this period from March to May 2017; of which 32 (45.7%) were males and 38 (54.3 %) were females (Figure 1).

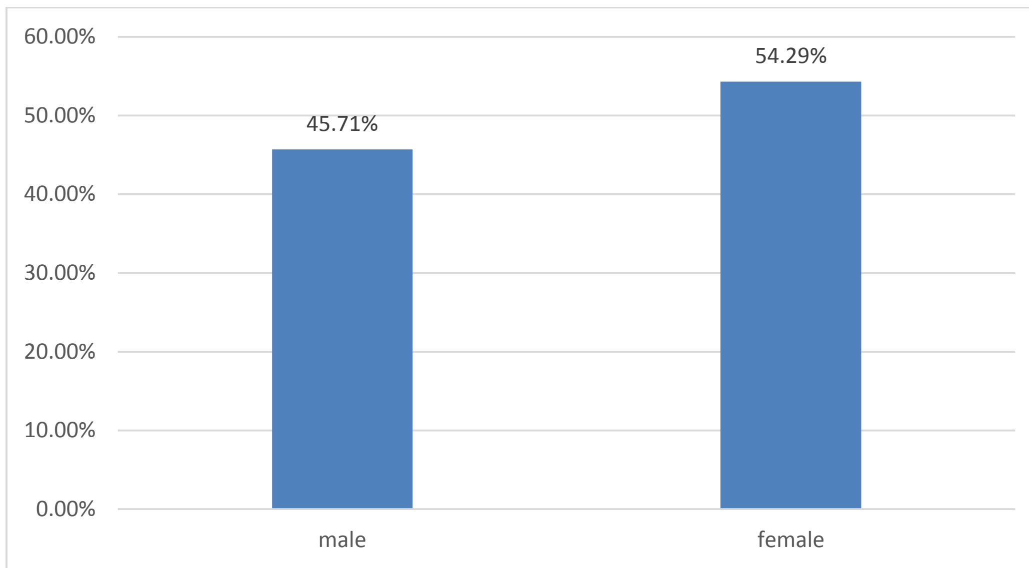

Figure 3.1: Gender distribution in study population.

Age of study population was ranged from 30-82 years (54± 11.9).

Duration of disease among patients was ranged from 5-30 years (11 ±5.9).

Twenty six (37%) of the patients with good control (HBA1c < 7) and 44 (63%) with bad control (HBA1c > 7) (Figure 2).

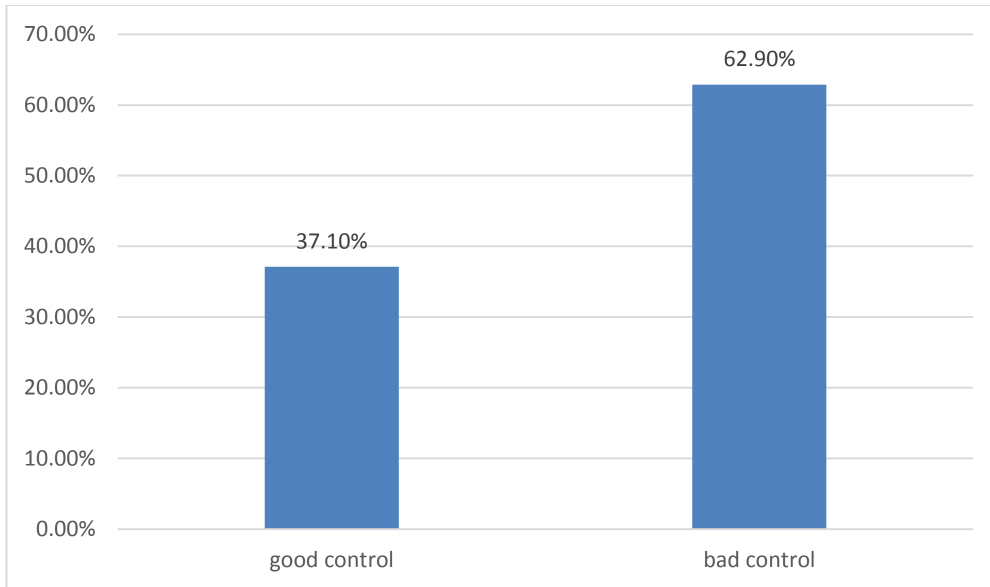

Figure 3.2: Distribution of study population according to glycemic control.

Fifty nine patients without complications while (5) with nephropathy, (3) with cardiopathy and (3) with retinopathy (Figure 3).

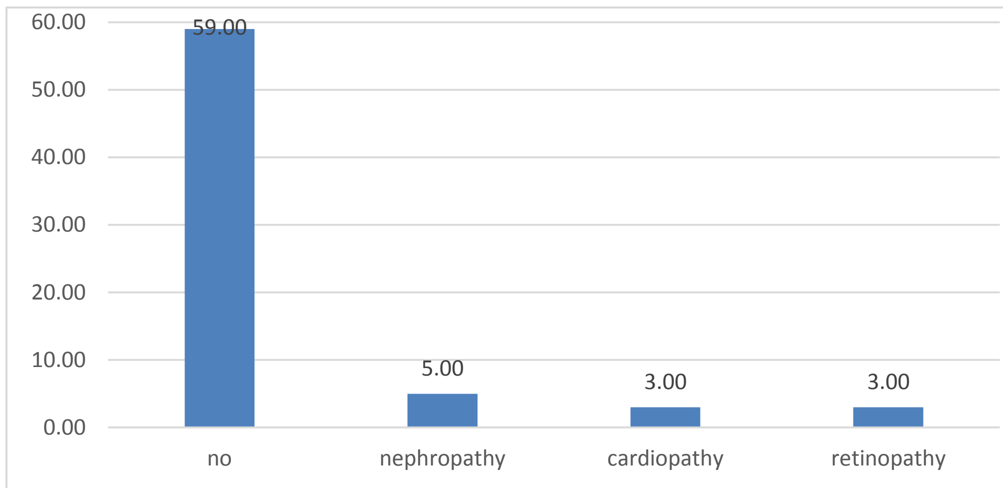

Figure 3.3: Frequency of complications among study subjects.

Twenty eight (40%) of patients were found to have normal fibrinogen level (180-450 mg/dl) and 42 (60%) with hyperfibrinogenemia (469 ± 91.3 mg/dl).

The results showed no statistically significant correlation between HbA1c and fibrinogen level (r =0.1, *P.value* = 0.30) (figure 4).

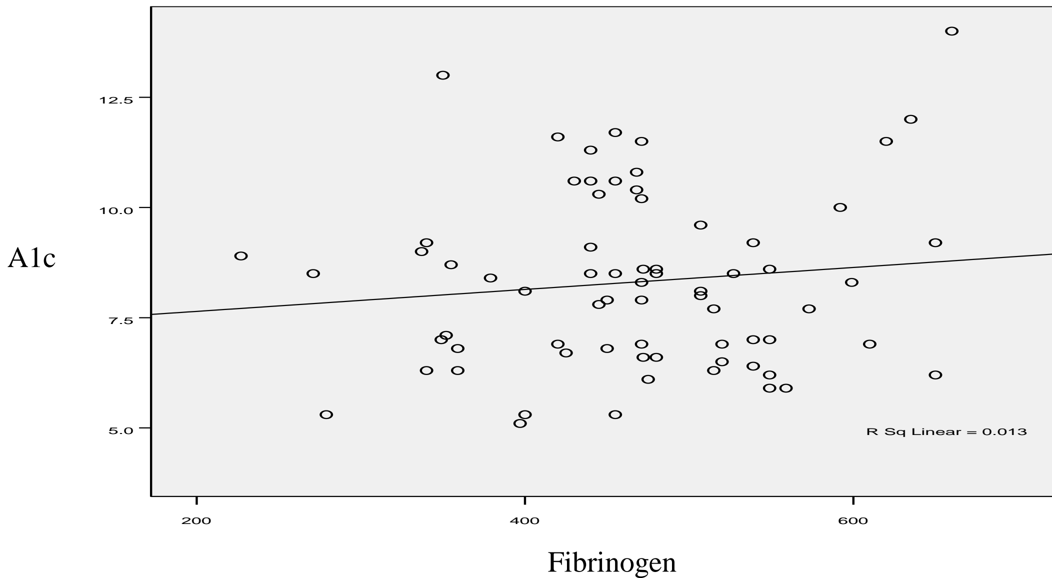

Figure (3.4): Correlation between HbA1c and fibrinogen level.

There was no statistically significant difference in mean fibrinogen level in patients with good control and those with bad control (Table 1).

Table 3.1: Comparison of mean fibrinogen level in patients with good and those with bad control.

Parameter	Sample	No.	Mean	SD	*P.value*
Fibrinogen level mg/dl	Good control	26	471.7	88.676	0.76
	Bad control	44	464.9	93.016	

There was no statistically significant difference in mean fibrinogen level when compared in diabetic patients with complications and those without complications (Table 2).

Table 3.2: Comparison of mean fibrinogen level in patients with complications and those without complications.

Parameter	Complication	No.	Mean	SD	*P.value*
Fibrinogen level mg/dl	Yes	11	489.82	105.326	0.38
	No	59	463.32	88.272	

The results showed no statistically significant correlation between fibrinogen level and age (r=0.3*, p. value*=0.3) (figure 5).

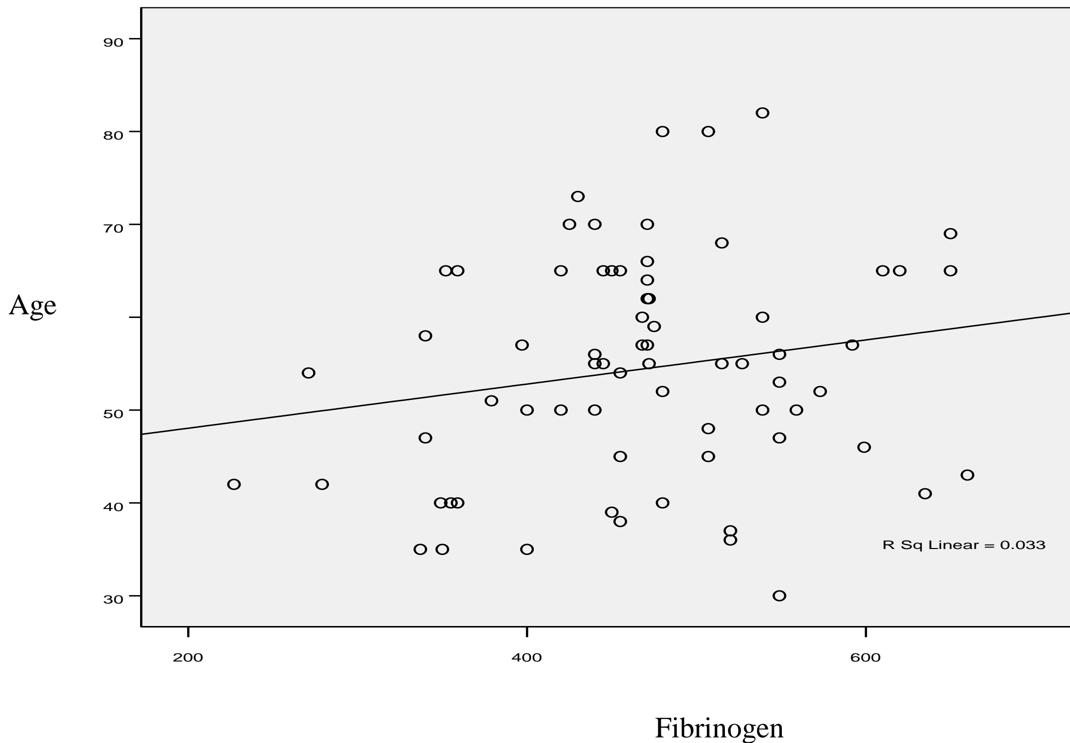

Figure (3.5): Correlation between fibrinogen level and age.

There was a statistically significant difference in mean fibrinogen level in males compared to females (Table 3).

Table 3.3: Comparison of mean fibrinogen level according to gender.

Parameter	Sex	No.	Mean	SD	*P.value*
Fibrinogen level mg/dl	Male	32	442	94.7	0.02
	Female	38	490	83.3	

Mean of fibrinogen level was found significantly increase with increasing duration of DM($r = 0.2$, *p. value* = 0.03) (figure 6).

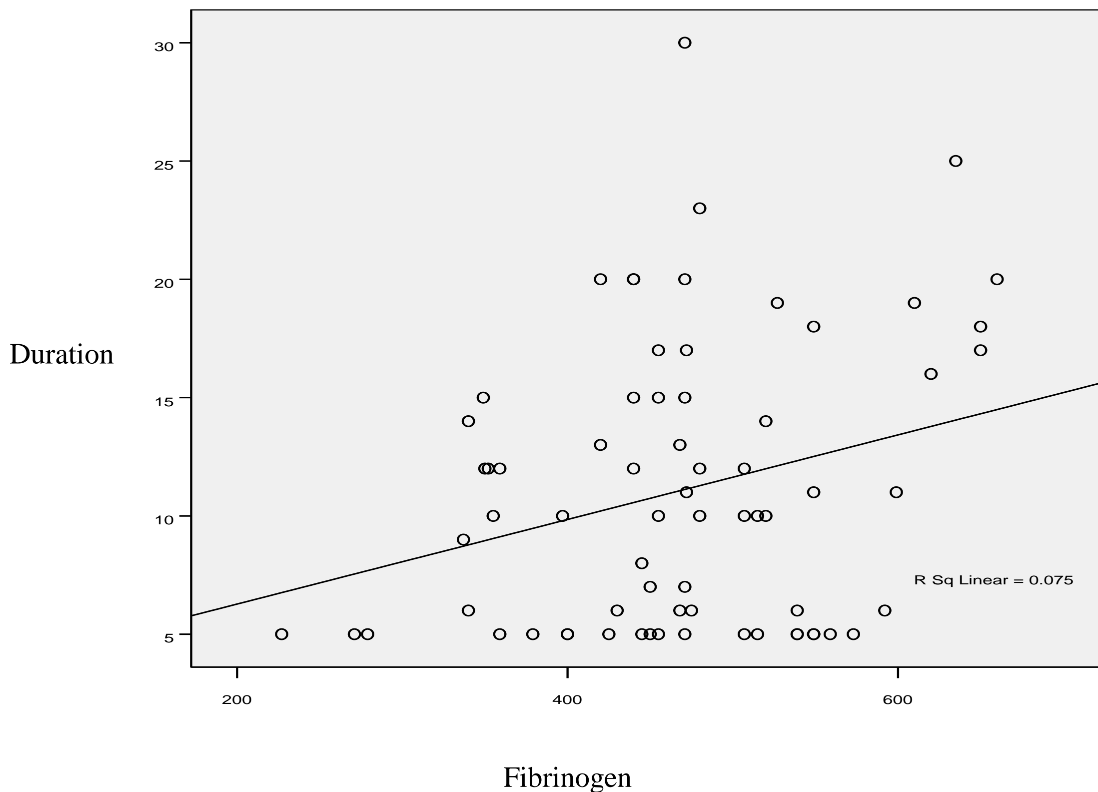

Figure (3.6): Correlation between duration of DM and fibrinogen level.

Chapter Four

Chapter Four

Discussion, Conclusion and Recommendations

4.1 Discussion

This is a descriptive cross- sectional study aimed to assess fibrinogen level among diabetic patients type(2) and correlate the results with glycemic control, patients gender and age, duration of disease and development of complications.

In the present study, mean fibrinogen level was high, 60% of patients with hyperfibrinogenemia and 40% with normal fibrinogen level and, this result disagree with Ganda *et al* in which mean fibrinogen level was normal (Ganda *et al.*, 1992).

In this study, no statistically significant correlation was found between Hb A1c and fibrinogen level, this result agree with a study in Sudan by Abdurahman *et al* but disagree with study in India by Bembde who reported a significant correlation .We found that there was no statistically significant difference in mean fibrinogen level in patients with good control and those with bad control, this finding disagree with Bembde who showed statistically significant association, this variation may due to nutritional habits in these countries (Abdulrahman *et al.*,2013; Bembde,2011).

Concerning complications and fibrinogen level we found that no statistically significant difference in mean fibrinogen level in patients with and without complications , this finding agrees with Abdulrahman *et al* in Sudan and Nguyen *et al* in Australia and there was no statistically significant association between hyperfibrinogenemia and complications, this finding disagrees with Pacilli *et al* study who reported statistically significant association of fibrinogen level with

coronary artery disease, this may occur due to large sample size in these previous studies (Abdulrahman *et al*.,2013; Nguyen *et al*.,2009 ; Pacilli *et al*.,2013).

In the current study, there was no statistically significant correlation between fibrinogen level and age, this finding disagrees with Ganda *et al* who showed statistically significant association with age (Ganda *et al*., 1992).

We found that there was statistically significant difference in mean fibrinogen level in males compared to females, mean fibrinogen level in females was higher than in males, this finding consistent with Soliman in Egypt, but Bruno *et al* in Italy reported higher fibrinogen level in males than females and Jain *et al* showed no statistically significant association (Soliman, 2005; Bruno *et al*., 1996; Jain *et al*., 2001).

Regarding fibrinogen level and duration of disease there was a statistically significant correlation, this disagree with Kafle *et al* and Jain *et al* who showed no statistically significant correlation, this may due to short duration in these studies (Kafle *et al*.,2010; Jain *et al*.,2001).

4.2 Conclusion

- The overall mean of fibrinogen level was found high in patients with DM type 2.
- Fibrinogen level increases with increasing duration of disease.
- Diabetic control has no effect on fibrinogen level.

4.3 Recommendations

- Fibrinogen level should be estimated in patients with DM type 2 with increase duration.

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Appendixes

Appendix One

Reagents

- Thrombin reagent, which was lyophilized preparation from bovine source 50 NIH units per vial.
- Fibrinogen calibrator, which was lyophilized preparation of human plasma equivalent to stated amount of fibrinogen on mg basis (refer FIBRINOGEN graph paper supplied with each kit for the value of each lot).
- Owren's buffer, ready to use (PH 7.35).

Appendix Two

Sudan Universityof Science and Technology

College of Graduate Studies

Questionnaire of evaluation of fibrinogen level among patients with diabetes mellitus type 2

Patient informations

Serial No. ……………..

Gender M□ F☐

Age …………………years

Duration of diabetes mellitus …………..years

Complications:

Cardiopathy □ Retinopathy □ Nerve damage □

Nephropahy□ Neuropathy □ Sexual dysfunction □

Foot problems □ Miscarriage and stillbirth □

Lab. Results:

HbA1c………………….. % fibrinogen level……………mg/dl.