Sudan University of Science and Technology
College of Graduate Studies

Estimation of Prothrombin Time, Activated Partial Thromboplastin Time and platelets count in Diabetes Type II patients in Khartoum State

تقدير زمن البروثرومبين والثرومبوبلاستين المنشط الجزئ وتعداد الصفائح الدموية لمرضي السكري من النوع الثاني في ولايه الخرطوم

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الآيـــة

قال تعالى :

أَفَحَسِبْتُمْ أَنّمَا خَلَقْنَاكُمْ عَبَثًا وَأَنّكُمْ إِلَيْنَا لَا تُرْجَعُونَ ﴿١١٥﴾ فَتَعَالَى اللّـهُ الْمَلِكُ الْحَقُّ لَا إِلَـهَ إِلّا هُوَ رَبُّ الْعَرْشِ الْكَرِيمِ ﴿١١٦﴾

صدق الله العظيم

سورة المؤمنون الآيات ***115-116***

Dedication

This work is dedicated to

My beloved mother

My beloved father

My beloved brother and sister.

To my faithful friends and colleagues.

And all Sudanese people.

Acknowledgement

In the Name of Allah, the most merciful, the most compassionate all praise is to Allah, the lord of the worlds, and prayers and peace upon Mohammed His servant and messenger.

First and foremost, I must acknowledge my limitless thanks to Allah, the ever magnificent; the ever thankful, for His help and bless. I am totally sure that this work would have never become truth, without His guidance.

I am a deep debt of gratitude to my university for giving my an opportunity to complete this work.

I am grateful to some people , who worked hard with me from the beginning till the completion of the present research specially my supervisor D. Munsour Mohammed Munsour , who has been always generous during all phases of the research , and I highly appreciate the efforts expended by him.

Last but not least, deepest thanks go to all specially, who took part in making this thesis real.

Abstract

This is an analytical case control study, conducted during the period from July to November in Best Care Hospital. The study aimed to evaluate prothrombin time, international normalized ratio (INR), activated partial thromboplastin time and platelets count among patients with diabetes type II. Thirty samples were collected from 12 males and 18 females their age range between 30—80 and a means of 51 years, and diabetic duration from 1—30 years. The patients were divided into diabetic controlling and un-controlling groups in respect to level of HbA1C (less than 8%, more than 8%) respectively.

Thirty control samples were collected from healthy non diabetic volunteers with same age ranges. All subjects were questionnaired during blood collection about some clinical information. Blood samples and controls were investigated for PT, INR, APTT and Platelets count using coagulometer and sysmex semi-automated, hematology analyzer respectively. There was no significant difference in PT, INR, APTT and platelets count in diabetic patients compare with control group (P-value 0.893, 0.971,0.321 and 0.703) respectively. The results obtained show that diabetic patients have normal coagulation mechanisms.

مستخلص البحث

هذه الدراسة التحليلية الوصفية اجريت في مستشفي بست كير في الفترة من يوليو الي نوفمبر. الهدف من هذة الدراسة هو تقويم زمن البروثرومبين، المعدل الدولي الطبيعي ،زمن الثرومبوبلاستين المنشط الجزئ وتعداد الصفائح الدموية لدي مرضي السكري جمعت ثلاثين عينة من مرضي السكري من النوع الثاني 12ذكر و18 انثي من النوع الثاني من اعمار تتراوح بين (30-80) سنة ومتوسط الاعمار 51 سنة وفترة المرض تتراوح ما بين سنة الي ثلاثون سنة، جمعت 30 عينة من متبرعين اصحاء للضبط، اجري تقويم زمن البروثرومبين ، المعدل الدولي الطبيعي ،زمن الثرومبوبلاستين المنشط الجزئ وتعداد الصفائح الدموية لكل العينات. اثبتت الدراسة ان متوسط نتائج مرضي السكري عند مقارنتها بمتوسط نتائج المشاركين الاصحاء ذات نتائج متقاربة ولكن دون قيمة معنوية. اثبتت الدراسة ان السكري ليس له تاثير علي معدل تجلط الدم .

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List of Abbreviations

`ADP	Adenosine Di Phosphate
APC	Activated protein C
APTT	Activated Partial Thromboplastin Time
AT	Antithromboin
ATP	Adenosine Tri Phosphate
BCE	Before Common E
Ca	Calcium
Cacl2	Calcium chloride
CE	Common Era
DIC	Disseminated Intravascular Coagulopathy
DM	Diabetes Mellitus
eAG	Estimated Average Glucose
EDTA	Ethylene Diamine Tetra Acetic acid
FDPs	Fibrin Degradation Products
FIA	Fluorescence Immune Assay
GP	Glycoprotein
HMWK	High Molecular Weight Kininogen
ICU	Intensive Care Unit
IDDM	Insulin Dependent Diabetes Mellitus
IFCC	International Federation of Clinical Chemistry
MW	Molecular Weight

NGSP	National Glycohemoglobin Standardization Program
NIDDM	Non-Insulin Dependent Diabetes Mellitus
NPH	Normal Pressure Hydrocephalus
PPP	Platelet Poor Plasma
PT	Prothrombine Time
PZI	Protein Z Inhibitor
SPSS	Statistical Package of Social Science
TAFI	Thrombin Activatable Fibrinolysis Inhibitor
TF	Tissue Factor
TGFa	Tumor Growth Factor alpha
TPA	Tissue Plasminogen Activator
TXA2	Thromboxane A2
U-PA	Urokinase Plasminogen Activator
VWF	Von Willebrand Factor

Chapter One

Introduction & Literature review

Chapter one

Introduction and Literature Review

1.1 Introduction:

The concept of blood coagulation dates back to 1960 when Davie, Ratnoff and Macfarlane described the "waterfall" and "cascade" theories outlining the fundamental principle of cascade of pro-enzymes leading to activation of downstream enzymes (Achneck *et al.*, 2010). Haemostasis, defined as arrest of bleeding, comes from Greek, haeme meaning blood and stasis meaning to stop (Thornton *et al.*, 2010). Thrombo-haemmorhagic balance is maintained in the body by complicated interaction between coagulation and fibrinolytic system as well as platelets and vessel wall .Usually, the coagulation processes under the inhibitory control of several inhibitors that limit the clot formation, thus avoiding thrombus propagation. This delicate balance is interrupted whenever the pro-coagulant activity of the coagulation factors is increased, or the activity of naturally occurring inhibitors is decreased (Previtali *et al.*, 2011). Diabetes mellitus (DM) is a metabolic disorder characterized by chronic hyperglycemia due to disturbances of carbohydrate, fat, and protein metabolism that are associated with absolute or relative deficiencies in insulin secretion, insulin action or both (Charles, 1998). Diabetes have three main types : type one diabetes mellitus which called IDDM (Insulin Dependent Diabetes mellitus) , type two diabetes mellitus which called NIDDM (Non -Insulin Dependent Diabetes mellitus) and gestational diabetes which is classified as type two diabetes mellitus. The long term affects and complications of diabetes include progressive development of the retinopathy, and neuropathy with micro vascular and macro vascular diseases. Macro

vascular disorders such as atherosclerosis are recognized as a major cause of mortality in diabetic population, and are implicated in the circulatory disturbances that are seen in diabetes. The circulatory disturbances are further complicated by alteration in platelet count and activity, coagulopathy, fibrinolytic aberration, haemorrheological factors, and changes endothelial metabolism (McFarlane, 1997). Many studies have shown that diabetes is a hypercoagulable state. Hypercoagulability results from enhanced vascular endothelial cell expression of tissue factor and Von Willebrand factor. Other factors include increased platelet adhesiveness, elevated level of procoagulant factor and decreased fibrinolytic activity (Alvin, 2001).

1:2: Literature review:

1.2.1Coagulation system:

The concept of blood coagulation dates back to 1960 when Davie, Ratnoff and Macfarlane described the "waterfall" and "cascade" theories outlining the fundamental principle of cascade of pro-enzymes leading to activation of downstream enzymes (Achneck *et al.*, 2010). Haemostasis, defined as arrest of bleeding, comes from Greek, haeme meaning blood and stasis meaning to stop (Thornton *et al.*, 2010). Thrombo-haemmorhagic balance is maintained in the body by complicated interaction between coagulation and fibrinolytic system as well as platelets and vessel wall .Usually, the coagulation processes under the inhibitory control of several inhibitors that limit the clot formation, thus avoiding thrombus propagation. This delicate balance is interrupted whenever the pro-coagulant activity of the coagulation factors is increased, or the activity of naturally occurring inhibitors is decreased (Previtali *et al.*, 2011).

Some of thrombogenic and antithrombogenic components are listed in Table (Previtali *et al.*, 2011):

Site	Thrombogenic	Anti thrombogenic
Vessel wall	- Exposed endothelium - TF - collagen	- Heparin - thrombomodulin - Tissue plasminogen activator
Circulating Elements	- Platelets - Platelets activating factor - Clotting factor - Prothrombin - Fibrinogen - VWF	- Antithrombin - Protein c and s - plasminogen

It is important for a perioperative physician to understand the intricacies of two (more so in a preexisting haematological disorder) that go side in maintaining the circulating blood in fluidic state. Pathological situations requiring surgery or anaesthesia or any other invasive procedure trigger the hemostatic system. This balance is also disturbed by trauma, cytokines or infectious agents. Thus, the perioperative period is at high risk for both pro-haemorrhagic and prothrombotic abnormalities. Hypoxia, hypothermia, metabolic acidosis and extracorporeal circulation may also further aggravate the situation (Bombeli and Pahn, 2004). Coagulopathy may also be encountered by the intensivist due to physiological disturbances, disturbances in the primary haemostasis, hemostasis, and abnormalities of blood, plasma or due to disseminated intravascular coagulation (Meybohm *et al*. 2013).

1.2.1.2 Primary homeostasis:

Primary haemostasis results from complex interactions between platelets, vessel wall and adhesive proteins leading to the formation of initial ' platelet plug'. The endothelial cells lining the vascular wall exhibit the antithrombotic properties due to multiple factors viz: negatively charged heparin-like glycosaminoglycan , neutral phospholipids , synthesis and secretion of platelet inhibitors , coagulation inhibitors and fibrinolysis activators .In contrast , sub endothelial layer is highly thrombogenic and contains collagen , Von Willebrand factor (Vwf) and other proteins like laminin , thrombospondin , and vitronectin that are involved in platelet adhesion . Any vascular insult results in arteriolar vasospasm, mediated by reflex neurogenic mechanisms and release of local mediators like endothelin and platelet-derived thromboxane A2 (TxA2) (Cines *et al*., 1998, Lasne *et al*., 2006, Triplett, 2000).

1.2.1.3 Platelets:

Platelets are disc shaped, a nucleate cellular fragments derived from megakaryocytes. They have a pivotal role in haemostasis by forming the initial haemostatic plug that provides a surface for the assembly of activated coagulation factors leading to formation of fibrin stabilized platelet aggregates and subsequent clot retraction. Platelets have two types of granules:

- Alpha granules contain P-selectin , fibrinogen, fibronectin,factor v ,factor VIII,factorIV,platelet derived growth factor and tumor growth factor-a (TGF-a) (HeemsKerk *et al*., 2002) .

- Gamma granules or dense granules-contain adenosine triphosphate (ATP), adenosine diphosphate (ADP), calcium (ca), serotonin, histamine and epinephrine (HeemsKerk *et al.*, 2002).Normally platelets do not adhere to intact vascular endothelium. Subsequent to the vascular injury, platelets adhere to collagen and Vwf in the sub endothelial tissue and undergo a morphological change by assuming irregular surface, forming numerous pseudopods thus drastically increasing their surface area (Andrews and Berndt, 2004). The formation of the platelet plug involves a series of steps:

1.2.1.3.1 Platelet adhesion:

After vascular injury Vwf acts as a bridge between endothelial collagen and platelet surface receptors Gp1b and promotes platelet adhesion. The platelet glycoprotein complex I (Gp-1b) is the principal receptor for Vwf (HeemsKerk *et al*., 2002).

1.2.1.3.2. Platelet secretion:

After adhesion, degranulation from both types of granules takes place with the release of various factors. Release of calcium occurs here. Calcium binds to phospholipids that appear secondary to the platelet activation and provides a surface for assembly of various coagulation factors (HeemsKerk *et al.*, 2002) .

1.2.1.3.3. Platelet aggregation:

Thromboxane A2 produced by activated platelets provide stimulus for further platelet aggregation. TxA2 along with ADP enlarge this platelet aggregate leading to the formation of the platelet plug, which seals off vascular injury temporarily. ADP binding also causes a conformational change in Gp11b /111a receptors presents on the platelet surface causing deposition of fibrinogen. Thrombin generation also catalyzes the conversion of this fibrinogen to fibrin which adds to the stability of the platelet plug and is now known as secondary homeostasis (HeemsKerk *et al.*, 2002). Prostacyclin inhibits platelet aggregation (platelet anti aggregation effect) and the balance between TxA2 and prostacyclin leads to localized platelet aggregation thus preventing extension of the clot thereby maintaining the vessel lumen patency (Cines *et al.*, 1998, Ashby *et al.*, 1990).

1.2.1.4 Clotting factors (coagulation proteins):

Majority of clotting factors are precursors of proteolytic enzymes known as zymogens that circulate in an inactive form. The activation of each zymogen is depicted by suffixing letter "a" to the Roman numeral identifying that particular zymogen. Most of the procoagulants and anticoagulants are produced by liver except factor III, IV and VIII. These

proteins undergo a post translation modification (vitamin k dependent Gama carboxylation of glutamic acid residues) which enables them to bind calcium and other divalent cations and participate in clotting cascade. Deficiency of vitamin k or administration of vitamin k antagonists (warfarin) lead to anticoagulation (Monroe *et al.*, 2010).

1.2.1.4.1 Nomenclature of coagulation proteins:

The first 4 of the 12 originally identified factors are referred to by their common names, i.e., fibrinogen, prothrombin, tissue factor (TF), and calcium and are not assigned any Roman numerals .F6 no longer exists. The more recently discovered clotting factors (e.g. prekallikrein and high-molecular-weight kininogen) have not been assigned Roman numerals. Some factors have more than one name. Factor V and FVIII are also referred to as the labile factors because their coagulant activity is not durable in stored blood. Prothrombin is a plasma protein formed by liver (MW 68700). It is an unstable protein, splitting into smaller proteins one of which is thrombin (MW 33700). Thrombin generated from prothrombin also has pro-inflammatory effects which are exerted by the activation of protease activating receptors present on monocyte, lymphocytes, endothelium and dendritic cells (Hall, 2010).Von Willebrand factor is a glycoprotein present in blood plasma and produced constitutively as ultra-large vWf in endothelium, megakaryocytes, and subendothelial connective tissue. It mediates platelet adhesion to subendothelial surface. It also acts as a carrier protein for coagulant activity of Factor VIII and is referred there as VIII: C. (Sadler, 1998,Barash *et al*,. 2006)]Fibrinogen is an essential coagulation protein produced by liver (MW340 kDa) and is the precursor of fibrin that ultimately defines the strength of clot.(Doolittle *et al.*, 1998, Kamath and

Lip, 2003)Factor III or TF is a membrane bound procoagulant glycoprotein (MW47-kDa) present in the subendothelial tissue and fibroblasts and is not exposed to blood until disruption of the vessel wall. (Monroe *et al*., 2010) It is the primary initiator of coagulation *in vivo*. TF is localized predominantly to the tunica media and tunica adventitia of blood vessels and a smaller quantity as circulating TF on monocytes. Tissue factor may be activated by physical injury (activation of Vessel wall TF), by direct vascular injury or functional injury (activation of circulating TF), by hypoxia, sepsis, malignancy, inflammation, etc.

Clotting factors can also be classified into three groups (Mackman *et al*., 2007, Many et al., 2001):

Fibrinogen Family	Vitamin K dependent	Contact Family
Fibrinogen	Factor II	Factor XI
Factor V	Factor VII	Factor XII
Factor VIII	Factor IX	HMWK
Factor XIII	Factor X	Prekallikerin

1.2.1.4.2 Classification of coagulation factors:

Fibrinogen Family

Vitamin K dependent proteins

Contact family.

Hypoxia up regulates the expression of P selectin present in the α granules of platelets on the endothelium leading to recruitment of monocytes containing TF, thus initiating coagulation.

With the exposure of TF to factor VII/VIIIa in the blood, it allows for the formation of TF-VIIIa complex and thus initiate the coagulation cascade (Myers *et al.*, 2003, Closse *et al.*, 1997).

1.2.1.5 Naturally occurring anticoagulants in the body:

The anticoagulant system exerts a regulatory role over the procoagulant activity in blood thus localizing the thrombus formation. The main anticoagulant mechanisms naturally present in the body include the following (Clovin, 2002):

1.2.1.5.1 Antithrombin:

Antithrombin (AT), previously known as AT III is the main inhibitor of thrombin. It is a serine protease inhibitor, which binds and inactivates thrombin, factor IXa, Xa, XIa and XIIa. The enzymatic activity of AT is enhanced in the presence of heparin. However, the plasma concentration of heparin is low and does not contribute significantly to the *in vivo* activation of AT. AT is activated by binding of heparin sulphate present on endothelial cell surface. AT binds coagulation factors in a ratio of 1:1 and this complex is removed by reticuloendothelial cells. Other thrombin inhibitors are heparin cofactor II, α2 macroglobulin and α1-antitrypsin (Opal *et al*., 2002, Ejiofor, 2013).

1.2.1.5.2 Tissue factor plasminogen inhibitor:

It is a polypeptide produced by endothelial cells. It acts as a natural inhibitor of the extrinsic pathway by inhibiting TF-VIIa complex. (Ejiofor, 2013, Price *et al.*, 2004)] Protein S enhances the interaction of factor Xa in the presence of calcium and phospholipids. (Dahm *et al.*, 2008)

1.2.1.5.3 ProteinC:

The propagation phase of the coagulation is inhibited by the Protein C pathway that primarily consists of four key elements:

- Protein C is a serine protease with potent anticoagulant, profibrinolytic and anti-inflammatory properties. It is activated by thrombin to form activated protein C (APC) and acts by inhibiting activated factors V and VIII (with Protein S and phospholipids acting as cofactors) Thrombomodulin - A transmembrane receptor on the endothelial cells, it prevents the formation of the clot in the undamaged endothelium by binding to the thrombin Endothelial protein C receptor is another transmembrane receptor that helps in the activation of Protein C.

- Protein S is a vitamin K-dependent glycoprotein, synthesized by endothelial cells and hepatocytes. It exists in plasma as both free (40%) and bound (60%) forms (bound to C4b-binding protein). The anticoagulant activity is by virtue of free form while the bound form acts as an inhibitor of the complement system and is up regulated in the inflammatory states, which reduce the Protein S levels thus resulting in procoagulant state. It functions as a cofactor to APC in the inactivation of FVa and FVIIIa. It also causes direct reversible inhibition of the prothrombinase (FVa–FXa) complex. (Rigby *et al*., 2004).

- Protein Z dependent protease inhibitor/protein Z (PZI):

It is a recently described component of the anticoagulant system that is produced in the liver. It inhibits Factor Xa in reaction requiring PZ and calcium (Corral *et al*., 2007).

1.2.1.6 Coagulation pathways:

The coagulation proteins are the core components of the coagulation system that lead to a complex interplay of reactions resulting in the conversion of soluble fibrinogen to insoluble fibrin strands (Bombeli and pahn, 2004).

1.2.1.6.1 Coagulation cascade:

It has been traditionally classified into intrinsic and extrinsic pathways, both of which converge on factor X activation. The classical theory of blood coagulation is particularly useful for understanding the *In vitro* coagulation tests, but fails to incorporate the central role of cell-based surfaces in *In vivo* coagulation process (Bombeli and pahn, 2004). Interestingly contact activation critical for *In vivo* haemostasis does not get support from following observations. Persons lacking FXII, prekallikrein, or high-molecular-weight kininogen do not bleed abnormally. Second, patients with only trace quantities of FXI can withstand major trauma without unusual bleeding, and those who completely lack factor XI (haemophilia C) exhibit mild haemorrhagic disorder. Deficiencies of FVIII and FIX (both intrinsic pathway factors) lead to haemophilia A and B, respectively, however the classic description of two pathways of coagulation leave it unclear as to why either type of haemophiliac cannot not simply clot blood via the unaffected pathway. To answer all this, the modern time-based structuring of blood coagulation provides more authentic description of the coagulation process. It is now appreciated that the classic theories may provide only a reasonable model of in vitro coagulation tests (i.e., APTT and PT) (Bombeli and pahn, 2004).

1.2.1.6.1.1 Extrinsic pathway:

It is considered as the first step in plasma mediated haemostasis. It is activated by TF, which is expressed in the sub endothelial tissue.(Lanse et al .,2006)Under normal physiological conditions, normal vascular endothelium minimizes contact between TF and plasma procoagulants , but vascular insult expose TF which binds with factor VIIa and calcium to promote the conversion of factor X to Xa (Owens and Mackman , 2010).

1.2.1.6.1.2 Intrinsic pathway:

It is a parallel pathway for thrombin activation by factor XII. It begins with factor XII, HMW kininogen, prekallekerin and factor XI, which results in activation of factor XI. Activated factor XI further activates factor IX, which then acts with its cofactor (factor VIII) to form tenase complex on a phospholipid surface to activate factor X.(Hall, 2010,Kumar *et al*., 2010)]

1.2.1.6.1.3 Common pathway:

Activated factor X along with its cofactor (factor V), tissue phospholipids, platelet phospholipids and calcium forms the prothrombinase complex which converts prothrombin to thrombin. This thrombin further cleaves circulating fibrinogen to insoluble fibrin and activates factor XIII, which covalently crosslinks fibrin polymers incorporated in the platelet plug. This creates a fibrin network which stabilises the clot and forms a definitive secondary haemostatic plug (Hall, 2010, Kumar *et al*., 2010).

1.2.1.6.1.4 Fibrinolytic system:

Fibrinolytic system is a parallel system which is activated along with activation of coagulation cascade and serves to limit the size of clot. Fibrinolysis is an enzymatic process that dissolves the fibrin clot into fibrin degradation products (FDPs) by plasmin originating from fibrin bound plasminogen in liver. This reaction is catalysed by TPA or urokinase plasminogen activator (u-PA) released from vascular endothelium. The release of t-PA is stimulated by tissue occlusion, thrombin, epinephrine, vasopressin and strenuous exercise .Plasmin activity is tightly regulated by its inhibitor (α-2 antiplasmin) thus preventing widespread fibrinolysis (Cesarman and Hajjar, 2005). *In vivo* activity of the fibrinolytic system is assessed clinically by measuring the FDP's. D dimers are produced by digestion of cross linked fibrin and are specific indicators of fibrinolysis used in the assessment and diagnosis of pulmonary embolism, DIC or deep vein thrombosis (Clovin, 2004).

1.2.1.6.1.4.1 Regulation of the fibrinolytic system:

Since plasmin has the potential to degrade fibrinogen leading to deleterious consequences, the fibrinolytic activity is limited by following factor:

1. Plasminogen activator inhibitor - It is the main physiological inhibitor of fibriolysis and acts by inhibiting t-PA and u-PA irreversibly

2. TAFI - It is a plasma proenzyme synthesized by liver and activated by thrombin. It decreases the affinity of plasminogen to fibrin and augments the action of anti-trypsin in inhibiting

3. Plasmin inhibitors - α2 antiplasmin and α2Macroglobulin are the glycoproteins that exert action by virtue of plasmin inhibition (Ejiofor, 2013).

1.2.1.6.2 Current concept of coagulation:

Current evidence supports the understanding that intrinsic pathway is not a parallel pathway but indeed it augments thrombin generation primarily initiated by the extrinsic pathway Newer model describes coagulation with following steps (Triplett, 2000):

1.2.1.6.2.1 Initiation:

It occurs by expression of TF in damaged vessel which binds factor VIIa to activate factor IX and factor X. This activation of factor IX by TF-VIIa complex serves as the bridge between classical extrinsic and intrinsic pathways. Factor Xa then binds to factor II to form thrombin (factor IIa). Thrombin generation through this reaction is not robust and can be effectively terminated by TF pathway inhibitor (Triplett, 2000).

1.2.1.6.2.2 Amplification:

Since the amount of thrombin generated is not sufficient, therefore numerous positive feedback loops are present that bind thrombin with platelets. Thrombin that is generated in the initiation phase further activates factor V and factor VIII, which serves as a cofactor in prothrombinase complex and accelerates the activation of Factor II by F Xa and of F Xa by F IXa, respectively (Triplett, 2000).

1.2.1.6.2.2.3 Propagation:

The accumulated enzyme complexes (tenase complex and prothrombinase complex) on platelet surface support robust amounts of

thrombin generation and platelet activation. This ensures continuous generation of thrombin and subsequently fibrin to form a sufficiently large clot (Triplett, 2000).

1.2.1.6.2.2.4 Stabilization:

Thrombin generation leads to activation of factor XIII (fibrin stabilizing factor) which covalently links fibrin polymers and provides strength and stability to fibrin incorporated in platelet plug. In addition, thrombin activates thrombin activatable fibrinolysis inhibitor (TAFI) that protects the clot from fibrinolysis. (Bombeli and pahn, 2004, Lanse *et al*., 2006).

1.2.2 Diabetes mellitus:

Diabetes mellitus (DM) is a metabolic disorder characterized by chronic hyperglycemia due to disturbances of carbohydrates, fat , and protein metabolism that are associated with absolute or relative deficiencies in insulin secretion , insulin action or both (Charles, 1998), over 170 million people world which and about 1.9- 7.0 y of the African population are affected. (wokoma, 2002, Fabiyi *et al.*,2002).Diabetes have three main types : type one diabetes mellitus which called IDDM (Insulin Dependent Diabetes Mellitus) , type two diabetes mellitus which called NIDDM (Non Insulin Dependent Diabetes Mellitus) and gestational diabetes which is classified as type two diabetes mellitus (McFarlane, 1997) .

1.2.2.1 Classification of diabetes mellitus:

1.2.2.1.1 Type one diabetes mellitus:

Type 1diabetes is also called insulin-dependent diabetes .It used to be called Juvenile-onset diabetes,because it often begins in child hood .Is an autoimmune condition .It is caused by the body attacking it is own pancreas with antibodies .In people with type 1 diabetes,the damaged pancreas does not make insulin .This type of diabetes may be caused by agenetic pre-disposition. It could also be the result of faulty beta cells in the pancreas that normally produce insulin .Anumber of medical risks are associated with type 1 diabetes .Many of them stem from damage to the tiny blood vessels in your eyes called (diabetic retinopathy), nerves (diabetic neuropathy) . Even more serious is the increase the risk of heart disease and stroke. Treatment of type 1 diabetes involves taking insulin, which needs to be injected through the skin in to the fatty tissue below .The methods of injecting insulin includes:

- Syrings insulin pensthe ture pre-filled cartidges and fine needle.
- set injection that use high pressure air to send aspray of insulin through the skin
- Insulin pumps that dispense insulin through flexible tubing to acatheter under the skin of the abdomen.

Aperiodic test called the A1C blood test estimates glucose level in your blood over the previous three months. It’s used to help identify over all glucose level control and the risk of complications from diabetes, including organ damage. Having type 1 diabetes dose require significant life style changes that include:

- Frequent testing of your blood sugar level.
- Careful meal planning.
- Daily exercise.

Takinginsulinand other medication needed.People with type 1 diabetes can lead long, active lives if they carefully monitor their glucose, make the needed life style changes, and adhere to the treatment plan (Dansinger, 2017).

1.2.2.1.2 Type 2 diabetes mellitus:

By far ,the most common form of diabetes is type 2 diabetes accounting for 95 % of diabetes cases in adults .Some 26 million American adults have been diagnosed with the disease .Type 2 diabetes used to be called adult-onset diabetes ,but with the epidemic of obese and overweight kids ,more teengagers are now developing type 2 diabetes .Type 2 diabetes also called non-insulin dependent diabetes .Is often a milder form of diabetes than type 1 .Nevertheless ,type 2 diabetes can still cause Major health complication ,particularly in the smallest blood vessels in the body that nourish the kidney , nerves ,and eyes .also increases your risk of heart disease and stroke . In which pancreas usually produces some insulin. But either the amount produced is not enough for the body's needs, or the body's cells are resistant to it .Insulin resistance , or lack of sensitivity to insulin , happens primarily in fat, liver, and muscle cells .And All test is a blood test that estimates average glucose level in your blood over the previous three months . Periodic A1C testing may be advised to see how well diet,exercise, and medication are working to control blood sugarand prevent organ damage. The A1C is typically done a few times a years (Dansinger, 2017).

1.2.2.1.3 Gestational diabetes mellitus:

Diabetes that's triggered by pregnancy is called gestational diabetes (pregnancy,to some degree, leads to insulin resistance). It is often diagnosed in middle or late pregnancy.Because high blood sugar level in a mother are circulated through the placenta to the baby,gestational diabetes must be controlled to protect the baby's growth and development. According to the National Institute of Health, the reported rate of gestational diabetes is between 2%to 10%of pregnancies. Gestational diabetes usually resolves itself after pregnancy. Having gestational diabetes dose, however, put mothers at risk for developing type 2 diabetes later in life .Up to 10%woman with gestational diabetes develop type 2 diabetes .It can occur anywhere from a few weeks after delivery to months or years later .With gestational diabetes, risks to the unborn baby are even greater than risks to the mother .Risks to the baby include abnormal weight gain before birth, breathing problems at birth,and higher obesity and ziabetes risk later in life . Risks to the mother include needing a cesarean section due to an overly large baby, as well as damage to heart ,kidney, nerves,and eye .Treatment during pregnancy includes working closely with your health care treatment and :

- Careful meal planning to ensure adequate pregnancy nutrients without excess fat and calories.
- Daily exercise.
- Controlling pregnancy weight gain.

- Taking diabetes insulin to control blood sugar levels if needed (Dansinger, 2017).

1.2.2.1.4 Other forms of diabetes mellitus:

After rare kinds of diabetes can result from specific condition. For examples, disease of pancreas,certain surgeries and medication, or infections can cause diabetes .These types of diabetes account for only 1% to 5% of all cases of diabetes (Dansinger, 2017).

1.2.2.2 Sign and symptoms of diabetes mellitus:

The signs and symptoms of untreated diabetes are weight loss , polyuria(increased urination), polydipsia (increased thirst), and polyphagia (increased hunger)(Cooke and Plotnick, 2008) .Symptoms may be develop rapidly (weeks or months) in type 1 DM,while they usually develop more slowly and may be subtle or absent in type 2 DM .Several other signs and symptoms can mark the onset of diabetes although they are not specific to the disease .In addition to the known ones above , they include blurry vision, headache ,fatigue ,slow healing of cuts ,and itchy skin . Prolonged high blood glucose absorption in the lens of the eye, which leads to changes in it is shape, resulting in vision changes. Anumber of skin rashes that can occur in diabetes are collectively known as diabetic dermadromes (Rockefeller, 2015).

1.2.2.3 Causes of diabetes mellitus:

Comparison of type 1 and type 2 diabetes (Shoback, 2011) :

Features	Type 1 diabetes	Type 2 diabetes
Onset	Sudden	Gradual
Age at onset	Mostly in children	Mostly in adults
Body size	Thin or normal	Often obese
Ketoacidosis	Common	Rare
Autoantibodies	Usually present	Absent
Endogenous insulin	Low or absent	Normal, decreased or increased
Concordance in identical	50%	90%
Prevalence	~10%	~90%

DM is classified in to four broad categories type 1, type 2,gestational diabetes and other specific types .The" other specific types " are collection of a few dozen individuals causes (Shoback, 2011).diabetes is amore variable disease than once thought and people may have combinations forms.The terms "diabetes", without qualification usually refers to diabetes mellitus (Tuomi *et al.*, 2014).

1.2.2.4 Pathophysiology:

Insulin is the principle hormone that regulates the uptake to glucose from the blood in to most cells of the body, especially liver, adipose tissue and muscle, expect smooth muscle, in which insulin acts via the IGF-1.Therefore,deficiency of insulin or the insensitivity of it is receptors play a central role in all forms of DM (Insulin Basics, American Diabetes, and Association-retrieved 24 April, 2014).The body obtains glucose from the main places: the intestinal absorption of food; the breakdown of the glycogen, the storage form of glucose found in the liver; and gluconeogenesis, the generation of glucose from non-carbohydrates substrates in the body. Insulin plays a criticalrole balancing glucose levels in the body. Insulin can inhibit the breakdown of glycogen or the process of gluconeogenesis , it can stimulate the transport of glucose into fact and muscle cells ,and it can stimulate the storage of glucose in form of glycogen (David and Dolors, 2011).Insulin is released in to the blood by beta cells(B-cells),found in the islets of Langerhans in the pancreas , in responseto rising levels of blood glucose ,typically after eating .Insulin is used by about two-thirds of the body's cells to absorb glucose from the blood for use as fuel , for conversion to other needed molecules , or for storage . Lower glucose levels result in decreased insulin release from the beta cells and in the breakdown of glycogen to glucose. This process is mainly controlled by the hormone glucagon, which acts in the opposite manner to insulin (Kim *et al.*, 2012). If the amount of insulin available is insufficient , if cells respond poorly to the effects of insulin (insulin insensitivity or insulin resistance), or if the insulin itself is defective , then glucose will not be absorbed properly by the body cells that require it , and will not be stored appropriatery in the liver and muscles . The net effect is persistently high levels of blood glucose, poor protein synthesis, and other metabolic derangements, such as acidosis (David and Dolores,

2011). When the glucose concentration in the blood remains high over time, the kidneys will reach a threshold of reabsorption, and glucose will be excreted in the urine (glycosuria) (Robert *et al*., 2012). This increases the osmotic pressure of the urine and inhibits reabsorption of water by kidney, resulting in increased urine production (polyuria) and increased fluid loss. Lost blood volume will be replaced osmotically from water held in the body cells and otherbody compartments, causing dehydration and increased thirst (polydipsia) (David and Dolores, 2011).

1.2.2.5 Diagnosis of diabetes mellitus:

DM is characterized by recurrent or persistent high blood sugar, and is diagnosed by demonstrating any one of the following (WHO, 2003):

- Fasting plasma glucose level >7.0 mmol/l (126 mg/dl).
- Plasma glucose >11.1 mmol/l (200mg/dl) two hours after a 75g oral glucose load as in glucose tolerance test .
- Symptoms of high blood sugar and casual plasma glucose >11.1mmol/l (200mg/dl).
- Glycated hemoglobin (HbA1C)>48mmol/mol (>6.5 DCCT %) (WHO, 2010).

A positive result, in the absence of unequivocal high blood sugar, should be confirmed by a repeat of any of the above methods on a different day. It is preferable to measure a fasting glucose level because of the case of measurement and considerable time commitment of formal glucose tolerance testing, which takes two hours to complete and offers no prognostic advantage over the fasting test(Saydah *et al*., 2001).According to the current definition, two fasting glucose measurements above

126mg/dl (7.0mmol/l) is considered diagnostic for DM .Per the world Health Organization people with fasting glucose from 6.1 to 6.9 mmol/l (110 to 125 mg/dl) are considered to have impaired fasting glucose (WHO,2006) . People with plasmaglucose at or above 7.8mmol/l (140mg/dl), but not over 11.1mmol/l (200mg/dl) two hours after a 75g oral glucose load are considered to have impaired glucose tolerance. Of these two pre diabeticstates, the latter in particular is a major risk factor for progression to full-blown DM, as well as cardiovascular disease (Santaguida et al., 2008). The American Diabetes associated since 2003 uses as slightly different range for impaired fasting glucose of 6.5 to 6.9 mmol/l (100 to 125 mg/dl) (Bartoil et al., 2011) Glycated hemoglobin is better than fasting glucose for determining risks of cardiovascular disease and death from any cause (Selvin et al., 2010).

1.2.2.6 Complications of diabetes mellitus:

All forms of diabetes increase the risk of long-term complication. These typically develop after many years (10-20) but may be the first symptom in those who have otherwise not received a diagnosis before that time. The major long-term complications related to damage to blood vessels (Sarwar et al., 2010). The primary complications of diabetes due to damage to the eyes , kidneys,and nerves .Damage to the eyes , known as diabetic retinopathy , is caused by damage to the blood vessels in the retina of the eye , and can result in gradual vision loss and blindness. Damage to the kidneys,known as diabetic nephropathy, can lead to tissue scarring, urine protein loss, and eventually chronic kidney disease, sometimes requiring dialysis or kidney transplantation. Damage to the nerves of the body, known as diabetic neuropathy,is most common complication of diabetes .The symptoms can include numbness, tingling,

pain, and altered pain sensation, which can lead to damage the skin. Diabetes related foot problems (such as diabetic foot ulcers) may occur, and can be difficult to treat, occasionally requiring amputation. Additionally, proximal diabetic neuropathy causes painful muscle atrophy and weakness. There is a link between cognitive deficit and diabetes. Compared to those without diabetes, those with the disease have a 1.2 to 1.5 fold greater rate of decline in cognitive function (Cukierman, 2005).Being diabetic , especially when on insulin , increase the risk offalls in older people (Yang *et al*., 2016) .

1.2.2.7 Hemoglobin A1C:

Glycated protein is formed post-transnationally through the slow, non-enzymatic reaction between glucose and amino groups on proteins. HbA1C is clinically useful index of mean glycaemia during the preceding 120 days, the average life of span of erythrocytes. Carefully controlled studies have documented as close relationship between the concentrations of HbA1C and mean glycaemia. HbA1C is considered as a more reliable parameter in monitoring glycaemia over the glycemic reading with the conventional glucometer.

1.3 Rationale:

Diabetes is a major source of morbidity in developing countries. In Sudan diabetes is an increasing problem, being responsible for 10% of hospital admission and mortality. Measurement of hemostatic parameter is important in the decrease development of complication of diabetes mellitus. Any disturbance in level of hemostatic parameter have role in pathogenesis and prognosis of diabetes. Diabetes patients have hypercoagulability in the blood, this study is designed to show the effect of diabetes in these hematological parameters (prothrombin time, Activated partial thromboplastin time Platelet count and International Normalized Ratio (INR).

1.4 Objectives:

1.4.1 General objective:

Estimate of coagulation profile and platelets count in diabetes Type II in Khartoum State.

1.4.2 Specific objectives:

1. To estimate level of PT, PTT, Platelet count and INR tests in patients with diabetes and compare with healthy individuals.
2. To determine the effect of diabetes exposed period in PT, APTT, platelet count and INR tests.
3. To assess relation between PT, PTT, INR and platelet count and gender.
4. To correlate between controlling of diabetes and /or duration of the disease and the tested parameters.

Chapter two

Materials and Methods

Chapter two

Materials and Methods

2.1 Study design:

This is a description cross-sectional study aimed to determine the effect of diabetes in coagulation profile (PT, APTT) .The study was conducted in Khartoum state during the period of July to November.

2.2Study population:

Samples taken from diabetic type II women and men including all ages.

2.3 Sample size and sampling:

30 samples were randomly selected for this study by using random selection method as case group and 30 samples collected from healthy individuals as control group.

2.4 Inclusion criteria:

Diabetes patients using insulin injections and tablets.

2.5 Exclusion criteria:

The patients with hypertension or used oral anticoagulant.

2.6 Ethical considerations:

The samples were taken under ethical circumstances after ethical approval from best care hospital administration.

2.7 Collection and processing of blood samples:

Sample of 2 ml of blood was collected in 20 microliter part citrate solution. After centrifugation (3000 r/ 15 minutes at room temperature) to

obtain platelet poor plasma (ppp), the plasma was collected and stored at -20c until analysis .All samples were analyzed for prothrombin time(PT) and activated partial thromboplastin time (APTT).

2.8 Data analysis and presentation:

Data analyzed by SPSS version 11.5 program and presented in form of tables and graphs (Independent t test, correlation and one way ANOVA..

2.9 Methodology:

Semi- automated method by coagulometer biobas 10.

2.10 Coagulometer:

It is automated coagulometer using magnetic sensor making the results accurate and more relianle than manual testing .It performs all coagulation tests like PT, APTT, etc.

Principle:

Measuring cuvette with small rod stainless steel magnetic stirrer inside , kept in coagulometer holes which was adjusted at 37c , the clotting reaction initiated through the test dependent added reagents , magnetic stirrer began to run rapidly around it is horizontal axis due to magnetic field .Surrounding and magnetic sensor was measuring the time , the clot which was formed stopped the movement of magnetic stirrer and the time which was spent up to the end point time was recorded on display as the clotting time of the performed test .

Sampling:

Platelets poor plasma (PPP), the blood was drawing using a needle with limited occlusion of the arm by the tourniquet. The blood was added to

the anticoagulant at a ration of 2ml of the blood to 0.2ml of citrate.Buffer sodium citrate and gently mixed, 3.2% buffer sodium citrate container was used.(Shapiross, 2003)The sample was centrifuge at 3000r/ 15 minutes to obtain platelets poor plasma (ppp), the plasma was placed in to eppendorf tube cap and frozen at -20c used for prothrombin time , activated partial thromboplastin time.

2.11 Methods:

2.11.1 Prothrombin time (PT) automated using biobas10 coagulometer.

Principle:

The prothrombin test measures the clotting time of the plasma in the presence of an optimal concentration of tissue extraction (thromboplastin) and indicates the overall efficiency of the extrinsic clotting system (Giangrande, 2003).

Procedure:

PT liquid thromboplastin brought to 37c and was well mixed, coagulometer was adjusted at 37c and cuvettes with magnetic stirrers inside were kept in holes at 37c for 2 minutes then 200ml of prothrombin liquid thromboplastin added to the plasma and simultaneously was measured at the end point, the clotting time determined directly from the display.

2.11.2 Activated partial thromboplastin time (APTT), automated using biobas10:

Principle:

The test measures the clotting time of plasma often the activation of contact factors but without added tissue thromboplastin, and so indicates the overall efficiency of intrinsic pathway. To standardized the activation of contact factors. The plasma is first pre-incubated with kaolin. Standardaized phospholipid is provided to allow the test to be performed on PPP. The test depends at only the contact factor and on factors VII and IX , but also on the reactions with factor X,V,II,I, it is also sensitive to the presence of circulating anticoagulants (inhibitors)and heparin (Giangrande, 2003) **.**

Procedure:

Activated partial thromboplastin reagent was brought to 37c and was well mixed , coagulometer was adjusted at 37c and cuvettes with magnetic stirrer inside werekept in holes at 37c , 100 ml of plasmas placed in cuvettes , 100ml of APTT reagent which was pre-warmed at 37c was added to plasma , mixed well and incubate for 3 minutes , 100ml of Cacl2 which was pre-formed at 37c was added and simultaneously the display started to measure the time , then the time of clot formation was observed directly from display.

2.11.3 I Chroma HBA1C:

Intended use:

Is a fluorescence Immunoassay (FIA) for the quantitative determination of HBA1C in human whole blood. It is useful as an aid in management

monitoring of the long-term glycemic status in patients with diabetes mellitus. For in vitro diagnostic only.

Introduction:

Glycated protein is formed post-transnationally through the slow, non-enzymatic reaction between glucose and amino groups on proteins. HbA1C is clinically useful index of mean glycaemia during the preceding 120 days, the average life of span of erythrocytes. Carefully controlled studies have documented as close relationship between the concentrations of HbA1C and mean glycaemia. HbA1C is considered as a more reliable parameter in monitoring glycaemia over the glycemic reading with the conventional glucometer.

Principle:

The test uses a sandwich immune detection method: the detector antibody in buffer binds to antigen in sample, forming antigen-antibody complexes, and migrates on to nitro cellulose matrix to be capture by other immobilized antibody in test strip. The more antigen in sample forms the more antigen-antibody complexes and leads to stronger intensity 0f fluorescence signal on detector antibody. Instrument for I Chroma tests displays the content of glycated hemoglobin in terms of percent of the total hemoglobin in blood.

Sample collection and processing:

-The sample is human whole blood (EDTA), it is recommended to test the sample within 12 hours after collection.

-Samples may be stored for up to a week at 2-8 c prior to being tested .

-If testing will be delayed more than a week, samples should be frozen at -70c or below. Samples stored frozen at -70c for 3 months showed no performance difference.

-Once the sample was frozen, it should be used one time only for test, because repeated freezing and thawing can result in the change the test values.

Procedure:

1-Draw 100ml of hemolysis buffer and transfer it into detection buffer.

2-Draw 5ml of fingertip blood or tube blood using 5ml capillary tube in to the detection buffer tube.

3-Close the lid of the detection buffer tube and mix the sample thoroughly by shaking it about 15 times.

4-Take out the cartridge half from i-chamber solt.

5-Pipette out 75ml of the sample mixture and load it into a sample well in the test cartridge.

6-Wait till the sample mixture flow appears in the windows (about 10 seconds).

7- Insert the cartridge into the I-chroma solt.

8-Leave the sample in i-chamber for 12 minutes before removing.

9-Press select button on the instrument for ichroma tests to start the scanning process.

10-Instrument for ichroma tests will start scanning the sample loaded cartridge immediately.

11-Read the test result on the display screen of the instrument for ichroma tests.

2.11.4. Platelet count:

A platelet count is used to detect the number of platelets in the blood. The test is included in a Complete Blood Count (CBC), a panel of tests often performed as part of a general health examination. Platelets are tiny fragments of cells that essential for normal blood clotting. A platelet count may be used to screen for or diagnose various diseases and conditions that can cause problems with clot formation. A platelet count may be performed in conjunction with one or more platelet function tests, which assess the function of platelets, and or tests that evaluate coagulation such as PT and PTT (WHO, 2017).

Chapter Three

Results

Chapter Three

Results

Population characteristic:

In this study 60 subjects were participated. 30(12 males and 18 females) of these were patients with type II diabetes patients and 30 were healthy control matched with age. The patients were divided according to HbA1C value in to diabetic controlling (13 patients) and uncontrolling (17 patients) groups. The age of subjects ranged from 30-80 years with a mean age of 50 years. All subjects were residing in Khartoum state, blood samples of patients were collected from Best Care Hospital.

Table (3-1): Means and STD of tested parameters in patients and controls

Parameters	Mean ± STD	P-value
PT	P 13.099 ± 1.219 C 13.113 ± 1.132	0.893
INR	P 1.113 ± 0.283 C 1.114 ± 0.312	0.971
APTT	P 33.853 ± 4.849 C 34.480 ± 4.671	0.321
Platelets count	P 297.53 ± 98.918 C 292.60 ± 97.293	0.703

P = patients (30 patients)

C = control (30 controls).

Figure (3-1) Relation between PT in seconds and age in years of patients. A no correlation between PT of the increase age of patients.

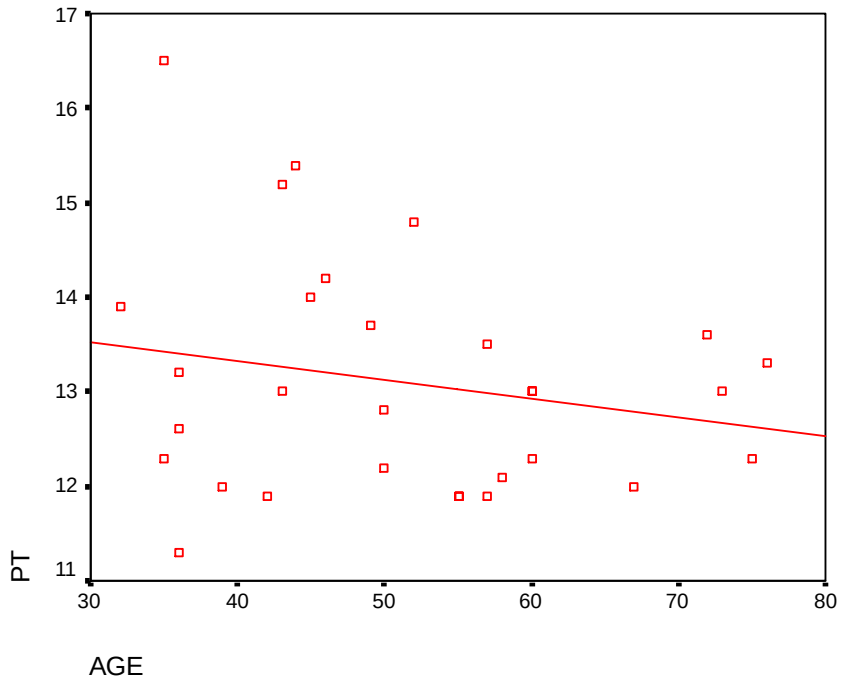


Figure (3-2) Relation between INR and age in years of patients. A no correlation between INR of the increase age of patients.

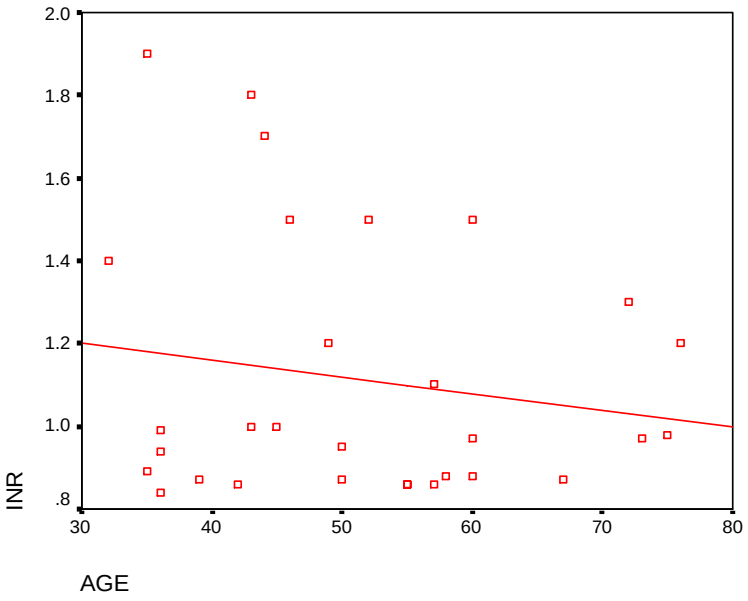


Figure (3-3) Relation between APTT in seconds and age in years of patients. A no correlation between APTT of the increase age of patient.

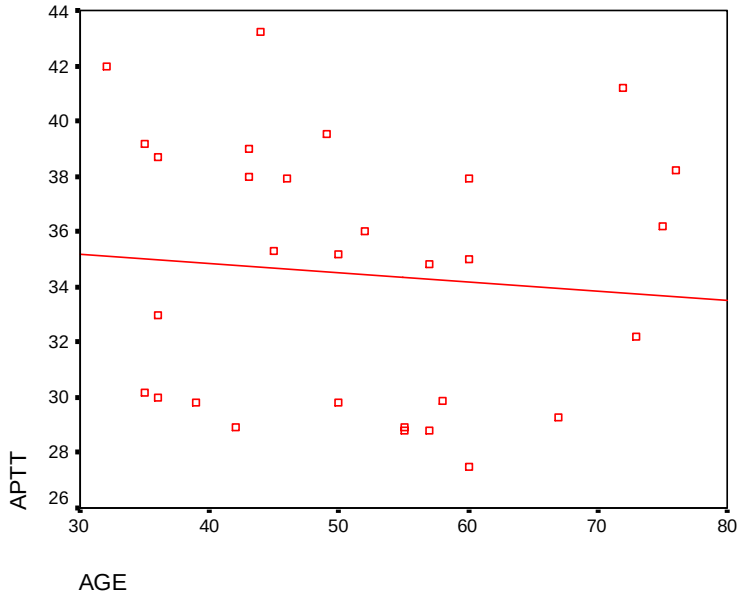


Figure (3-4) Relation between platelets count and age in years of patients. A no correlation between platelets count of the increase age of patient.

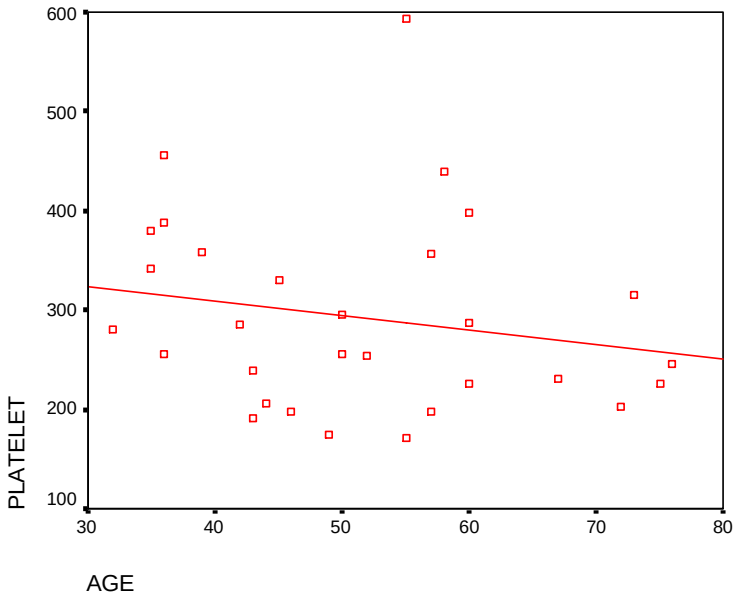


Table (3-2): Relation of parameters with gender of patients

Parameters	Mean ± STD	P-value
PT	M 13.2 ± 1.0 F 13.0 ± 1.3	0.703
INR	M 1.18 ± 0.29 F 1.00 ± 0.32	0.312
APTT	M 35.99 ± 5.19 F 33.47 ± 4.12	0.151
Platelets count	M 232 ± 57.0 F 333 ± 98.6	0.003

M = Males (12)

F = Females (18)

Table (3-3): Association between duration of the diabetes typeII and tested parameters as analyzed by ANOVA

Parameters	NO	Mean ± STD	P-value
PT	23	(1-10) years 13.13 ± 1.36	0.769
	7	More than 10 years 12.97 ± 0.61	
INR	23	(1-10) years 1.12 ± 0.34	0.568
	7	More than 10 years 1.07 ± 0.15	
APTT	23	(1-10) years 33.70 ± 4.71	0.100
	7	More than 10 years 37.02 ± 3.73	
Platelets count	23	(1-10) years 306 ± 97	0.166
	7	More than 10 years 247 ± 89	

Table (3-4): Parameters among diabetic controlling and uncontrolling groups

Parameters	NO	Mean ± STD	P-value
PT	13	Less than 8% 12.91 ± 1.14	0.834
	17	More than8% 13.22 ± 1.29	
INR	13	Less than 8% 1.05 ± 0.25	0.918
	17	More than 8% 1.15 ± 0.34	
APTT	13	Less than 8% 34.37±5.13	0.380
	17	More than 8% 34.55 ± 4.44	
Platelets count	13	Less than 8%275 ± 73	0.419
	17	More than 8% 307 ± 112	

Less than 8% = Controlling diabetes (13 patients)

More than 8% = Uncontrolling diabetes (17 patients)

Chapter Four

Discussion, Conclusion, Recommendations

Chapter Four

4.1 Discussion

The investigation of PT and INR showed no variation between patients (13.0933±1.21993) (1.1147±0.31291) and healthy individual (13.1333±1.05841) (1.0453±0.20549). The Estimation of APTT among diabetic patients showed an increased in the mean of APTT in patients (34.80±4.6714) compared to healthy individuals (33.210±5.0399) with statistically non-significant relation. The mean of platelets count of diabetic patient was decreased (292.60±97.293) when compared to healthy individuals (298.83±98.852) but this variation was not statistically significant relation.The results disagree with the study conducted in southeast Asian,by Acang and Jalil,1993,who found in significantly short PT, APTT in diabetic Patients especially who suffered from diabetes for a long time and followed by chronic complication (Acane and Jalil,1993).sec). In other hand the results of this study agreed with the study conducted in SUST by Abdeen and Ayman , in that study insignificant prolongation of PT and APTT were observed in patients compared to control but the difference within the normal range. According to disease duration , PT of patients was within normal range , APTT was prolonged progressively with increase period of disease on set (Abdeen and Ayman,2014).Estimation of platelets count among diabetic patients showed mild decreased level (292.60 ± 97.29) compared with normal healthy individuals (298.83 ± 98.85), The results of this study disagreed with the study conducted in SUST by Hassan, when she reported that there was significant increased in platelets count in the diabetic patient when compared with non diabetic control(Hassan, 2011).

Estimation of PT and APTT among diabetic male and female showed that increased PT and APTT in diabetic males (13.2 ± 1.06 , 35.99 ± 5.19) compared to diabetic females (13.02 ± 1.33 , 33.47 ± 4.12). But platelets count decreased in males (232 ± 57.01) compared to females (333 ± 98.61) .This results agreed with the study conducted in SUST by Hassan , as she reported a significant shortened APTT in diabetic male , significant prolongation of PT in diabetic male and insignificant increased of Platelets count in diabetic male when compared to diabetic female(Hassan,2011).

All parameters have no correlation with age. When show parameters among controlled diabetes were compared with non controlled diabetes mellitus, the results show that the mean of PT, INR and APTT in uncontrolled diabetes (13.3 ± 1.2, 0.985 ± 0.084, 34.97±5.15) are more increased than the mean of controlled diabetes (12.8 ±1.1, 0.94 ± 0.06, 33.97 ± 5.15), but the mean of platelets count of uncontrolled diabetes is decreased (269.6 ±73.8) than mean of controlled diabetes (312.06 ±113.1).The results also show that all parameters increased with duration of the disease but no data were available to compare the observation with similar study.

4.2 Conclusion:

1. PT among study group showed no variation when compared with mean of normal control this variation no clinical significance. APTT showed mild increased in diabetic when compared with normal but this increase not clinical significant. Platelet count showed mild decreased when compared with normal control, but this decreased have no clinical significant.

2. All parameters (PT, INR, APTT and Platelet count) increased in uncontrolled diabetic patients but is not clinical significant. The parameters PT, INR and PTT are increased with duration, this increased not clinical significant, but the platelets count decreased with duration and have clinical significant.

3. All parameter decreased with age. No variation of PT between male and female, but APTT and INR the male is more affected than female, and Platelet count and HBA1C the female is more affected than male .However in this study association between diabetes and hemostatic mechanism has been observed.

4.3 Recommendation:

1. The study provides an experimental evidence indicated that regular estimation of coagulation profile and platelet count for diabetes mellitus.
2. Diet control among diabetic patient is necessary as to avoid complication resulting from hyperglycemia.
3. Health program should be implemented to explain the complications associated with diabetes mellitus, and how to avoid these complications.
4. Further study with large sample size should be done to compare these parameters in diabetic patient and healthy individuals.

References

- Achneck HE, Sileshi B, Parikh A, Milano CA, Welsby IJ, Lawson JH. (2010) Pathophysiology of bleeding and clotting in the cardiac surgery patient: from vascular endothelium to circulatory assist device surface. Circulation.
- Alvin CP. (2001) Diabetes. In : principles of internal medicine , Braunwald E, Fauci causes : AS, Kasper DL, Havser SL, Longo DL , Jambon JL: harrinson(eds), 15 edition, USA, New york, MCgraw. Hill, page: 2109-2138.
- Andrews RK, Berndt MC (2004).Platelet physiology and thrombosis.
- Ashby B, Daniel JL, Smith JB (1990). Mechanisms of platelet activation and inhibition.HematolOncolClin North Am.; 4:1–26.
- Barash PG, Cullen BF, Stoelting RK (2006). 5th ed. Philadelphia: Lippincott Williams and Wilkins;. Clinical Anesthesia; pp. 224–6.
- Bartoli E, Fra GP, Carnevale Schianca GP (2011). "The oral glucose tolerance test (OGTT) revisited". European Journal of Internal Medicine. 22 (1): 8–12.
- Bombeli T, SpahnDR (2004). Updates in perioperative coagulation: Physiology and management of thromboembolism and hemorrhage. Br J Anaesth.; 93:275–87.
- American Association for clinical chemistry C 2001-2017.
- Cesarman-Maus G, Hajjar KA (2005). Molecular mechanisms of fibrinolysis. Br J Haematol.;129:307–21.
- Charles FA, (1998) endocrinology, in: mayo internal medicine board review. Udaya B.S.P, millhouse O.E, leann MS (eds), 3rd edition Raven, Lippincott: 187-278.

- Cines DB, Pollak ES, Buck CA, Loscalzo J, Zimmerman GA, McEver RP, et al (1998). Endothelial cells in physiology and in the pathophysiology of vascular disorders. Blood; 91:3527–61.
- Closse C, Seigneur M, Renard M, Pruvost A, Dumain P, Belloc F, et al (1997). Influence of hypoxia and hypoxia-reoxygenation on endothelial P-selectin expression. Thromb Res.;85:159–64.
- Colvin BT (2004). Physiology of haemostasis.Vox Sang.;87(Suppl 1):43–6.
- Cooke DW, Plotnick L (November 2008). "Type 1 diabetes mellitus in pediatrics". Pediatr Rev. 29 (11): 374–84; quiz 385.
- Corral J, González-Conejero R, Hernández-Espinosa D, Vicente V (2007). Protein Z/Z-dependent protease inhibitor (PZ/ZPI) anticoagulant system and thrombosis. Br J Haematol; 137:99–108.
- Cukierman, T (8 Nov 2005). "Cognitive decline and dementia in diabetes—systematic overview of prospective observational studies". Springer-Verlag.
- "Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications" (PDF). World Health Organization. 1999.
- "Diabetes Care" January 2010". American Diabetes Association.
- "Diabetes Programme". World Health Organization. Archived from the original on 26 April 2014.
- "Insulin Basics". American Diabetes Association. Archived from the original on 14 February 2014.
- Dahm AE, Sandset PM, Rosendaal FR (2008). The association between protein S levels and anticoagulant activity of tissue factor pathway inhibitor type 1. J Thromb Haemost.;6:393–5.

- Definition and diagnosis of diabetes mellitus and intermediate hyperglycemia (2006): report of a WHO/IDF consultation (PDF). World Health Organization. 2006.
- Doolittle RF, Spraggon G, Everse SJ (1998). Three-dimensional structural studies on fragments of fibrinogen and fibrin.CurrOpinStruct Biol.;8:792–8.
- Editor, Leonid Poretsky, (2009). Principles of diabetes mellitus (2nd ed.). New York: Springer.
- Ejiofor JA (2013). Anticlotting mechanisms I: Physiology and pathology. ContinEducAnesthCrit Care Pain; 13:87–92.
- Giangrande PL (2003). "Six characters in search of an author: The history of nomenclature of coagulation factors" *Br.J.Haematol* . **121**(5): 703-12.
- Hall JE (2010). Guyton and Hall Textbook of Medical Physiology: Enhanced E-Book. 11th ed. Philadelphia: Elsevier Health Sciences;.Hemostasis and blood coagulation; pp. 457–9.
- Heemskerk JW, Bevers EM, Lindhout T (2002). Platelet activation and blood coagulation.Thromb Haemost.;88:186–93.
- htt://europempc.org/abstract/med/7886593.
- htt://repository-sustech.edu/handle/123456789/16813.
- htt://repository-sustech.edu/handle/123456789/1979.
- Kamath S, Lip GY (2003). Fibrinogen: Biochemistry, epidemiology and determinants. QJM.;96:711–29.
- Kim E. Barrett ... (2012). Ganong's review of medical physiology (24th ed.). New York: McGraw-Hill Medical.
- Kumar V, Abbas AK, Fausto N, Aster JC (2010). Robbins and Cotran Pathologic Basis of Disease. 8th ed. Philadelphia, PA: Saunders

Elsevier;. Hemodynamic disorders, thromboembolic disease and shock; pp. 118–20.

- Lasne D, Jude B, Susen S (2006). From normal to pathological hemostasis. Can J Anesth; 53:S2–11.
- Mackman N, Tilley RE, Key NS (2007). Role of the extrinsic pathway of blood coagulation in hemostasis and thrombosis.ArteriosclerThrombVasc Biol.; 27:1687–93.
- Manly DA, Boles J, Mackman N (2011). Role of tissue factor in venous thrombosis.Annu Rev Physiol.; 73:515–25.
- McFarlane IA (1997). Endocrine disease and diabetes mellitus. In Williams JC, (Ed), textbook of diabetes (2nd Edition) oxford: Blackwell, pp 640-660.
- Meybohm P, Zacharowski K, Weber CF (2013). Point-of-care coagulation management in intensive care medicine.Crit Care. 17:218.
- Monroe DM, 3rd, Hoffman M, Roberts HR (2010). Williams Hematology. 8th ed. New York NY: McGraw-Hill Professional Publishing; .Molecular biology and biochemistry of the coagulation factors and pathways of hemostasis; pp. 614–6.
- Myers DD, Hawley AE, Farris DM, Wrobleski SK, Thanaporn P, Schaub RG, et al (2003). P-selectin and leukocyte microparticles are associated with venous thrombogenesis. J Vasc Surg.; 38:1075–89.
- Opal SM, Kessler CM, Roemisch J, Knaub S (2002). Antithrombin, heparin, and heparan sulfate. Crit Care Med.;30:S325–31.
- Owens AP (2010), 3rd, Mackman N. Tissue factor and thrombosis: The clot starts here. ThrombHaemost. 104:432–9.
- Previtali E, Bucciarelli P, Passamonti SM, Martinelli I (2011). Risk factors for venous and arterial thrombosis. Blood Transfus.; 9:120–38.

- Price GC, Thompson SA, Kam PC (2004). Tissue factor and tissue factor pathway inhibitor. Anaesthesia; 59:483–92.
- Rigby AC, Grant MA (2004). Protein S: A conduit between anticoagulation and inflammation. Crit Care Med.;32:S336–41.
- Robert K. Murray ... (2012). Harper's illustrated biochemistry (29th ed.). New York: McGraw-Hill Medical.
- Rockefeller, J. D. (2015). Diabetes: Symptoms, Causes, Treatment and Prevention. J.D. Rockefeller.
- Sadler JE (1998). Biochemistry and genetics of von Willebrand factor. Annu Rev Biochem.;67:395–424.
- Santaguida PL, Balion C, Hunt D, Morrison K, Gerstein H, Raina P, Booker L, Yazdi H (2008). "Diagnosis, Prognosis, and Treatment of Impaired Glucose Tolerance and Impaired Fasting Glucose". Summary of Evidence Report/Technology Assessment, No. 128. Agency for Healthcare Research and Quality.
- Saydah SH, Miret M, Sung J, Varas C, Gause D, Brancati FL (August 2001). "Postchallenge hyperglycemia and mortality in a national sample of U.S. adults". Diabetes Care. 24 (8): 1397–402.
- Selvin E, Steffes MW, Zhu H, Matsushita K, Wagenknecht L, Pankow J, Coresh J, Brancati FL (2010). "Glycated hemoglobin, diabetes, and cardiovascular risk in nondiabetic adults". N. Engl. J. Med. 362 (9): 800–11.
- Shapiross (2003). "Treating thrombosis in the 21 st century". *N.Engl.J. Med*. **349** (18): 1762-4.
- Shoback, edited by David G. Gardner, Dolores (2011). "Chapter 17". Greenspan's basic & clinical endocrinology (9th ed.). New York: McGraw-Hill Medical.

- Shoback, edited by David G. Gardner, Dolores (2011). Greenspan's basic & clinical endocrinology (9th ed.). New York: McGraw-Hill Medical.
- Thornton P, Douglas J. Coagulation in pregnancy (2010). Best Pract Res ClinObstet Gynaecol.; 24:339–52.
- Triplett DA (2000). Coagulation and bleeding disorders: Review and update. Clin Chem.;46:1260–9.
- Tuomi T, Santoro N, Caprio S, Cai M, Weng J, Groop L (2014). "The many faces of diabetes: a disease with increasing heterogeneity". Lancet. 383 (9922): 1084–94.
- WebMD medical reference Reviewed by Michael Dansinger (2017)."Type 1 diabetes mellitus in pediatrics". Pediatr Rev. 29 (11): 374–84; quiz 385. doi:10.1542/pir.29-11-374.
- Yang, Y; Hu, X; Zhang, Q; Zou, R (November 2016). "Diabetes mellitus and risk of falls in older adults: a systematic review and meta-analysis". Age and ageing. 45 (6): 761–767.

Appendices

Appendix (1): Questionnaire

Sudan University of Science and Technology

College of Graduate studies

Hematology Department

Name :…….

Age : ……

Gender :……

Sample No ( ) :……

Drug and Treatment : ……..

Duration :…….

Diabetic : yes ( ) No ( )

Control the Treatment : yes ( ) No ( )

Aspirin Taken: yes ( ) No ( )

Treatment for Hypercoagulability :

yes ( ) No ( )

Date: ……. Sign: ……

Appendix (2): materials supplied:

Features of coagulometer:

- One channel open system with all kind of quality reagents.
- Photometric system for the detection of stable polymer fibrin.
- Incorporated magnetic stirrer system in order to mix well the reagent and plasma.
- Temporary controlled block for storing 10 reaction cuvettes and a reagent vial.Performs PT, APTT, etc.

Prothrombin time (PT) automated using biobas 10 coagulometer.

Reagent:

1. Pooled normal control plasma.
2. Prothrombin time kit.

Equipment:

1. Coagulometer biobas with its cuvette.
2. Magnetic (ball).
3. Automatic pipette.
4. Yellow and blue tips.

Requirements:

Thromboplastin reagent which contains tissue factor and phospholipid obtained from human or rabbet brain or recompenant DNA, automatic pipette, ppp, curettes, magnetic stirrers and coagulometer.

Normal Values:

The normal range of PT is between 11-16 seconds (Shapiross, 2003).

Interpretation:

The common cause of prolonged PT is:Administration of oral anticoagulant drugs (vit k antagonists), liver disease, vit k deficiency, DIC, or prothrombin deficiency or defect.

Activated partial thromboplastin time (APTT), automated using biobas

Reagent:

1. Polled normal control plasma.
2. Activated partial thromboplastin time kit.

Equipment:

1. Coagulometer biobas and it cuvette.
2. Magnetic ball.
3. Automatic pipette.
4. Yellow and blue tips.

Requirements:

PPP, kaolin-cephalin reagent, Cacl2, 0.025 %water bath, automatic pipette , coulometer , cuvettes and magnetic stirrers .

Normal range:

The normal range is typically within 26-40 seconds (Shapiross, 2003).

Interpretation:

The common cause of prolonged APTT is : DIC , liver disease , massive transfusion with plasma deplete , administration of or contamination with heparin or other anticoagulants red blood cell, a circulating anticoagulant (inhibitor)and deficiency of coagulation factor other than factor (VII) .

Materials supplied in i-chroma tests:

Components of ichroma HbA1C:

Cartidge Box:

-Cartidges.

- ID chip.

- Instruction for use.

Detection buffer Box:

- Detection buffer Box

- Hemolysis buffer vial (3ml).

Sample collection and processing:

-The sample is human whole blood (EDTA), it is recommended to test the sample within 12 hours after collection.

-Samples may be stored for up to a week at 2-8 c prior to being tested .

-If testing will be delayed more than a week, samples should be frozen at -70c or below. Samples stored frozen at -70c for 3 months showed no performance difference.

-Once the sample was frozen, it should be used one time only for test, because repeated freezing and thawing can result in the change the test values.

Interpretation of the result:

-Instrument for ichroma tests calculates the test result automatically and displays HbA1C concentration of the test sample in terms of %(NGSP), mmol/mol (IFCC), mg/dl (eAG).

The cut-off (reference range) :

-NGSP (%): 4.5-6.5%.

-IFCC (mmol/mol) : 26-48 mmol/mol .

Working range :

-NGSP (%): 4-15%.

-IFCC (mmol/mol): 20.2-140.4mmol/mol.

- eAG (mg/dl): 68.1-383.8mg/dl.

Appendix (4): Results

NO	Sample	Sex	Plts	HBA1C	Age	PT	PTT	INR	Control	Duration
1	Case	M	357	6.1	57	13.5	34.8	1.10	Yes	3
2	Case	F	256	13.0	50	12.8	35.2	0.95	NO	12
3	Case	F	255	7.2	36	11.3	30.0	0.84	Yes	7
4	Case	M	202	6.0	72	13.6	41.2	1.30	Yes	15
5	Case	M	226	6.9	75	12.3	36.2	0.98	Yes	15
6	Case	F	191	9.6	43	13.0	39.0	1.00	NO	15
7	Case	F	342	10.9	35	16.5	39.2	1.90	NO	8
8	Case	F	439	7.0	58	12.1	29.9	0.88	Yes	30
9	Case	M	198	8.1	57	11.9	28.8	0.86	NO	8
10	Case	F	379	10.4	35	12.3	30.2	0.89	NO	1
11	Case	F	594	10.9	55	11.9	28.8	0.86	NO	3
12	Case	F	359	10.9	39	12.0	29.8	0.87	NO	3
13	Case	F	330	7.7	45	14.0	35.3	1.00	Yes	3
14	Case	F	245	8.5	76	13.3	38.2	1.20	NO	13
15	Case	M	225	11.5	60	13.0	37.9	1.50	NO	6
16	Case	F	239	11.1	43	15.2	38.0	1.80	NO	5
17	Case	M	280	7.2	32	13.9	42.0	1.40	Yes	4
18	Case	M	231	7.9	67	12.0	29.3	0.87	Yes	10
19	Case	M	172	8.0	55	11.9	28.9	0.86	NO	1
20	Case	F	456	11.0	36	12.6	33.0	0.94	NO	3
21	Case	F	254	9.0	52	14.8	36.0	1.50	NO	7

22	Case	F	398	9.7	60	12.3	27.5	0.88	NO	1
23	Case	M	315	7.9	73	13.0	32.2	0.97	Yes	4
24	Case	F	287	5.9	60	13.0	35.0	0.97	Yes	1
25	Case	F	286	5.6	42	11.9	28.9	0.86	Yes	1
26	Case	F	296	8.2	50	12.2	29.8	0.87	NO	5
27	Case	M	198	9.5	46	14.2	37.9	1.50	NO	7
28	Case	F	388	9.9	36	13.2	38.7	0.99	NO	3
29	Case	M	206	5.9	44	15.4	43.2	1.70	Yes	8
30	Case	M	174	9.7	49	13.7	39.5	1.20	NO	11
31	Control	F	333	5.4	36	12.1	30.2	0.88		
32	Control	F	440	5.2	40	13.9	39.1	1.30		
33	Control	F	380	4.5	33	12.5	28.9	1.20		
34	Control	M	350	6.8	46	12.9	32.3	0.99		
35	Control	M	284	5.3	55	14.9	41.2	1.50		
36	Control	M	282	5.3	43	13.9	39.0	1.20		
37	Control	F	450	6.3	32	12.0	28.0	0.85		
38	Control	M	150	5.1	50	11.9	27.9	0.86		
39	Control	F	308	4.9	36	14.5	43.9	1.40		
40	Control	F	198	5.3	35	12.5	27.7	0.95		
41	Control	F	221	5.2	30	12.4	35.0	0.91		
42	Control	F	203	5.0	42	11.9	28.9	0.96		
43	Control	M	199	5.3	33	12.8	31.5	0.94		
44	Control	F	148	4.8	55	13.9	40.2	1.20		

45	Control	F	232	5.1	49	13.1	29.8	0.97		
46	Control	F	254	4.9	31	13.0	29.4	1.50		
47	Control	F	301	5.5	65	12.1	30.3	0.88		
48	Control	F	324	4.9	32	12.1	31.1	0.88		
49	Control	M	193	7.0	39	12.2	31.9	0.90		
50	Control	M	348	5.9	44	15.9	35.9	1.20		
51	Control	F	413	5.7	50	13.0	33.2	0.97		
52	Control	F	255	5.4	65	12.9	33.5	0.94		
53	Control	F	326	4.8	57	14.0	29.4	1.50		
54	Control	M	316	4.5	38	13.1	29.8	0.97		
55	Control	M	244	4.4	42	12.8	30.0	0.97		
56	Control	M	558	4.9	40	12.2	29.8	0.87		
57	Control	M	530	6.1	59	15.4	43.2	1.70		
58	Control	F	254	6.5	63	13.0	35.0	1.50		
59	Control	F	239	6.6	34	12.9	28.7	0.97		
60	Control	M	341	5.2	45	14.2	42.0	1.50		

Appendix (5): Procedure for determination of HbA1C

BIO TECHNOLOGY boditech

Boditech HbA1c Calibrator REF CFPO-108
5 µL Capillary tube REF CFPO-19

MPLE COLLECTION AND PROCESSING

mple type for **ichroma™ HbA1c** is human whole blood.
t is recommended to test the sample within 12 hours after collection.
Samples may be stored for up to a week at 2-8 °C prior to being tested.
f testing will be delayed more than a week, samples should be frozen at -70 °C or below. Samples stored frozen at -70 °C or below for 3 months showed no performance difference.
Once the sample was frozen, it should be used one time only for test, because repeated freezing and thawing can result in the change of test values.

T SETUP

Check the components of the **ichroma™ HbA1c** as described below. : Cartridge, ID chip, instruction for use, detection buffer tube and hemolysis buffer vial.
Ensure that the lot number of the test cartridge matches that of ID chip, detection buffer as well as hemolysis buffer.
Keep the sealed cartridge (if stored in refrigerator), detection buffer and hemolysis buffer at room temperature for at least 30 minutes just prior to the test. Place the cartridge on a clean, dust-free and flat surface.
Turn on the instrument for ichroma™ test.
Insert the ID chip into the "ID chip port".
Press the 'Select' button on the instrument for ichroma™ test.
(Please refer to the 'Instrument for ichroma™ tests Operation Manual' for complete information and operating instructions.)
Insert a cartridge into i-Chamber slot.

T PROCEDURE

Draw 100 µL of hemolysis buffer and transfer it into detection buffer tube.
Draw 5 µL of fingertip blood or tube blood using 5 µL capillary tube and put the capillary tube into the detection buffer tube.
Close the lid of the detection buffer tube and mix the sample thoroughly by shaking it about 15 times.
Take out the cartridge half form i-Chamber slot.
Pipette out 75 µL of the sample mixture and load it into a sample well in the test cartridge.
Wait till the sample mixture flow appears in the windows. (about 10 seconds)
Insert the cartridge into i-Chamber slot.
Leave the cartridge in i-Chamber for 12 minutes before removing.
⚠ *Scan the sample-loaded cartridge immediately when the incubation time is over. If not, it will cause inexact test result.*
To scan the sample-loaded cartridge, insert it into the cartridge holder of the instrument for ichroma™ tests. Ensure proper orientation of the cartridge before pushing it all the way inside the cartridge holder. An arrow has been marked on the cartridge especially for this purpose.
Press 'Select' button on the instrument for ichroma™ tests to start the scanning process.
Instrument for ichroma™ tests will start scanning the sample-loaded cartridge immediately.
Read the test result on the display screen of the instrument for ichroma™ tests.

ERPRETATION OF TEST RESULT

Instrument for ichroma™ tests calculates the test result automatically and displays HbA1c concentration of the test sample in terms of % (NGSP), mmol/mol (IFCC), mg/dL (eAG).
The cut-off (reference range)

NGSP (%): 4.5-6.5 %

IFCC (mmol/mol): 26-48 mmol/mol

Working range

NGSP (%): 4-15%

IFCC (mmol/mol): 20.2-140.4 mmol/mol

eAG (mg/dL): 68.1-383.8 mg/dL

LITY CONTROL

Quality control tests are a part of the good testing practice to confirm the expected results and validity of the assay and should be performed at regular intervals.
The control tests should be performed immediately after opening a new test lot to ensure the test performance is not altered.

- Quality control tests should also be performed whenever there is any question concerning the validity of the test results.
- Control materials are not provided with **ichroma™ HbA1c**. For more information regarding obtaining the control materials, contact Boditech Med Inc.'s Sales Division for assistance. (Please refer to the instruction for use of control material.)

PERFORMANCE CHARACTERISTICS

- **Analytical Specificity**

Cross-reactivity

There was no significant cross-reactivity from these materials with the **ichroma™ HbA1c** test measurements.

Cross-reactivity material	Standard material conc.		
	5.2 %	6.5 %	10.5 %
	Recovery (%)		
HbA0 (20 mg/mL)	99.9	96.1	99.0
HbA1a,A1b (20 mg/mL)	100.9	96.8	101.0
Acetylated hemoglobin (100 mg/mL)	101.0	98.4	99.7
Carbamylated hemoglobin (100 mg/mL)	100.5	97.8	100.0
Glycated h-Albumin (100 mg/mL)	100.3	97.4	100.6
HbA1d (100 mg/mL)	100.9	97.0	100.3
Acetylaldehyde hemoglobin (100 mg/mL)	100.8	95.6	99.1

- Interference

There was no significant interference from these materials with the **ichroma™ HbA1c** test measurements.

Interference material	Standard material conc.		
	5.2 %	6.5 %	10.5 %
	Recovery (%)		
Non-interference	101.0	96.2	98.7
Acetaminophen (20 mg/dL)	100.4	97.8	100.9
L-ascorbic acid (500 mg/dL)	101.0	97.8	99.8
Bilirubin (2 g/dL)	100.8	97.8	100.4
D-glucose (1,000 mg/dL)	100.9	97.6	99.8
Intralipid (800 U/L)	100.8	96.2	100.6
Triglyceride (327 M)	100.9	96.1	99.6
Urea (10 g/dL)	100.1	98.1	99.7

- **Precision**

The intra-assay precision was calculated by one evaluator, who tested different concentration of control standard five times each with three different lots of **ichroma™ HbA1c.**

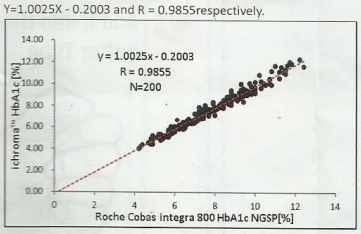
HbA1c (%)	Lot 1	Lot 2	Lot 3	AVG	SD	CV (%)	Accuracy (%)
5.2	5.28	5.18	5.24	5.23	0.12	2.36	100.6
6.5	6.46	6.48	6.34	6.43	0.13	1.99	98.9
10.5	10.4	10.56	10.58	10.51	0.19	1.83	100.1

The inter-assay precision was confirmed by 3 different evaluators with 3 different lots, testing five times each different concentration.

HbA1c (%)	Between-person			Between-lot		
	AVG	SD	CV (%)	AVG	SD	CV (%)
5.2	5.19	0.03	0.61	5.23	0.05	0.96
6.5	6.51	0.02	0.36	6.43	0.07	1.12
10.5	10.50	0.01	0.10	10.51	0.10	0.92

- **Comparability:**

HbA1c concentrations of 200 clinical samples were quantified independently with **ichroma™ HbA1c** and Roche Cobas integra800 as per prescribed test procedures. Test results were compared and their comparability was investigated with linear regression and coefficient of correlation (R). Linear regression and coefficient of correlation between the two tests were Y=1.0025X - 0.2003 and R = 0.9855respectively.



REFERENCES

1. Goldstein DE,Little RR, Lorenz RA, Malone JI, Nathan D, Peterson CM. Tests of glycemia in diabetes. Diabetes Care 1995; 18:896-909.
2. Bunn HF. Nonenzymatic glycosylation of protein: relevance to diabetes. Am J Med 1981; 70:325-30.
3. Jovanovic L, Peterson CM. The clinical utility of glycosylated hemoglobin. Am J Med 1981; 70:331-8.
4. Nathan DM, Singer DE, Hurxthal K, Goodson JD. The clinical information value of the glycosylated hemoglobin assay. N Engl J Med 1984; 310:341-6.
5. Goldstein DE, Little RR, Wiedmeyer HM, England JD, McKenzie EM. Glycated hemoglobin: methodologies and clinical applications. Clin Chem 1986; 32:B64-70.
6. Goldstein DE, Little RR, England JD, Wiedemeyer H-M, McKenzie E. Methods of glycosylated hemoglobins: high performance liquid chromatography and thiobarbituric acid colorimetric methods. In: Clarke WL, Larner J, Pohl SL, eds. Methods in diabetes research, Vol. 2. New York: John Wiley, 1986:475-504.
7. Tahara Y, Shima K. The response of GHb to stepwise plasma glucose change over time in diabetic patients. Diabetes Care 1993; 16:1313-4.
8. Svendsen PA, Lauritzen T, Soegaard U, Nerup J. Glycosylated haemoglobin and steady-state mean blood glucose concentration in type 1 (insulin-dependent) diabetes. Diabetologia 1982; 23:403-5.
9. Cefalu WT, Wang ZQ, Bell-Farrow A, Kiger FD, Izlar C. Glycohemoglobin measured by automated affinity HPLC correlates with both short-term and long-term antecedent glycemia. Clin Chem 1994; 40:1317-21.
10. Singer DE, Coley CM, Samet JH, Nathan DM. Tests of glycemia in diabetes mellitus. Their use in establishing a diagnosis and in treatment. Ann Intern Med 1989; 110:125-37.
11. Molnar GD. Clinical evaluation of metabolic control in diabetes. Diabetes 1978; 27:216-25.
12. UK Prospective Diabetes Study. Reduction in HbA1c with basal insulin supplement, sulfonylurea or biguanide therapy in maturity-onset diabetes. Diabetes 1985; 34:793-8.
13. Baker JR, Johnson RN, Scott DJ. Serum fructosamine concentrations in patients with type II (non-insulin-dependent) diabetes mellitus during changes in management. BMJ (Clin Res Ed) 1984; 288:1484-6.
14. Tahara Y, Shima K. Kinetics of HbA1c, glycated albumin, and fructosamine and analysis of their weight functions against preceding plasma glucose level. Diabetes Care 1995; 18:440-7.
15. Brooks DE, Devine DV, Harris PC, et al. RAMP(TM): A rapid, quantitative whole blood immunochromatographic platform for point of care testing. Clin Chem 1999; 45:1676-1678.

Note: Please refer to the table below to identify various symbols.

	Sufficient for <n> tests
	Read instruction for use
	Use by Date
LOT	Batch code
REF	Catalog number
	Caution
	Manufacturer
EC REP	Authorized representative of the European Community
IVD	In vitro diagnostic medical device
	Temperature limit
	Do not reuse
CE	This product fulfills the requirements of the Directive 98/79/EC on in vitro diagnostic medical devices

For technical assistance; please contact:
Boditech Med Inc.'s Technical Services
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E-mail: sales@boditech.co.kr

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CE

Document No. : INS-AA-EN (Rev. 13)
Revision date : July 20, 2016

Diabetes ichroma™ HbA1c

INTENDED USE

ichroma™ HbA1c is a fluorescence Immunoassay (FIA) for the quantitative determination of HbA1c(Hemoglobin A1c) in human whole blood. It is useful as an aid in management and monitoring of the long-term glycemic status in patients with diabetes mellitus.

For *in vitro* diagnostic use only.

INTRODUCTION

Glycated protein is formed post-translationally through the slow, nonenzymatic reaction between glucose and amino groups on proteins. HbA1c is a clinically useful index of mean glycemia during the preceding 120 days, the average life span of erythrocytes. Carefully controlled studies have documented a close relationship between the concentrations of HbA1c and mean glycemia. HbA1c is considered as a more reliable parameter in monitoring glycemia over the glycemic reading with the conventional glucometer.

PRINCIPLE

The test uses a sandwich immunodetection method; the detector antibody in buffer binds to antigen in sample, forming antigen-antibody complexes, and migrates onto nitrocellulose matrix to be captured by the other immobilized-antibody on test strip.

The more antigen in sample forms the more antigen-antibody complex and leads to stronger intensity of fluorescence signal on detector antibody. Instrument for ichroma™ tests displays the content of glycated hemoglobin in terms of percent of the total hemoglobin in blood.

COMPONENTS

ichroma™ HbA1c consists of 'Cartridges', 'Detection Buffer Tubes', 'Hemolysis Buffer Vial' and an 'ID chip'.

- The cartridge contains a test strip, the membrane which has anti human HbA1c at the test line, while rabbit IgG at the control line.
- Each cartridge is individually sealed in an aluminum foil pouch containing a desiccant. 25 sealed cartridges are packed in a box which also contains an ID chip.
- The detection buffer contains anti human HbA1c-fluorescence conjugate, anti rabbit IgG-fluorescence conjugate, bovine serum albumin (BSA) as a stabilizer and sodium azide in phosphate buffered saline (PBS) as a preservative.
- The detection buffer is pre-dispensed in a separate tube.
- The hemolysis Buffer contains nonionic detergent and sodium azide as preservative in PBS.
- 25 detection buffer tubes and hemolysis buffer vial are packaged in a box and further packed in a Styrofoam box with ice-pack for the shipment.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- Carefully follow the instructions and procedures described in this "Instruction for use".
- It is recommended to use fresh samples.
- It is possible to use frozen samples. Please refer to "SAMPLE COLLECTION AND PROCESSING".
- Do not expose ichroma™ HbA1c test kit to direct sunlight.
- Lot numbers of all the test components (cartridge, ID chip, detection buffer and hemolysis buffer) must match each other.
- Do not interchange the test components between different lots or use the test components after the expiration date, either of which might yield misleading of test result(s).
- Do not reuse. A detection buffer tube should be used for processing one sample only. So should a cartridge.
- The cartridge should remain sealed in its original pouch before use. Do not use the cartridge, if is damaged or already opened.
- Frozen sample should be thawed only once. For shipping, samples must be packed in accordance with the regulations. HbA1c Sample with severe hemolytic and hyperlipidemia cannot be used and should be recollected.
- Just before use, allow the cartridge, detection buffer and sample to be at room temperature for approximately 30 minutes.
- **ichroma™ HbA1c** as well as the instrument for ichroma™ tests should be used away from vibration and/or magnetic field. During normal usage, it can be noted that instrument for ichroma™ tests may produce minor vibration.
- Used detection buffer tubes, pipette tips and cartridges should be handled carefully and discarded by an appropriate method in accordance with relevant local regulations.
- The mixture of Detection Buffer and Hemolysis buffer must be used within 1 hour after mixing.
- An exposure to larger quantities of sodium azide may cause certain health issues like convulsions, low blood pressure and heart rate, loss of consciousness, lung injury and respiratory failure.
- **ichroma™ HbA1c** will provide accurate and reliable results subject to the following conditions.
  - Use **ichroma™ HbA1c** should be used only in conjunction with instrument for ichroma™ tests.
  - Any anticoagulants other than **EDTA, sodium heparin, sodium citrate** should be avoided.

STORAGE AND STABILITY

- The cartridge is stable for 20 months (while sealed in an aluminum foil pouch) if stored at 4-30 °C.
- The detection buffer pre-dispensed in a tube is stable for 20 months if stored at 2-8 °C.
- The hemolysis buffer dispensed in a vial is stable for 20 months if stored at 4-30 °C.
- After the cartridge pouch is opened, the test should be performed immediately.

LIMITATIONS OF THE TEST SYSTEM

- The test may yield false positive result(s) due to the cross-reactions and/or non-specific adhesion of certain sample components to the capture/detector antibodies.
- The test may yield false negative result. The non-responsiveness of the antigen to the antibodies is most common where the epitope is masked by some unknown components, so as not to be detected or captured by the antibodies. The instability or degradation of the antigen with time and/or temperature may cause the false negative as it makes antigen unrecognizable by the antibodies.
- Other factors may interfere with the test and cause erroneous results, such as technical/procedural errors, degradation of the test components/reagents or presence of interfering substances in the test samples.
- Any clinical diagnosis based on the test result must be supported by a comprehensive judgment of the concerned physician including clinical symptoms and other relevant test results.
- The test conditions for **ichroma™ HbA1c** are as follow.
  - Temperature: 20-30 °C
  - Humidity: 10-70 %

MATERIALS SUPPLIED

REF CFPC-38

Components of **ichroma™ HbA1c**

- Cartridge Box:
  - Cartridges 25
  - ID Chip 1
  - Instruction For Use 1
- Detection Buffer Box
  - Detection Buffer Tubes 25
  - Hemolysis Buffer Vial (3 mL) 1

MATERIALS REQUIRED BUT SUPPLIED ON DEMAND

Following items can be purchased separately from **ichroma™ HbA1c**. Please contact our sales division for more information.

- Instrument for ichroma™ tests
  - **ichroma™ Reader** REF FR203
  - **ichroma™ II** REF FPRR021
  - **ichroma™ D** REF 13303
- **i-Chamber** REF FPRR009
- **ichroma™ Printer** REF FPRR007
- **Boditech HbA1c Control** REF CFPO-96