Sudan University of Science and Technology

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Estimation of Anti-thrombin III Level in Sudanese Patients with Myocardial Infarction in Khartoum State

تقدير مستوى مضاد الثرومبين الثالث في المرضى السودانيين المصابين باحتشاء عضلة القلب في ولاية الخرطوم

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بسم الله الرحمن الرحيم

قال تعالى: (سَنُرِيهِمْ آيَاتِنَا فِي الْآفَاقِ وَفِي أَنْفُسِهِمْ حَتَّىٰ يَتَبَيَّنَ لَهُمْ أَنَّهُ الْحَقُّ ۗ أَوَلَمْ يَكْفِ بِرَبِّكَ أَنَّهُ عَلَىٰ كُلِّ شَيْءٍ شَهِيدٌ {٥٣})

صدق الله العظيم

سورة فصلت الآية (٥٣)

Dedication

To the one who was always there, supporting me,

to my husband….

To my candles when it dims,

to my parents….

To my sky sparkle,

To my child….

To my strength and support in this life,

To my brothers….

To the beauty of my life,

To my friends….

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Abstract

This is an analytical case control study which conducted in Khartoum state in the period from October 2018 to January 2019 to evaluate the antithrombin III in Sudanese patients with myocardial infarction (MI). For this purpose 70 individuals were chosen (40 patients with Myocardial infarction which considered as cases and 30 healthy persons and considered as controls).

One ml point eight of fresh venous blood were collected from each volunteer after filling the non-self questionnaire, in vacutainer tube containing 0.2 ml from 3.2% tri-sodium citrate solution for anticoagulation. Then the content of the tube were mixed and centrifuged at 3000 round/min for 15 minutes for preparation of platelet poor plasma (PPP). The PPP were tested for Antithrombin III by using fully automated Biosystem device (A15). The results were analyzed by independent T-test and one way anova of the SPSS computer programme.

The result showed that there was significant decreased ATIII level in patients with MI (15.442 mg/dl) when compared with healthy individuals (27.840 mg/dl) (P.value 0.000).

There was insignificant increased ATIII level in males (22.208 mg/dl) when compared with females (18.929 mg/dl) (P. value 0.237).

Also there was insignificant increased ATIII in MI patients with diabetes mellitus when compared with MI patients without D.M (P. value 0.782).

Also insignificant increased ATIII in MI patients with hypertension when compared with MI patients without hypertension (P. value 0.885).

There was significant different in the level of ATIII between different age groups (P. value 0.029).

الخلاصـــــــة

هذه دراسة تحليلية أجريت في ولاية الخرطوم في الفترة من أكتوبر ٢٠١٨ إلى يناير ٢٠١٩ لتقييم مضاد الثرومبين ٣ في المرضى السودانيين الذين يعانون من احتشاء عضلة القلب. لهذا الغرض تم اختيار ٤٠ عينة دم {٤٠ من المرضى الذين يعانون من احتشاء عضلة القلب.

تم جمع ١.٨ مليلتر من الدم الوريدي من كل متطوع بعد ملء الاستبيان ، في أنبوب يحتوي على ٠.٢ مليلتر من ٣.٢ ٪ محلول ثلاثي ستراث الصوديوم لمنع تخثر الدم. ثم تم خلط محتوى الأنبوب واستخدام جهاز الطرد المركزي عند ٣٠٠٠ دورة في الدقيقة لمدة ١٥ دقيقة لإعداد عينة البلازما فقيرة الصفائح الدموية. التي تم اختبارها لتحديد مضاد الثرومبين باستخدام جهاز (A15 biosysteme) . وقد تم تحليل النتائج عن طريق اختبار الفرق بين المتوسطين غير المعتمدين واختبار التباين الأحادي (ANOVA) في برنامج الحزم الإحصائية للعلوم الاجتماعية المحوسب.

تُظهر النتيجة نقصان ذا دلالة إحصائية في مستوى مضاد الثرومبين ٣ في المرضى الذين يعانون من احتشاء عضلة القلب (١٥.٤٤٢ملغ/ديسيلتر) بالمقارنة مع الأفراد الأصحاء (٢٧.٨٤٠ملغ/ديسيليتر).

لم يكن هناك زيادة ذات دلالة إحصائية في مستوى مضاد الثرومبين ٣ في الذكور (٢٢.٢٠٨ ملغ / ديسيلتر) عند مقارنتها بالإناث (١٨.٩٢٩ ملغ / ديسيلتر).

أيضا لم يكن هناك زيادة ذات دلالة إحصائية في مستوى مضاد الثرومبين ٣ في مرضى احتشاء عضلة القلب المصابين بمرض السكري بالمقارنة مع المرضى الغير مصابين بالسكري.

كذلك لم يكن هناك زيادة ذات دلالة إحصائية في مستوى مضاد الثرومبين ٣ في مرضى احتشاء عضلة القلب المصابين بارتفاع ضغط الدم بالمقارنة مع المرضى الغير مصابين بارتفاع ضغط الدم.

كان هناك اختلاف كبير في مستوى مضاد الثرومبين ٣ بين مختلف الفئات العمرية.

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List of abbreviations:

ADP	Adenosine diphosphate
AMI	Acute myocardial infarction
APC	Activated protein C
AST	Aspartate aminotransferase
ATIII	Antithrombin III
ATP	Adenosine triphosphate
C5a	Complement5a
CHD	Coronary heart disease
CK	Creatinine kinase
CK-MB	Creatinine kinase-Muscle/Brain
cTn	Cardiac troponin
DIC	Disseminated intravascular coagulation
DM	Diabetes mellitus
DVT	Deep vein thrombosis
ECG	Electrocardiogram
ECM	Extracellular matrix
FDP	Fibrin degradation products
FV	Factor V
GPIIb /IIIa	Glycoprotein IIb/IIIa
H&E	Haematoxylin and eosin
HMWK	High molecular weight kininogen
HTN	Hypertension
IHD	Ischemic heart disease
IL-1	Interleukin-1
LAD	Left anterior descending artery
LCX	Left circumflex artery

LDH	Lactate dehydrogenase
LV	Left ventricular
MB	Myocardial band
MI	Myocardial infarction
NSAIDs	Non-steroidal anti-inflamatory drugs.
PA	Plasminogen activator
PA-I	Plasminogen activator inhibitor-I
PC	Protein C
PCI	Percutaneous coronary intervention
PDGF	Platelet derived growth factor
PS	Protein S
RCA	Right coronary artery
RV	Right ventricular
SPSS	Statistical Package for Social Sciences
STEMI	ST segment elevation myocardial infarction
TF	Tissue factor
TFPI	Tissue factor pathway inhibitor
TGF-B	Transforming growth factor-B
TM	Thrombomodulin
TNF	Tumor necrosis factor
t-PA	Tissue plasminogen activator
TXA2	Thromboxane A2
vWF	Von Willebrand factor
WHO	World health organization
HCII	Heparin cofactor II

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Chapter one

Introduction and Literature review

1.1 Introduction:

The hemostatic system consists of blood vessels, platelets, and the plasma coagulation system including the fibrinolytic factors and their inhibitors. When a blood vessel is injured, three mechanisms operate locally at the site of injury to control bleeding: vessel wall contraction, platelet adhesion and aggregation (platelet plug formation), and plasmatic coagulation to form a fibrin clot. All three mechanisms are essential for normal hemostasis. Abnormal bleeding usually results from defects in one or more of these three mechanisms (Munker *et al.*, 2007). Tendency to thrombosis or molecular abnomalities of hemostasis that predisposes to thromboembolic diseas defined as thrombophilia (Schved, 2003). For a better understanding of the pathogenesis of pathological bleeding, it is customary to divide hemostasis into two stages (i.e., primary and secondary hemostasis). Primary hemostasis is the term used for the instantaneous plug formation upon injury of the vessel wall, which is achieved by vasoconstriction, platelet adhesion, and aggregation. The fibrin formation is not required for hemostasis at this stage. Primary hemostasis is, however, only temporarily effective. Hemorrhage may start again unless the secondary hemostasis reinforces the platelet plug by formation of a stable fibrin clot. Finally, mechanisms within the fibrinolytic system lead to a dissolution of the fibrin clot and to a restoration of normal blood flow (Munker *et al.*, 2007).

The heart muscle requires a constant supply of oxygen-rich blood to nourish it. The coronary arteries provide the heart with this critical blood supply. If you have coronary artery disease , those arteries become narrow and blood cannot flow as well as it should. Fatty matter, calcium, proteins and inflammatory cells build up within the arteries to form plaques of different sizes. The plaque deposits are hard on the outside and soft and mushy on the inside (Mathis, 2004).

When the plaque's hard, outer shell cracks (plaque rupture), platelets (disc-shaped particles in the blood that aid clotting) come to the area, and blood clots form around the plaque. If a blood clot totally blocks the artery, the heart muscle becomes "starved" for oxygen. Within a short time, death of heart muscle cells occurs, causing permanent damage. This is called a myocardial infarction (MI), or heart attack. While it is unusual, a heart attack can also be caused by a spasm of a coronary artery. During coronary spasm, the coronary arteries restrict or spasm on and off, reducing blood supply to the heart muscle (ischemia). It may occur at rest and can even occur in people without significant coronary artery disease. Each coronary artery supplies blood to a region of heart muscle. The amount of damage to the heart muscle depends on the size of the area supplied by the blocked artery and the time between injury and treatment.Healing of the heart muscle begins soon after a heart attack and takes about eight weeks. Just like a skin wound, the heart's wound heals and a scar will form in the damaged area. But, the new scar tissue does not contract or pump as well as healthy heart muscle tissue. So, the heart's pumping ability is lessened after a heart attack. The amount of lost pumping ability depends on the size and location of the scar (Mathis, 2004).

1.2 Problem of the study:-

This study is concerned with the way the level of antithrombin iii effects myocardial infarction occurrence due to the important role of ATIII as inhibitor of coagulation by using chromogenic analysis in the MI patients who are admitted in Khartoum state. Also the study investigates the use of antithrombin III level in the prediction of MI. Also the possibility of a correlation between the level of ATIII and age, gender and chronic diseases including diabetes mellitus and hypertension.

1.3 Literature review

1.3.1 Hemostasis

Hemostasis is the physiological process that stops bleeding at the site of an injury while maintaining normal blood flow elsewhere in the circulation. Blood loss is stopped by formation of a hemostatic plug. The endothelium in blood vessels maintains an anticoagulant surface that serves to maintain blood in its fluid state, but if the blood vessel is damaged components of the subendothelial matrix are exposed to the blood. Several of these components activate the two main processes of hemostasis to initiate formation of a blood clot, composed primarily of platelets and fibrin. This process is tightly regulated such that it is activated within seconds of an injury but must remain localized to the site of injury (Gale, 2011).

Hemostasis is devided in to primary hemostasis (platelets plug formation), secondary hemostasis (which is refers to formation of a stabilized fibrin clot through the coagulation cascade) and tertiary hemostasis (refers to formation of plasmin for breakdown of fibrin via fibrinolysis) (York, 2013).

The fibrinolysis pathway also plays a significant role in hemostasis. Fibrinolysis efficiency is greatly affected by several factors including clot structure, fibrinogen isoforms and polymorphisms, the rate of thrombin generation, the reactivity of thrombus-associated cells such as platelets, and the overall biochemical environment(Chapin and Hajjar 2015).

Multiple anticoagulant mechanisms regulate and control these systems to maintain blood fluidity in the absence of injury and generate a clot that is proportional to the injury. The proper balance between procoagulant systems and anticoagulant systems is critical for proper hemostasis and the avoidance of pathological bleeding or thrombosis (Gale, 2011).

1.3.1.1 Platelets:

Platelets are produced from bone marrow precursors known as megakaryocytes. Normal platelet count ranges from 1,50,000-4,00,000/cu mm (150-400×10^9/L) and it has a normal life span of 8-10 days (Nayak, Rai and astha, 2017).

Platelets are activated upon contact with subendothelial matrix proteins, including collagen, von Willebrand factor, and fibronectin, in response to vascular injury (Broos *et al*., 2011). Activation of platelet leads to exposure of cell surface anionic phospholipids, which serve as a nidus for the assembly of procoagulant proteins. In the ensuing activation of the coagulation cascade, a sequential series of serine protease-mediated cleavage events, thrombin is activated from its zymogen prothrombin (de witt *et al*., 2014).

There are two main platelet membrane receptors: Glycoprotein (GP) IIb-IIIa which is the main receptor on cell surfa**,** and Glycoprotein Ib-IX which is a receptor for binding vWF with platelets. Platelets cytoplasmic granules including alpha, dense, and lysosomal granules.Alpha granules, unique in platelets and contain hundreds of proteins, including integral membrane proteins, pro-coagulant molecules, chemokines, mitogenic, growth factors (platelets derived growth factor (PDGF) and transforming growth factor-B (TGF-B)), adhesion molecule (P-selectin), and microbicidal proteins (Manne, Xiang and Rondina, 2017). Dense granules, are smaller than α-granules. Contain potent aggregating molecules include calcium (which is essential for activation of the coagulation cascade), polyphosphates, adenine nucleotides, and bioactive amines, serotonin, histamine, CD63, and ADP/ATP (Manne, Xiang and Rondina, 2017). Lysosomal granules, contain acid hydrolases include cathepsins, hexosaminidase, β-galactosidase, arylsulfatase, β- glucuronidase and acid phosphatase), and they express CD63 and LAMP-1/2 (Nayak, Rai and astha, 2017). Lysosomes play a role in the digestion of phagocytic and cytosolic components, also may have an

important extracellular functions, such as degradation of extracellular matrix components, supporting receptor cleavage and fibrinolysis, and remodeling of the vasculature (Nickel and Rabouille, 2009).Platelet also has contractile cytoskeleton which consists of dense microtubules and circumferential microfilaments, that maintain the disk shape of platelets (Nayak, Rai and astha, 2017).

1.3.1.1.1 Roles of Platelets in Hemostasis:

Platelets have a key roles in hemostasis as they can quickly adhere and aggregate at sites of vascular injury, forming the platelet plug (the first wave of hemostasis). Activated platelets can also provide negatively charged phosphatidylserine-rich membrane surface that enhances cell-based thrombin generation, which facilitates blood coagulation (the second wave of hemostasis) (Hou *et al*., 2015).

1.3.1.2 Blood vessel wall

Blood vessels, particularly their endothelial lining, play an important role in the maintenance of vascular fluidity, arrest of hemorrhage (hemostasis), prevention of thrombosis, and regulation of inflammatory cell processes. The endothelium extends to all recesses of the body and maintains an intimate association with flowing blood and blood cells. Endothelial cells in post capillary venules are responsible for mediating adhesion and transmigration of leukocytes, whereas arteriolar endothelium is important for regulation of vasomotor tone. Proteomic studies have revealed that endothelial cells have the unique capacity to express and elaborate thromboregulatory molecules, which can be classified according to their chronologic appearance following vascular injury into Early thromboregulators appear prior to thrombin formation which include: nitric oxide (NO), prostacyclin and prostaglandin D2, Endothelial cell CD39/ENTPDase1and endothelin. Late thromboregulators arrive after thrombin has formed which include: endothelin, antithrombin, endothelial cell/heparin proteoglycans , tissue

factor pathway inhibitor, thrombomodulin-proteinC-proteinS pathway, plasminogen activators, inhibitors and receptors. Inflammatory thromboregulator which include: thrombomodulin-proteinC-proteinS pathway, cellular adhesion molecules, selectins (Kaushansky *et al.*, 2016).

1.3.1.2.2 Vasoconstriction:-

Under normal circumstances, the endothelium maintains comparatively low basal vascular tone by the production and release of factors that relax vascular smooth muscle, including prostacyclin, endothelium-derived relaxing factor, and endothelium-derived hyperpolarizing factor . With progressive dysfunction or injury, the endothelial cell supports increased vessel tone by the synthesis and release of the vasoconstrictor substances which include: endotlhelin and endothelium-derived constricting factor(s), and by reduced elaboration of vasodilator products (Loscalzo, 1995). Thromboxane A$_2$, a vasoconstrictor and stimulator of platelet activation (Mitchell *et al.*, 2008). Angiotensin II (ANG II) also produces vasoconstriction by a direct action on smooth muscle cells via AT1 receptors. These receptors are also present in the endothelium (Pueyo *et al.*, 1998).

1.3.1.3 Primary hemostatic plug:-

Primary hemostasis occurs when platelets adhere to an injured or disrupted endothelial surface. Adherence is followed by activation, or the release of platelet granule contents. The agonists released from platelet granules recruit additional platelets and induce their activation and aggregation. Adhesion, activation, and aggregation are mediated by different receptors and ligands depending on the local blood flow conditions (Mcmichael, 2005) (Fig 1.1) (Schoorl *et al.*, 2013).

Platelet adhesion:the mechanism of platelets adhesion is supported by certain interactions between the receptors on the platelet membrain and absorbed plasma proteins. The platelet membrane receptors including tyrosin kinase receptors, integrins, leucine rich receptors; G-protein coupled transmembrane receptors,

selectins and immunoglobulin domain receptors. This receptors are enriched with glycoprotein receptors embedded in the phospholipid bilayer and they are involved to facilitate hemostatic function by mediating the interactions within cell-platelet and platelet-substrates (Periayah *et al*., 2017). The link occur through the interaction of the subendothelial von Willebrand factor (vWF) with the platelet glycoprotein Ib (GPIb) receptor (Reininger *et al*., 2006). VWF is a large multimeric protein secreted from endothelial cells and megakaryocytes that is always present in the soluble state in the plasma as well as in the immobilized state in subendothelial matrix blood plasma and platelets. VWF mediates adhesion and aggregation of platelets at sites of vascular injury, processes that are critical for both haemostasis and thrombosis (Ruggeri, 2007).

Platelets activation: when platelets adhere to the endothelial cells they get activated and change in shape from a discoid ball to a sphere (Vorchheimer and Becker, 2006). This change result in increase the adhesion area and second, stimulate other platelets resting in plasma to become activated through biological mechanisms (include signaling pathways in the extracellular membrane of platelets) (Jackson, Nesbitt and Kulkarni, 2003). Activated platelets secret their granules contents, ($\propto$ granules and dense granules) to the plasma, which increase the local concentration of agonists necessary for atherosclerosis (Huo and Ley, 2004). There are constituents involved in platelets activation include collagene, adenosine diphosphate (ADP), Thromboxane A2 (TXA2), thrombin (Siljander and Lassila, 1999), fibrinogen and vWF. Calcium is also released and is required for the coagulation (Nayak, Rai and astha, 2017) Collagene activate platelets by surface contact (Siljander and Lassila, 1999). The activation require GPVI on the platelets membrane (Knight *et al*., 1999).ADP released by dense granules from activated platelets (Weiss, 1995). ADP makes platelet activation possible without their direct contact with the vessel wall (Jung and Moroi, 2000). Thrombin formed through proteolysis process and transformation of factor II (prothrombin)

to IIa (thrombin) during platelets activation (Fitzgerald and Fitzgerald, 1989; Wagner and Hubbell, 1990).

Platelets aggregation: after platelets activation exciting the GpIIb/IIIa receptor which attach to vWF or Fibrinogen occur. Each activated platelet extends pseudopods and aggregated. Generation of thrombin via the hemostasis mechanism lead to heightened activations. Platelet aggregation promote a primary platelet plug. The ADP receptor could be detected on platelets as helping with aggregation. P2Y1 receptors assist in stimulating the initial platelet shape changes and platelet aggregation. At the same time, P2Y12 is an important mediator for blood clotting. It increases significantly, responding to ADP to complete the aggregation process. finally, the formed platelet plug must be stabilized by the formation of fibrin (Kumar *et al*., 2013; Coller *et al*., 1991; Offermanns, 2006).
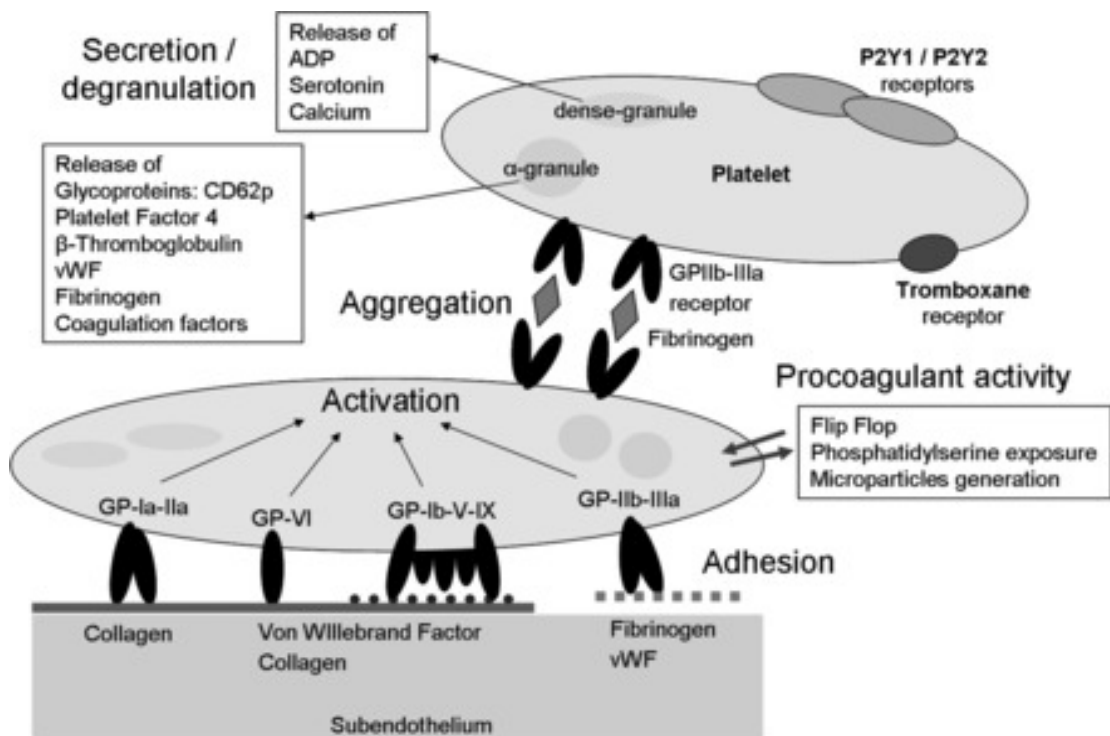
Figure (1.1) (Schoorl *et al*., 2013): Primary hemostasis; the role of platelets in primary hemostasis. exposure of the subendothelial matrix including collagen at the site of injury. Platelets adhesion is mediated by vWF. Collagen binding to

GPVI results in PLT activation. platelet-derived agonists, platelet-derived adhesive proteins and plasma-derived adhesive proteins important for firm PLT adhesion and aggregation. Platelet aggregation at the site of injury is mediated by platelet receptors(Schoorl *et al*., 2013).

1.3.1.4 Secondary hemostasis:

Secondary hemostasis consists of the cascade of coagulation serine proteases that culminates in cleavage of soluble fibrinogen by thrombin Falati *et al*., 2002; Furie, 2009), (Figure 1.2) (Myers and Wakefield, 2005) . Thrombin cleavage generates Insoluble fibrin that forms a crosslinked fibrin mesh at the site of an injury. Fibrin generation occurs simultaneously to platelet aggregation (Falati *et al*., 2002; Furie, 2009).
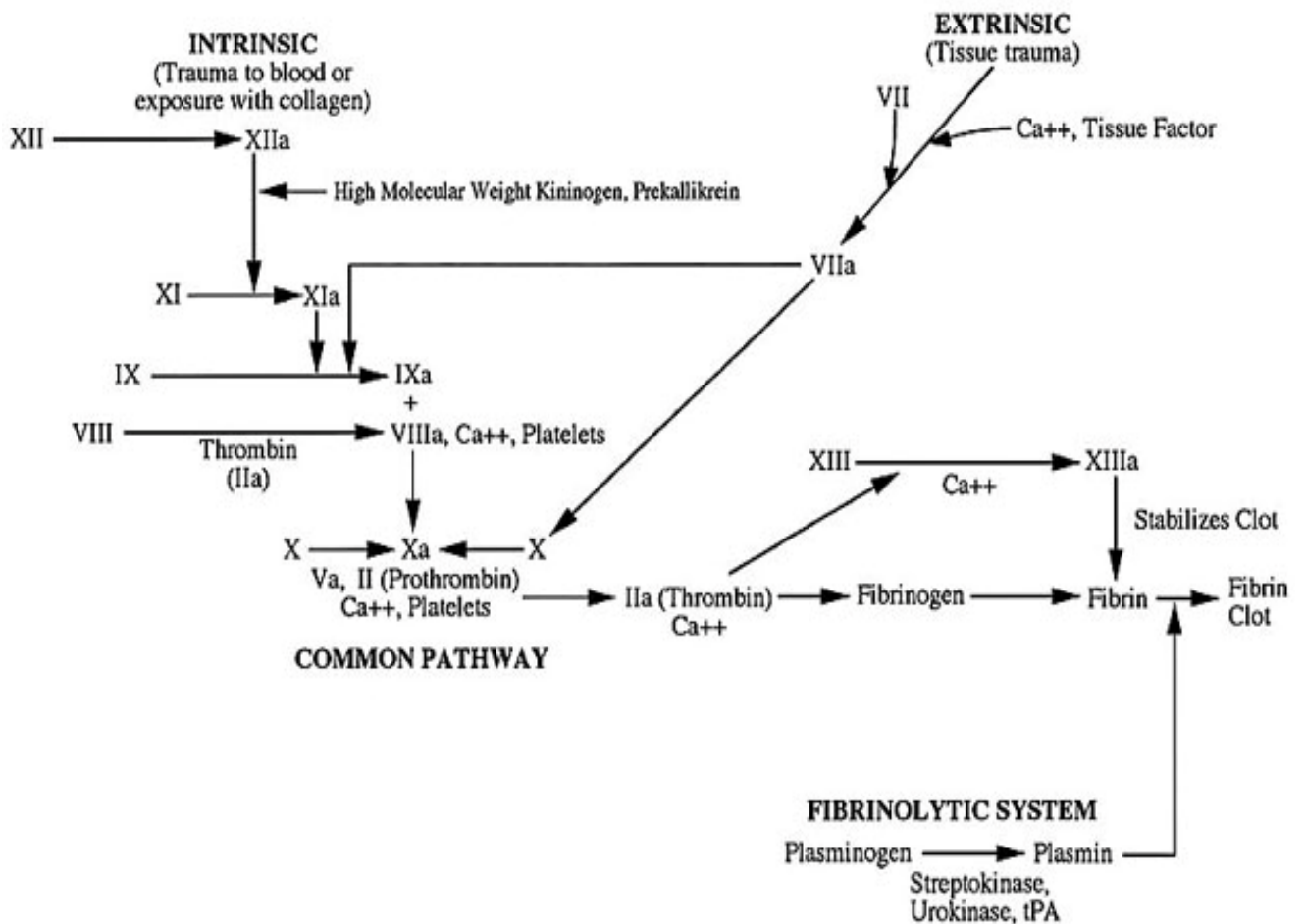
Figure (1.2) (Myers and Wakefield, 2005): Secondary Hemostasis: Coagulation factors that activate both the Intrinsic and Extrinsic coagulation pathways (Myers and Wakefield, 2005).

1.3.1.5 Coagulation mechanism:

The main constituents of coagulation system are the coagulation proteins that lead to a series of cascading reactions resulting in the conversion of soluble fibrinogen to insoluble fibrin strands (Palta *et al*., 2014). Majority of clotting factors are precursors of proteolytic enzymes known as zymogens that circulate in an inactive form. This procoagulants and anticoagulants are produced by liver except factor III, IV and VIII (Monroe *et al*., 2010).

Clotting factors can be classified into three groups: Fibrinogen Family which include fibrinogen, factor V, factor VIII and factor XIII. Vitamin K dependent proteins include factor II, factor VII, factor IX and factor X. Contact family include factor XI, factor XII, High molecular weight kininogen (HMWK) and Prekallikerin (Palta *et al*., 2014).

The coagulation mechanism occur by three pathways: extrinsic pathway, intrinsic pathway and common pathway.

1.3.1.5.1 The extrinsic pathway:-

The extrinsic pathway consists of the transmembrane receptor tissue factor (TF) and plasma factor VII/VIIa (Mackman, Tilley and Key, 2007). It is activated by TF, which is expressed in the subendothelial tissue (Lasne *et al*., 2006). TF binds with factor VIIa, calcium and phospholipid provided by activated platelets to promote the conversion of factor X to Xa (Owens and Mackman, 2010).TF can be produced in vitro by stimulation of endothelial cells and monocytes (that are normally in contact with plasma) with different stimuli such as endotoxin, complement C5a,immune complexes, interleukin-1(IL-1) and tumor necrosis

factor (TNF). Furthermore, monocytes have been reported to synthesize TF in vivo. Therefore, in addition to normal haemostasis, the TF pathway of coagulation may be involved in several pathologic conditions associated with disordered coagulation and thrombosis (Camerer, Kolsto and Pryds, 1996).

1.3.1.5.2 The intrinsic pathway:-

In this pathway thrombin activated by factor XII. It begins after exposure of contact factors (factor XII, HMWK, prekallekerin) and factor XI, which results in activation of factor XI. Activated factor XI activates factor IX. Activated factor IX in the presence of its cofactor (factor VIII), calcium ions and phospholipids that is provided by platelets surface activate factor X (Hall, 2010; Kumar, Abbas and Aster, 2010).

1.3.1.5.3 The common pathway: the intrinsic and extrinsic pathways converge at factor X to a single common pathway which leads to the generation of thrombin. Activated factor X in the presence of factor Va, phospholipid and Ca++, converts prothrombin to thrombin which eventually induces the formation of fibrin from fibrinogen. Exposure of fibrinogen to thrombin results in rapid proteolysis of fibrinogen and the release of fibrinopeptides A and B. the remainder of the molecules (fibrin monomers) polymerizes spontaneously to form an insoluble fibrin polymer. Factor XIII activated by the action of thrombin in to XIIIa, in the presence of calcium ions, will stabilize the insoluble fibrin polymer, thereby inducing the formation of a solid clot (Camerer, Kolsto and Pryds, 1996).

1.3.1.6 Fibrinolytic system:

The fibrinolytic system is activated simultaneously with the coagulation cascade and functions to maintain the fluidity of blood during coagulation (Krutsch, 2011). Fibrinolysis is the physiological process in which insoluble fibrin clot removed by enzymatic digestion and result in production of fibrin degradation products (FDP) (Turgeon, 1999). When a clot is formed, plasminogen is

incorporated and then converted to plasmin by tissue plasminogen activator (tPA) and fragments of factor XII. Endothelial cells release t-PA in response to thrombin (Krutsch, 2011). Plasmin degrades fibrin and fibrinogen through hydrolysis to produce smaller fragments. The gradual process occurs at the same time that healing is occurring. Finally, the particulate products of the hydrolytic digestion phagocytozed by the cell of mononuclear phagosytic system (Turgeon, 1999).

1.3.1.7 Inhibitory mechanism:-

Serpins are the predominant protease inhibitors and are responsible for the control of many highly regulated processes including blood coagulation and fibrinolysis. The serpin inhibitory mechanism has recently been revealed by the solution of a crystallographic structure of the final serpin–protease complex. The serpin mechanism, in contrast to the classical lock-and-key mechanism, involves dramatic conformational change in both the inhibitor and the inhibited protein. The final result is a stable covalent complex in which the properties of each component are altered so as to allow clearance from the circulation. Several serpins are involved in hemostasis: antithrombin (AT) inhibits many coagulation proteases, most importantly factor Xa and thrombin; heparin cofactor II (HCII) inhibits thrombin; protein C inhibitor (PCI) inhibits activated protein C and thrombin bound to thrombomodulin; plasminogen activator inhibitor 1 inhibits tissue plasminogen activator; and α_2-antiplasmin inhibits plasmin. Nearly all of these reactions are accelerated through interactions with glycosaminoglycans (GAGs) such as heparin or heparan sulfate (Huntington, 2003).

1.3.1.8 Antithrombin III:

Antithrombin (AT) is a plasma-derived, single-chain glycoprotein with a molecular weight of 58 kDa. It belongs to the serine proteinase inhibitor (serpin) superfamily of inhibitors (Roemisch *et al*.,2002). ATIII is the major plasma

inhibitor of thrombin and also inhibits free plasmatic factors include factor Xa, IXa, VIIa (Gaman and Gaman, 2014), XIa, XIIa, kallikrein, and HMWK. Heparin and heparan sulfate potentiate these reactions, and heparin is used for the prevention and treatment of thrombosis. When antithrombin is complexed with heparin, its rate of inhibition of several coagulation proteases is accelerated by up to 10,000-fold. The general mechanism of inhibition involves reaction of the active site of the enzyme with a peptide loop structure (the reactive center loop) of antithrombin, forming a tight, equimolar (1 : 1) complex (Hoffman *et al*., 2018).

1.3.1.8.1 Antithrombin III Deficiency:

Antithrombin deficiency is a blood disorder characterized by the tendency to form clots in the veins (thrombosis). Defeciency of ATIII may be inherited or acquired. In people with congenital antithrombin deficiency, there is a reduced amount of this substance in the blood due to a genetic abnormality. an inherited tendency to thrombosis is known as thrombophilia. Antithrombin III deficiency may also be acquired; in such cases, the disorder may be reversible with resolution improvement in the disease process responsible for the deficiency (Bauer *et al*., 2009), in such cases the defficiency due to highly consumption of ATIII during coagulation mechanism (Ioannis *et al.*, 2010; Tousoulis *et al*., 2007). It is also initially manifested by recurrent venous thromboembolism (Ozdemir and Ozcan, 2017). most of ATIII deficient relatives their clinical problem (such as venous thrombosis) often occurs at an earlier age before 55 years (Boekholdt and Kramer, 2007).

1.3.1.8.2 Hereditary antithrombin deficiency:

Inherited anti- thrombin III deficiency is a recognized risk factor for the early development of venous thrombo embolism, which has now been shown to be caused by a spectrum of molecular defects (Lane *et al*., 1994).

Molecular analysis of SERPINC1, the gene encoding antithrombin, has been restricted so far to cases with confirmed or familial antithrombin deficiency. However, some pathogenic mutations are not detected by current functional methods and other gene defects may have functional consequences only observed under specific conditions. Thus, molecular analysis may be the best method to identify antithrombin deficiency. Up to 80% of patients with antithrombin deficiency have SERPINC1 gene defects, mostly (90% of the 315 gene defects described so far) point mutations or small deletions or insertions affecting the 7 exons or flanking regions. The description of new SERPINC1 gene defects may reveal new residues with functional or structural relevance and new mechanisms causing deficiency of this endogenous anticoagulant. Moreover, other genes and mechanisms may also be involved in antithrombin deficiency. Thus, disorders of N-glycosylation explain up to 5% of cases with antithrombin deficiency. However, there are still up to 10-15% of cases with antithrombin deficiency of unknown cause (Corral *et al*., 2018). Treatment of AT III deficiency is by using of AT III concentrates and heparinotherapy. The treatment with AT III concentrates is for patients which faced major surgical interventions and in pregnant women with AT III deficiency(Gaman and Gaman, 2014).

1.3.2 Myocardial Infarction:-

Commonly referred to as (heart attack) is necrosis of heart muscle resulting from ischemia. The major underling cause of IHD is atherosclerosis while MI can occur at any age, the frequency rises progressively with increasing age and with increasing atherosclerotic risk factors (Kumar, Abbas and Aster, 2013).

In the early 1970s, the World Health Organization (WHO) had defined the term myocardial infarction by the presence of 2 of the 3 following characteristics: i) Symptoms of acute ischemia (chest pain), ii) development of Q waves in electrocardiogram (ECG) and iii) increase of enzymes in the blood and these are

combination of total creatine kinase (CK), CK-myocardial band (MB), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) (Mythili and Malathi, 2015).

However, in 1999, the Joint European Society of Cardiology and the American College of Cardiology Committee jointly proposed the new definition for myocardial infarction, emphasizing the importance of sensitive and serological biomarkers for the diagnosis of acute myocardial infarction (AMI), and the cardiac troponins (cTn) which considered as gold standard were introduced (Mythili and Malathi, 2015).

myocardial infarcts may take one of the following patterns:-

Transmural infarctions: thrombus formation is adynamic process regulated by flow, blood cells, and plasma proteins, and a crucial event in the development of coronary occlusion. Without it, coronary artery disease would rarely be fatal. Coronary thrombi in patients with ST-segment elevation myocardial infarction (STEMI) contain not only platelets and fibrin, but also inflammatory blood cells, releasing a vast number of cytokines (Luscher, 2015).

Subendocardial infarctions: Occur in situations of universal decrease in cardiac blood flow due to hypotension (shock). Necrosis of the myocardium limited to the inner third of the muscle but also can expand to the region of more than one coronary artery (Bass, Burroughs and Way, 2009). these infarcts typically do not exhibit ST segment elevations or Q waves on the ECG tracing. As already mentioned (Fig 1.3) (Kumar, Abbas and Aster, 2013).
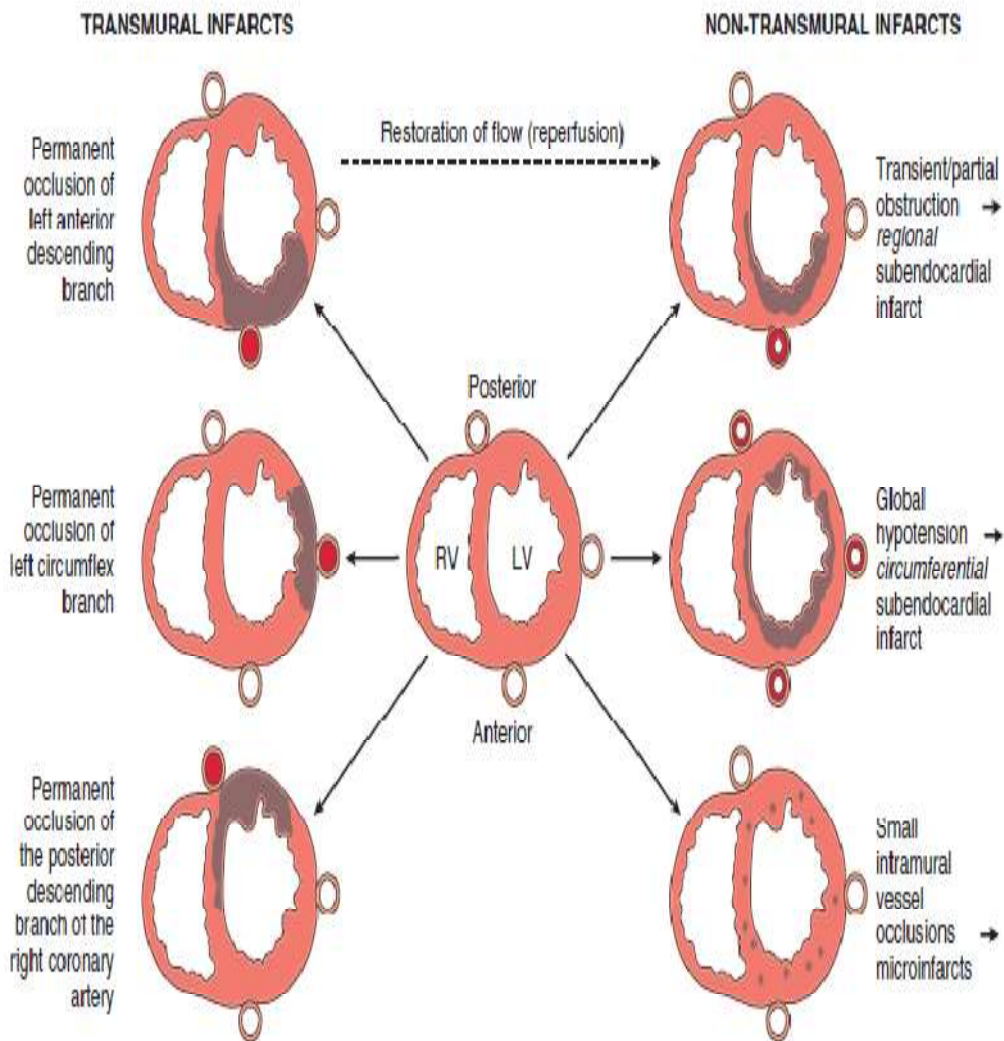
Figure (1.3) (Kumar, Abbas and Aster, 2013): Dependence of myocardial infarction on the location and nature of the diminished perfusion. Left, Patterns of transmural infarction resulting from major coronary artery occlusion. Right ventricle may be involved with occlusion of right main coronary artery (not depicted). Right, Patterns of infarction resulting from partial or transient occlusion (top), global hypotension superimposed on fixed three-vessel disease (middle), or occlusion of small intramyocardial vessels (bottom) (Kumar, Abbas and Aster 2013).

1.3.2.1 Classification of myocardial infarction:-

In 2007 the clinical classification of myocardial infarction into 5 types was introduced as an important component of the universal definition. The spontaneous type 1 myocardial infarction is related to a coronary plaque rupture, fissuring, or dissection with resulting intraluminal thrombosis. Type 2 is secondary to myocardial ischemia resulting from increased oxygen demand or decreased supply. Type 3 is linked to unexpected cardiac death when cardiac biomarkers are unavailable (Saaby *et al.*, 2013). Type 4 presenting after percutaneous coronary intervention. Type 5 presenting after coronary artery bypass grafting (Shah *et al.*, 2015). The launch of this classification has raised clinical interest particularly in type 2 myocardial infarction (Saaby *et al.*, 2013).

1.3.2.2 Common causes of myocardial infarction:-

Some of the medical causes of Myocardial infarction may include: Atherosclerotic plaques: Rupture of an atherosclerotic plaque on an artery supplying heart muscle is the most common cause of a myocardial infarction (Van de Werf *et al.*, 2008; Reed *et al.*, 2017). Diet: Saturated fat has an important role in the development of myocardial infarctions. Eating polyunsaturated fat instead of saturated fats has been shown in studies to be associated with a decreased risk of MI (Hooper *et al.*, 2015). Alcohol intake: Acute and prolonged intake of high quantities of alcoholic drinks (3–4 or more daily) increases the risk of a heart attack (Krenz and Korthuis, 2012). Genetics: Family history of ischemic heart disease or MI, particularly if one has a male first degree relative (father, brother) who had MI before age 55 years, or a female first-degree relative (mother, sister) less than age 65 increases a person's risk of MI (Perk *et al.*, 2012). Genome-wide association studies have found 27 genetic variants that are associated with an increased risk of myocardial infarction (Donnell and Nabel, 2011). Oral contraceptive pills: Women who use combined oral contraceptive pills have a modestly increased risk

of MI, especially in the presence of other risk factors (Roach *et al*., 2015). Non-steroidal anti inflammatory drugs (NSAIDs): The use of non-steroidal anti inflammatory drugs (NSAIDs), even for as short as a week, increases risk (Bally *et al*., 2017). Short-term exposure to air pollution: such as carbon monoxide, nitrogen dioxide, and sulfur dioxide (but not ozone) have been associated with MI (Mustafic *et al*., 2012). Infections: A number of acute and chronic infections including *Chlamydophila,pneumoniae*, influenza, *Helicobacter pylori*, and *Porphyromonas gingivalis* among others have been linked to atherosclerosis and myocardial infarction (Chatzidimitriou *et al*., 2012). Calcium deposition: Calcium deposits in the coronary arteries can be detected with CT scans. Calcium seen in coronary arteries can provide predictive information beyond that of classical risk factors (Hulten *et al*., 2011).

In 10% of cases, MI is not associated with atherosclerosis and is caused by other mechanisms include: vasospasm which is Intense, relatively prolonged, with or without coronary atherosclerosis and platelet aggregation. Also emboli May arise from left atrium due to atrial fibrillation, left-sided mural thrombosis, vegetative endocarditis, paradoxical embolus from right side of heart or peripheral veins. MI may also be unexplained In one-third patients, small intramural coronary vessel disease or hematological abnormalities, e.g. hemoglobinopathies, may lead to acute coronary episodes (Bhattacharya, 2009).

1.3.2.3 Symptoms of myocardial infarction:-

Signs and symptoms of MI include: Chest pain which may be adiating pain (left arm and/or shoulder, right arm and/or shoulder, both arms and/or shoulder, neck, back, epigastric) or oppressive pain. Also include shortness of breath, sweating, nausea, vomiting, abnormal heart beating, anxiety, fatigue, weakness, stress and depression (Lu *et al*., 2015).

1.3.2.4 Risk factors of myocardial infarction:-

The risk factors for MI include: Abnormal lipids, Current smokers which were defined as individuals who reported smoking cigarettes or other forms of tobacco (beedies, cigars, pipes, sheesha) (Teo *et al*., 2006), Abdominal obesity also considered as one of the risk factors of MI and was defined using the waist to hip ratio incorporating sex-specific cut-offs for women and men comparing the upper tertile to the lowest tertile (Yusuf *et al*., 2005), Hypertension and diabetes,Regular alcohol use: that was defined as consumption of alcohol at least three times a week, A dietary risk score using seven food items (meats, fried foods, salty snacks, green leafy vegetables, other raw vegetables, other cooked vegetables, and fruits) (Iqbal *et al*., 2006), Psychosocial stress factors: (depression, locus of control, global stress, financial stress, and life events) (Rosengren *et al*., 2004).Women experience their first acute MI on average 9 years later than men. The difference in age of first MI is largely explained by the higher risk factor levels at younger ages in men compared to women (Anand *et al*., 2008).

1.3.2.5 Pathophysiology of myocardial infarction:-

An AMI occurs when there is a reduction in myocardial perfusion, which is sufficient to cause cell necrosis. This is most commonly due to thrombus formation in a coronary artery. The inciting event is rupture or fissuring of an atherosclerotic plaque, which exposes the blood to thrombogenic lipids and leads to activation of platelet and clotting factors. The coronary plaques that are most prone to rupture are those with a rich lipid core and thin fibrous cap (Boateng and Sanborn, 2013). It can embolize distally or can obstruct coronary blood flow and result in myocardial ischemia or necrosis(Turgut and Bates, 2000). Other rare causes of a myocardial infarct include coronary artery embolism from a valvular vegetation or intracardiac thrombi, cocaine use, coronary artery dissection, hypotension, and anemia (Boateng and Sanborn, 2013).

1.3.2.6 Complications of myocardial infarction:

Complications of MI include Arrhythmias, cardiac failure and cardiogenic shock (associated with large infarcts) which occur within minutes to hours. And during days thrombotic complications are developed including mural thrombosis, atrial thrombosis and leg vein thrombosis.Within weeks to months chronic heart failure, Dressler's syndrome (an auto-immune disorder with pericarditis and pleurisy) and cardiac aneurysm. Also recurrence of infarction due to further thrombotic occlusion may occure. This complications are frequent, producing serious and often fatal effects (Macfarlane *et al*., 2000)

1.3.2.7 Morphological events:

1. ATP depletion lead to loss of contractility within a few minutes.

2. A state of irreversible injury sets in 20-40 min.

3. Microvascular injury begins within 1hour (Bhattacharya, 2009).

1.3.2.8 Diagnosis of myocardial infarction:

Electrocardiography (ECG) and measurement of cardiac troponins are used for diagnosis of MI. ECG by itself is often insufficient to diagnose an acute coronary syndrome or acute myocardial infarction, since ST-segment deviation may be observed in other conditions, such as acute pericarditis, left ventricular hypertrophy, and the Brugada syndrome (Luscher, 2015).

The troponins are a complex of three protein subunits that found on the thin filaments of the skeletal and cardiac muscle fibers, which are troponin C (calcium-binding component), troponin T(tropomyosin-binding component) and troponin I(inhibitory component). troponin C is not specific for myocardial injury because it's isoforms similar in cardiac and skeletal muscle (Lewandrowski, Chen and Jaunzzi, 2002; Ruseva, 2005). While the isoforms of troponin T and I in the skeletal and cardiac muscle are differ, and thus are extremely specific for cardiac tissue necrosis (Apple, 1999). Troponin I is an ideal marker of myocardial injury

because it is extremely specific for the cardiac muscle and has not been isolated from the skeletal muscle. (Higgins and Higgins, 2003). Troponin T is present bound to the contractile elements of the myocardial cells and free in the cytoplasm and exhibits a dual release initially of the cytoplasmic component and later of the bound component (Katus *et al*., 1991). They are released into the circulation 6–8 h after myocardial injury, peak at 12–24 h and remain elevated for 7–10 days (Tucker *et al*., 1997). Troponin is the gold-standard for diagnostic evaluation of patients with suspected myocardial infarction (MI) (Neumann *et al*., 2019). Also they are superior to all other clinically available cardiac biomarkers (such as myoglobin, CK-MB, heart fatty acid-binding protein and myeloperoxidase) (Reichlin *et al*., 2009).

The only disadvantage of cTn is the late clearance that makes it difficult to identify a recurrent myocardial infarction (Mythili and Malathi 2015).

1.3.2.9 Early intervention and prevention of myocardial infarction: the major risk factors are tobacco smoking, elevated apolipoprotein A, hypertension, diabetes mellitus, abdominal obesity, psychosocial factors, low fruit and vegetable intake, physical inactivity and alcohol consumption. Strategies for prevention by reducing risk factors are applicable universally. Individual healthcare providers can implement primary and secondary preventive measures to individual patients. Primary prevention involves the avoidance of disease in high-risk subjects free of disease, whereas the purpose of secondary prevention is to avoid recurrence of myocardial infarction. The general principle is to encourage improved and proven lifestyle measures and to prescribe evidence-based effective medications. Primary prevention requires greater investment and planning to identify people at high risk, plus the implementation of life-style intervention and pharmacological prevention. In both situations, strategies will have to be tailored to suit individual countries and economies. Life-style measures (i.e. sensible diet, physical exercise and smoking cessation) are

effective and need to be promoted. Compliance with preventive measures is achievable. Primordial prevention, which involves reducing the prevalence of risk factors, rests mainly on public education, media, legislation and government policy, and is very dependent on individual governments' commitment and determination. It requires promoting a healthier life-style in the population as a whole by encouraging people to seek alternatives and making them available (Pais, 2006).

1.3.2.10 Treatment of myocardial infarction:

Decrease the size of infarct is the principal role of treatment . fibrinolytic therapy (with streptokinase and tissue plasminogen activator (t-PA)) is the mainstay of treatment which is given to dissolve the thrombus in the artery and restore flow (Aylward, 1996). Nitroglycerin may be administered to relief of ongoing ischemic discomfort and is indicated as a vasodilator in patients with STEMI associated with left ventricular (LV) failure (Antman, Anbe and Armstrong, 2004).Aspirin (antiplatelet agent), which keeps blood clots from forming by preventing blood platelets from sticking together. The painkillers such as morphine or meperidine can be administered to relieve pain (Lu *et al*., 2015). Treatment with direct thrombin inhibitors during percutaneous coronary intervention (PCI) is non-inferior to unfractionated heparin and glycoprotein IIb/IIIa receptor antagonists and is associated with a significant reduction in bleeding. The intra-coronary use of a glycoprotein IIb/IIIa antagonist can reduce infarct size (Reddy *et al*., 2015). Beta-Blockers have been used after (AMI) as part of primary therapy (emergency treatment) and in secondary prevention specialy if the patient is tachycardic or hypertensive. Beta blockers reduce myocardial workload, and thus oxygen demand, via a reduction in heart rate and blood pressure. They reduce catecholamine levels, decrease myocardial ischemia and limit infarct size, and may prevent the development of definite infarction in acute coronary syndrome (ACS) patients (Kezerashvili, Marzo and Deleon, 2012).

1.4 Previous studies:-

In 2012 a study was conducted in Italy where the level of antithrombin III was low in patients with MI compared to normal subjects.

Other study was conducted in Greece in 2010 showed decrease in plasma levels of ATIII found in patients with AMI compared to control group.

 Also a study was conducted in 1998 in Boston, U.S.A showed higher levels of ATIII in the MI subjects than in controls, It is more likely that at the time of infarction, these inhibitor is higher possibly due to a homeostatic response to previous plaque rupture and thrombosis.

1.5 Rationale:

Myocardial infarction is one of the serious diseases and has high a mortality rate (Chi and Kloner, 2003), this study was been conducted due to the important role of the antithrombin III in the early detection of MI due to its importance as an inhibitor of the hemostatic mechanism and it's decrease is a well known risk factor for thrombosis.

1.6 Objectives:

1.6.1 General objective:

To estimate the anti-thrombin III level in sudanese patients with myocardial infarction in khartoum state.

1.6.2 Specific objectives:

1- To determine whether there is a difference between ATIII level in patients with MI and healthy individuals.

2- To estimate the antithrombin III level within gender and age in MI patients.

4- To correlate between ATIII in MI patients with diabetes mellitus and without diabetes mellitus.

5-To correlate between ATIII in MI patients with hypertension and without hypertension.

Chapter two

Materials and Methods

Material and Methods

2.1 Study design:

This study was been designed as an analytical case control study, the level of Antithrombin III in patients with myocardial infarction who represent a case group matched to the level of Antithrombin III in individuals with no history of cardiovascular disease or myocardial infarction who represent a control group by chromogenic assay.

2.2 Study area:

The study was been conducted at Alshab hospital, Khartoum state.

2.3 Study period:

This study was been achieved in the period from (October,2018 to January,2019).

2.4 Study population:

Sudanese patients with myocardial infarction in emergency department and ICU in Alshab hospital. Healthy individuals who had no myocardial infarction (volunteer).

2.5 Inclusion criteria:

For a case group any patient diagnosed with myocardial infarction was been included in the study. And for a control group any healthy and normal individual was been included in the study.

2.6 Exclusion criteria:

Patients with leukemias, DIC, cancers, inflammations and patients treated with heparin was been excluded that can effect coagulation cascade.

2.7 Sample size:

Forty patient with myocardial infarction and thirty healthy person as control.

2.8 Data collection method and tool:

- The data was collected using laboratory investigation to measure ATIII level.

- Non-self questionnaire which were specifically designed to obtain information.

2.9 Data analysis:

All the variables of the study were numerical variables and was been analyzed by using statistical package for social sciences(SPSS) version 16 and the p value will be considered statistically significant at 0.05 by using independent t-test which determine if the antithrombin III level have effect on myocardial infarction, frequencies were obtained and independent t-test was used to compare between the parameters of case and control.

2.10 Ethical considerations:

Each volunteer or patient were informed with the aims of the study and it's importance in the future, and informed assort of the procedure and the amount of the blood sample that withdrawn from them to perform tests then a verbal and writin consent was obtained.

2.11 Methodology:

2.11.1 Preparation of platelet poor plasma:

All blood samples were collected under antiseptic equation. One point eight ml venous blood collected in a vacutainer tubes containing 0.2 ml of 3.2% Tri-sodium citrate (as anti-coagulent) as quickly as possible and mixed gently with

adequate mixing to avoid hemolysis and clots, then centrifuged at 3000 rpm for 15 minutes to measure platelet poor plasma for ATIII value.

2.11.2 Antithrombin III estimation:-

The ATIII was been measured using autoanalyzer device (A15 biosystem).

2.11.2.1 Principle:

Measurement of antigen-antibody reaction by the end point method.

2.11.2.2 Procedure:

Control, calibrator and samples(PPP) placed in the rack according to the positioning screen, also reagents (antiserum and buffer) placed in the rack according to the positioning screen then sample and reagent racks placed in the device also according to the positioning screen. After that according to instructions of the reagents included in the kit the information about the reagents and samples were been entered included the amount taken from samples and reagents (5 μL from the plasma sample, 250 μL from the buffer as reagent 1 and 30 μL from antiserum as reagent 2) and choose the ATIII from the list of tests and finally start the running. The result took about 11minutes.

Chapter three

Results

3.1 Results:

Demographic data of study population:

This study is conducted on 40 patients with MI and 30 healthy individuals (volunteer). Their mean ages is (55.13) for cases and controls.

Table (3.1) comparison between the ATIII of test and control.

Parameters	Study group	N	Mean ± Std. Deviation	P.value
ATIII	Case	40	15.442 ± 10.5091	
	Control	30	27.840 ± 8.5237	0.000

Table (3.1) show significant decrease in mean of ATIII of study group when compared with control (P.value < 0.05).

Table (3.2) comparison between the ATIII in males and females.

Parameters	Study group	N	Mean ± Std.Deviation	P.value
ATIII	Male	22	17.095 ± 10.4024	
	Female	18	13.422 ± 10.5753	0.277

Table (3.2) show insignificant increase in mean of ATIII in males when compared with females (P.value > 0.05).

Table (3.3) comparison between the ATIII in the patients with diabetes mellitus and patients without D.M.

parameters	Patients with D.M	N	Mean ± Std.Deviation	P.value
ATIII	Yes	17	15.988 ± 10.9391	
	No	23	15.039 ± 10.4093	0.782

Table (3.3) show insignificant increase in mean of ATIII in patients with DM when compared with patients without DM (P.value > 0.05).

Table (3.4) comparison between the ATIII in the patients with hypertension and patients without HTN.

Parameters	Patients with HTN	N	Mean ± Std.Deviation	P.value
ATIII	Yes	19	15.700 ± 9.4546	
	No	21	15.210 ± 11.6099	0.885

Table (3.4) show insignificant increase in mean of ATIII in patients with HTN when compared with patients without HTN (P.value > 0.05).

Table (3.5) age groups and their frequency and percent:

Age group	Categories	Frequency	%
Age I	27- 55	37	52.9
Age II	56 – 83	33	47.1

Table (3.6) Comparison between ATIII of different age groups:

Parameter	Age group	N	Mean ± Std.Deviation	P.value
ATIII	27-55	37	22.124 ± 12.4091	
	56-83	33	19.221 ± 10.2446	0.293

Table(3.6) show there was insignificant increase in mean of ATIII level in the age group (27-55) when compared with age group (56-83).

Chapter four

Discussion, Conclusion and

Recommendations

4.1 Discussion:

This analytical study was carried out in Khartoum state during the period from October 2018 to January 2019 to evaluate the effect of ATIII on myocardial infarction.

The mean of ATIII result of study group was (15.442 mg/dl) which significantly decreased than that of control group (27.840 mg/dl) (P.value 0.000). This result was consistent with a study conducted in 2008 in Skopje, Macedonia which indicates significant decrease in ATIII result. Also consistent with the study of 1991 in china which showed that the AT III level in AMI patients was much lower than that in normal controls using the methods of chromogenic substrate.

The mean of antithrombin III in male was (22.208 mg/dl) which insignificantly higher than females (18.929 mg/dl) (P.value 0.237). this consistent with the study conducted in 1978 in Nerway which indicate women have insignificant lower levels of ATIII than men. And not consistent with a study in 1994 in Sheffield, UK which showed males had significantly higher ATIII concentration than females and the level increased with age in women.

The mean of ATIII in patient with diabetes mellitus was (15.988 mg/dl) which insignificantly higher than in patient without DM (15.039 mg/dl)   (P. value 0.782). This agree with the study conducted in Italy at 1984 which indicate insignificant decrease in the ATIII level in patients without DM.

The mean of ATIII in patient with hypertension was (15.700 mg/dl) which insignificantly higher than in patient without HTN (15.210 mg/dl). (P. value 0.885) .this disagree with the study of Junker Ralf in 1998 in Munster, Germany which show ATIII level significantly higher in hypertensive patients than normotensive individuals.

There was significant difference between the levels of ATIII in defferent age groups (P. value .029).This disagree with the study of Ole Rasmus in Oslo, Nerway, which indicate significant difference in the ATIII concentration among age groups.

4.2 Conclusion:

- This analytical study concluded that a hypercoagulable state is associated with significant decreasing of ATIII level in Sudanese patient with myocardial infarction.

- When compared MI patients with healthy persons (control) found real significant different in the values of ATIII.

- This study also show insignificant increase in the ATIII level in males when compared with females.

- Insignificant increase of ATIII level in patients with diabetes and hypertension.

- Also show insignificant increase of ATIII level in the age group (27-55) when compared with age group (56-83).

4.3 Recommendations:

This study recommend that :-

1. Further studies should be conducted using large sample size to end up with more accurate results.

2. ATIII study should be included routinaly in the investigation work up .

3. full coagulation profile, protein C and protein S must be conducted to confirm the state.

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Appendices

Apendix (1)

Informed consent

(الموافقة المستنيرة)

الإسم: خديجة التجاني حسن

الجامعة: طالبة ماجستير في أمراض الدم ومبحث المناعة بجامعة السودان للعلوم والتكنولوجيا

عنوان البحث: تقدير مستوى مضاد الثرومبين الثالث في المرضى السودانيين المصابين بالذبحات القلبية في ولاية الخرطوم.

عزيزي المشارك الهدف من قيامي بهذا البحث هو معرفة مدى تأثير مستوى مضاد الثرومبين الثالث في حدوث الذبحات القلبية. وللوصول لذلك سأقوم بأخذ عينات دم منك ومن عدد ٣٩ من المرضى المصابين بالذبحة القلبية وسيكون أخذ العينات بأمان تام ولا توجد أي مخاطر متوقعة عليك. بعد ذلك سأجري عليها التحاليل المخبرية لمعرفة مستوى مضاد الثرومبين الثالث ومدى تأثيره على حدوث الذبحة.

سأقوم بأخذ المعلومات المتعلقة بالدراسة بملء استبيان منك ومن ملفك الموجود بالمستشفى وذلك بعد أخذ الموافقة من المستشفى وسوف أقوم بالمحافظة على الملفات وارجاعها لمكانها بعد أخذ المعلومات وكل ذلك سيكون في سرية تامة.

ستكون مشاركتك في البحث طوعية ومن حقك الانسحاب من البحث في أي وقت والتوقيع على طلب الإنسحاب، كما يمكنني في ظروف معينة إيقافك من البحث دون أخذ موافقتك.

لأي استفسار يمكنك الاتصال بي على الرقم:

٠٩١٦٩٣٦٩٥٣

أنا المشارك .... بعد إطلاعي علي فكرة البحث و أهدافه قبلت أن أشارك في البحث وأن يتم أخذ عينة دم مني.

التوقيع:.............................

شكرا لتعاونكم

توقيع الباحث:.............................

Appendix (2)

Sudan university of science and technology

Collage of medical laboratory science

Department of hematology

Estimation of ATIII in Sudanese patients with MI

Questionnaire

Name:……………………………………

Age:……………………………………

Sex:

Male ☐

Female ☐

Other diseases:

Diabetic   Yes ☐   No ☐

Hypertensive   Yes ☐   No ☐

Result:

ATIII:…………

Appendix (3):

A15 Biosystem (full automation device)