**SUDAN UNIVERSITY OF SCIENCE AND TECHNOLOGY**

**COLLEGE OF GRADUATE STUDIES**

# Genetic Variant of Ribosomal protein S20 Gene among Sudanese Patients Diagnosed with Myocardial Infarction

# التغير الجيني لبروتين الرايبوسوم بين المرضى السودانيين المصابين بإحتشاء عضلة القلب

**A thesis submitted for Partial Fulfillment of the Requirements for M.Sc. Degree in Medical Laboratory Science (Hematology and Immunohematology)**

Submitted by: Dalya Sulaiman Musa Alawad B.Sc.

in Medical Laboratory Science (Hematology and Immunohematology)

in Omdurman Islamic university 2017

Supervisor

**Prof. Fathelrahman M Hassan**

**January 2023**

بسم الله الرحمن الرحيم

قَالَ تَعَالَىٰ: ﴿ سَنُرِيهِمْ ءَايَٰتِنَا فِى ٱلْءَافَاقِ وَفِىٓ أَنفُسِهِمْ حَتَّىٰ يَتَبَيَّنَ لَهُمْ أَنَّهُ ٱلْحَقُّ ۗ أَوَلَمْ يَكْفِ بِرَبِّكَ أَنَّهُۥ عَلَىٰ كُلِّ شَىْءٍ شَهِيدٌ ﴿٥٣﴾ ﴾ فصلت: ٥٣

# DEDICATION

All thanks for Allah for the guidance, strength, power of mind, protection and skills and for giving me a healthy life. I dedicate this study to my beloved parents, who have been my source of inspiration and who continually provide their moral, spiritual, emotional, and financial support.

To my brothers, sisters, relatives, friends, and colleagues who shared their words of advice and encouragement to finish this study**.**

## ACKNOWLEDGEMENT

In the beginning, I thank Allah for the success and completion of this research. I would like to thank my supervisor Prof. Fathelrahman.M. Hassan professor of hematology and immunohematology who was very learner and his door office was always open whenever and checked my examples, assisted me in a myriad way with writing and helpfully commented on earlier draft of project. I had a question about my research and writing. I would also like to acknowledge all staff team work of molecular biology laboratory at National research's institute for helping me in this research specially Dr. Salah .G. El-zaki who has carefully answered my questions in Bioinformatics, provided me with materials.

# Abstract

Arterial thrombosis and venous thrombosis are major causes of morbidity and mortality in the world and myocardial infarction is the common cause of disability and death in the world, cause of 10-25%of death in Sudan and its occupied 75% from cardiovascular disease in sub Saharan in Africa. Ribosomal proteins disorder associated with different disease like cancers and cardiovascular diseases. Ribosomal protein S20gen located in chromosome 8 P13.1 in the second intron which belong to 40 S subunit to S10P family and act as activator to protein **53** so it may have acritical role in coagulation modulation and any abnormality of this gen may affect the role of cardio and endothelial cell which response to protein **53** expression. ribosome protein S20 (A88T) polymorphism was detected in Diamond black fan anemia. And this study aimed to detect ribosome protein S20 polymorphism in Acute myocardial infarctions.

this study was descriptive cross sectional hospital based conducted in Alshaab hospital in Khartoum state from March 2022 to January 2023 with 47 patient diagnosed with myocardial infraction by ECG and cardiac enzymes, Clinical data were collected by non-self-questionnaire and recording forms, two ml of venous blood was collected from each patient with myocardial infarction in EDTA tube. And DNA was extracted by ammonium chloride method (salting out method). blood was genotyped by PCR enzymes fragmentation method and result was analyzed by SPSS version 26 program to calculate mean, standard deviation, for correlation T test and Chi -square.

Mean age of myocardial infarction patient is 58 ± 10 and 76% most of them were males, the majority of patient came from Khartoum State, the common clinical symptoms were chest pain 86.2% and about 44% of patient had a pervious history of myocardial infarction. And in chronic disease the hypertension represents 31% and diabetes mellitus 27% both 21%, risk factors under study included: obesity29% and smoking42%. all study population had a wild type of allele AA so all study population is negative for RPS20 polymorphism, and significant increase in total white blood cell in MI patient whom had a chronic disease that ribosomal protein S 20 A88T polymorphism is not associated with myocardial infarction.

# مستخلص البحث


يعد الخثار الشرياني والتخثر الوريدي من الأسباب الرئيسية للمرضى والوفيات في العالم واحتشاء عضلة القلب هو السبب الرئيسي للعجز والوفاة في العالم ويسبب 10–25٪ من الوفيات في السودان واحتلاله 75٪ من أمراض القلب والأوعية الدموية في مناطق الصحراء الوسطى في أفريقيا. اضطراب بروتينات الرايبوسوم المرتبط بأمراض مختلفة مثل السرطانات وأمراض القلب والأوعية الدموية،بروتين الرايبوسومS20 في الكروموسوم الثامن والانترون الثاني وينتمي لعائلة البروتين و21 ويعمل كمحفز ل بروتين 53 وقد يكون له دور مهم في امراض التخثر واي خلل فيه قد يؤثر على دور القلب والخلايا البطانية التي تستجيب لبروتين 53, تم اكتشاف بروتين الريبوسوم S20A88T متعدد التشكل في فقر الدم الماسة السوداءالمسطحة. وهدفت هذه الدراسة إلى الكشف عن تعدد أشكال بروتين الريبوسوم S20 في حالات احتشاء عضلة القلب الحاد.

كانت هذه الدراسة عبارة عن دراسة وصفية مقطعية ومقرها مستشفى الشعب بولاية الخرطوم في الفترة من مارس 2022 إلى يناير 2023 مع تشخيص إصابة 47 مريضًا بخرق عضلة القلب بواسطة تخطيط القلب وأنزيمات القلب، تم جمع البيانات السريرية بواسطة الاستبيان الغير ذاتي واستمارات تسجيل المرضى، وحوالي 2 مل من الدم الوريدي جمعت من كل مريض مصاب باحتشاء عضلة القلب في أنبوب EDTA واستخلص الحمض النووي بطريقة كلوريد الأمونيوم

تم التنميط الجيني للدم بطريقة تجزئة إنزيمات تقييد تفاعل البوليميراز المتسلسل وتم تحليل النتيجة بواسطة برنامج SPSS الإصدار 26 لحساب المتوسط والانحراف المعياري ولاختبار الارتباط T و square . متوسط عمر مريض احتشاء عضلة القلب هو 58± 10 و 76٪ منهم من الذكور ، وغالبية المرضى من ولاية الخرطوم وأكثر الأعراض السريرية هي آلام الصدر 86.2٪ وحوالي 44٪ من المرضى لديهم تاريخ سابق من احتشاء عضلة القلب أما الأمراض المزمنة فهم ارتفاع ضغط الدم ويمثل 31٪ ومرض السكري 27٪ كلاهما 21٪. وشملت عوامل الخطر قيد الدراسة: السمنة 29٪ والتدخين 42٪. كان لدى جميع أفراد الدراسة نوع بري من الأليل AA ، لذا فإن جميع أفراد الدراسة سلبيون بالنسبة لتعدد الأشكال RPS20 ، وزيادة ملحوظة في إجمالي مرض خلايا الدم البيضاء لدى أصحاب الامراض المزمنة.

## Table of content

## List of tables

## List of figures

## Abbreviation

| | |
|---|---|
| **AMI** | **Acute myocardial infarction** |
| **APC** | **Activated protein C** |
| **APS** | **Antiphospholipid syndrome** |
| **AT III** | **Antithrombin III** |
| **BFA** | **Diamond black fan anemia** |
| **CAD** | **Coronary artery syndrome** |
| **COC** | **Combination oral contraceptive** |
| **CRC** | **Colorectal cancer** |
| **CTn** | **Cardiac troponin** |
| **DVT** | **Deep venous thrombosis** |
| **ECG** | **Electrocardiogram** |
| **HMWK** | **High molecular weight kinenogenic** |
| **IHD** | **Iischemic heart disease** |
| **MDM2** | **Mouse douple minute2homolog** |
| **NSTEMI** | **Non ST elevated myocardial infarction** |
| **P53** | **Protein 53** |
| **PAI** | **Plasminogen activator inhipitor** |
| **PCR** | **Polymerase chain reaction** |
| **RP** | **Ribosomal protein** |
| **SPSS** | **Statistic package for social science** |
| **STEMI** | **ST elevated myocardial infarction** |
| **TF** | **Tissue factor** |
| **TFPI** | **Tissue factor plasminogen inhibitor** |
| **TPA** | **Tissue plasminogen activator** |
| **VTE** | **Venous thromboembolism** |

# Chapter I

# Introduction

# Introduction

## 1.1. Overview

Arterial and venous thrombosis are major causes of morbidity and mortality worldwide (Boekhold, S,2007).

Myocardial infarction, also known as a heart attack, is a potentially fatal condition that occurs when blood flow to the heart muscle is suddenly split off, causing tissue damage that is usually caused by a buildup of plaque. Its main symptoms are chest pain and shortness of breath (Balingit A, 2021). It can also cause heart failure, an irregular heartbeat, cardiogenic shock, or cardiac arrest. . (Mustafa, A .2015)

The majority of myocardial infarctions are caused by underlying coronary artery disease CAD, which is the leading cause of death in the United States, accounting for approximately 805000 deaths between 2005 and 2014. (OjhaN and Dhamoon,2022) . (Hilliard A et al., 2021)

Myocardial infarction can be classified as follows:

Myocardial infarction is classified into two types based on ischemic myocardial injury: Type 1 MI is caused by coronary thrombosis on the side of the plaque rupture, which partially (NSTEMI) obstructs blood flow. In the absence of known or suspected acute plaque rupture and thrombosis, type 2 MI results from a critical imbalance between myocardial oxygen supply and demand. Myocardial ischemia may be associated with ECG changes and elevated biochemical markers such as cardiac troponins (Newboy K and Perez R,2020). ( Ojha N and D. Dhamoon D, 2022) Troponin is the gold standard for diagnosing suspected myocardial infarction (MI) patients (Neumann et al , 2019).

Although no definitive cause of myocardial infarction has been identified, risk factors such as Obesity, smoking, and hereditary factors such as protein 53 elevation has a pro-thrombotic effect. There is not found definitive cause of myocardial infarction but risk factor such as Obesity, smoking beside hereditary factor like elevation of protein 53has pro thrombotic effect with DNA signaling and repair function :over expression of P53 in endothelial cell which found to reduce the expression of nitric oxide synthase and thrombomodulin, to increase the expression of plasminogen activator inhibitor -1(PAI-1) so the change in endothelial protein 53expression

lead to increase cardiovascular risk in arterial system and DVT in venous thrombosis.(Magdalena L &,Bauer T, 2018) .

Ribosomes are organelles that help to facilitate the production of proteins and composed of small subunits of the 40S as well as 60S formed of .large subunit These proteins ribosomal are genes encoding of the 60S subunit placed in the cytoplasm, and their structure is stable which is known as ribonucleoprotein molecules (RNP) which is important to transport the nucleolus under ribosomes via non-ribosome-associated cytoplasm 5S rRNA (Hassan F, 2021) ribosomal proteins (RPs) have extra ribosomal functions that are involved in cell proliferation, differentiation, apoptosis, DNA repair, and other cellular processes. The dysfunction of RPs has been linked to the development and progression of hematological, metabolic, cardiovascular diseases and cancer. (Wang W, Nag S, Zhang X…et al,2015). Ribosomal protein s20gen located in chromosome 8P12.1in the second intron belong to 40subunit to S10P family act as activator to protein **53** so it may have acritical role in coagulation modulation and any abnormality of this gen may affect the role of cardio and endothelial cell which response to protein **53** expression. (ZhouX,2015) (Leider B et al ,2022) (Saftan M et al,2022)

Low expression of RPS20 can cause Shwachman diamond syndrome (SDS) is a rare autosomal recessive disorder manifested as bone marrow failure, skeletal deformities, and exocrine pancreatic hypoplasia and leukemia's (Wang W, Nag S and Zhang X, 2016).

RPS20 A88T polymorphism lead to un functional protein due to substitution of Asparagine to isoleucine (Saftan M et al,2022).

## 1.2. Rational

thrombophilia (hypercoagulability or prothrombotic state) is an abnormality of blood coagulation that increases the risk of thrombus formation. And myocardial infarction is the most cause of disability and death in the world and cause of 10-25of death in Sudan (Bahaaldein A,2016). Established genetic disorder associated with thrombophilia include factor V Leiden, prothrombin gene mutation, protein C or S deficiency, and antithrombin III (AT III) deficiency and the ribosomal protein have rule in hemostasis by P53 which reduce the expression of nitric oxide synthase and thrombomodulin, to increase the expression of plasminogen activator inhibitor -1(PAI-1).

now the recent studies involved around the extra ribosomal function and association of ribosomal protein gens polymorphism in hematological disease like RPL5&9.(Hassan F,2021)

According to my best knowledge there was no study about association of ribosome protein S20 gen polymorphism and myocardial infarction but must study here about malignancy like colorectal cancers and madulloplastoma. (Bortoli M et al,2006) (Nieman et al, 2014).

Therefor this study may add a new knowledge about RPS20 genetic polymorphism and determined the frequency of it among myocardial infarction patient to point out the association and its use full in clinical diagnosis and management

## 1.3. Objective

### 1.3.1. General objective

To detect genetic variant of ribosomal protein s20 gene among Sudanese patient diagnosed with myocardial infarction

### 1.3.2. Specific objective

1 -To estimate genetic variant of ribosomal protein s20 in Sudanese patient diagnosed with myocardial infarction

2 -To correlate ribosomal protein s20 polymorphism have diagnostic role for myocardial infraction Sudanese patient and their thrombophilia history

3 -To correlate the frequency of age gender, residence and clinical symptoms of patient with myocardial infraction

4 -To correlate the frequency of chronic disease and risk factor of myocardial infraction patient

# Chapter II

# Literature review

# Literature review

## 2.1. Hemostasis

Hemostasis is the arrest of bleeding from an injured blood vessel, requires the combined the activity of vascular factor.

Hemostasis is divided 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)

### 2.1.1. Primary homeostasis

#### 2.1.1.1. Vascular system

Endothelial cell which lying in Blood vessels especially their play a major role in maintains the vascular fluidity, arrest of hemorrhage and prevent of thrombosis, Endothelial cells are particularly important in the prevention of coagulation. Endothelial cells express several molecules on their surface membranes that are important in regulation of coagulation. Examples are heparin sulfate and thrombomodulin, which activate anticoagulant systems (antithrombin and the protein C protein S system, respectively).Endothelial cells produce a variety of metabolic products that are critical in the prevention of thrombosis, including tissue plasminogen activator (t-PA), the primary initiator of the fibrinolytic system; tissue factor pathway inhibitor (TFPI), which inhibits coagulation via the TF-VIIa-Xa complex; and prostacyclin, a potent vasodilator and platelet antagonist. Endothelial cells also produce nitric oxide (NO; originally called the endothelial-derived relaxing factor [EDRF]), which is a potent vasodilator and platelet antagonist, and endothelin, which is a potent vasoconstrictor (William, 2015),

#### 2.1.1.2 Platelet

Platelets are small a nuclear cell fragments that bud off from megakaryocytes, specialized large polyploidy blood cells that originate in the bone marrow. Platelets are present at 150 to 400 million per milliliter of blood and circulate for about ten days. In a healthy blood vessel, and under normal blood flow, platelets do not adhere to surfaces or aggregate with each other. However, in the event of injury platelets are exposed to subendothelial matrix, and adhesion and

activation of platelets begins. Multiple receptors on the surface of platelets are involved in these adhesive interactions and these receptors are targeted by multiple adhesive proteins. The key for all of these receptors is that the adhesive interaction only takes place in the event of an injury to the blood vessel. This restriction is maintained in several different ways. Receptor GPIb-IX-V binds to immobilized von Willebrand factor (VWF) specifically through an interaction between GPIbα and the A1 domain of VWF. 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 sub endothelial matrix, However the soluble VWF in the circulation does not bind with high affinity to GPIbα The high affinity interaction may be dependent upon high sheer stress exerted by flowing blood on immobilized VWF, whether that VWF is immobilized on subendothelial matrix or other activated platelets. GPVI and GPIb-IX-V are critical for adhesion of platelets to subendothelial matrix at the site of injury and for their subsequent activation and Activation of platelets is critical for aggregation. In particular, the integrin's, αIIbβ3, α2β1 and αvβ3 are normally present on the platelet surface in an inactive form, but platelet activation induces a conformational transition in these receptors that exposes ligand binding sites. αIIbβ3 is arguably the most important of these receptors as it is present at the highest density on the platelet surface. In addition, αIIbβ3 binds to multiple ligands that promote platelet-platelet aggregation. These include fibrinogen, VWF, collagen, fibronectin and vitronectin. though other ligands have also been identified for each of these. All of the integrin's are maintained in an inactive state on quiescent platelets. Feedback activation of nearby platelets surrounding a new site of injury is critical for further aggregation and propagation of the platelet plug. This activation is mainly mediated by agonists released by activated platelets themselves acting on G protein-coupled receptors. ADP is released from platelet dense granules and binds to receptors P2Y1 and P2Y12. Thromboxane A2 is synthesized de novo by activated platelets and binds to the thromboxane receptor primarily, and other prostanoid receptors to a lesser degree. Serotonin is also secreted from dense granules and contributes to platelet activation. Another critical mechanism of platelet activation that links secondary hemostasis to platelet function is activation by thrombin. Thrombin is the terminal serine protease of the coagulation cascade (Gale A, 2011).

### 2.1.2 Secondary hemostasis

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

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 occurs by three pathways: extrinsic pathway, intrinsic pathway and common pathway.

**2.1.2.1 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 sub-endothelial 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)

**2.1.2.2. 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).

**2.1.2.3. 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 (Gale A,2011).

### 2.1.3. Tertiary hemostasis

Tertiary hemostasis is defined as the formation of plasmin, which is the major enzyme responsible for fibrinolysis at the same time as the coagulation cascade is activated, tissue plasminogen activator (tPA) is released from endothelial cells. Release is stimulated by a variety of factors, including hypoxia and bradykinin. Tissue plasminogen activator binds to plasminogen within the clot, converting it into plasmin. Plasmin lyses both fibrinogen and fibrin (soluble and cross-linked) in the clot, releasing fibrinogen) degradation products. This process is outlined in the image below, which also illustrates the inhibitors of the fibrinolytic pathway (antiplasmin and plasminogen activator inhibitor). The activation of plasminogen by tPA requires fibrin, thus limiting the production of plasmin to the site of thrombus formation. This localization of plasmin is important, since it is fairly non-specific in its activity and will not only destroy fibrin, but also other factors, including factors V and VIII and fibrinogen. There are other plasminogen activators independent of tPA. These include urokinase, factor XII and kallikrein. Activated factor XII and kallikrein can activate plasminogen into plasmin directly. Furthermore, factor XII and kallikrein produce bradykinin from high-molecular weight kininogen in the contact portion of the intrinsic pathway of coagulation; bradykinin being the most specific and potent stimulus for tPA release. This emphasizes the role of the early components of the intrinsic pathway (high-molecular weight kininogen, prekallikrein and factor XII) in fibrinolysis and bradykinin generation rather than coagulation. (Shaz B et al, 2019)

## 2.2. Thrombosis

Normal hemostasis comprises a series of regulatory process that culminate in the formation of blood clot that limit bleeding of injured vessel. One of the pathological counter part of hemostasis is thrombosis. (Kumar, Abbas and Aster, 2018)

Thrombosis define as a blood clot within blood vessel that limit a blood flow. Acute venous and arterial thrombosis are the most common cause of death in developed country. (Ashoropi D, Ameer MA and Fernand R, 2022).

### 2.3. Thrombophilia

Thrombophilia define as an abnormality of coagulation or fibrinolytic system that result in hypercoagulable state and increase the risk an individual for thrombotic event, where intravascular thrombus formation may arterial or venous. (Wahed A, Quesada A et al., 2019). Thrombophilia can be caused inherited or acquired condition. (Dautary Aand Krasi, G ,2019).

#### 2.3.1. Inherited cause of thrombophilia:

genetic factors associated with thrombophilia include factor V Leiden, prothrombin gene mutation, protein C or S deficiency, and antithrombin III (AT III) deficiency, whereas rare genetic defects such as hyperhomocysteinemia also established causes of thrombophilia, Intermediate genetic factors related to thrombophilia include elevated coagulation factors such as elevated factor VIII activity. Increase activities of factors IX and XI may also be associated with thrombophilia (Wahed and Disgupta, 2015).

##### 2.3.1.1. Factor V leiden:

Factor V Leiden is also known as Leiden type**,** APC resistance, and hereditary resistance to activated protein C **(**Rachal R,2020).is an inherited disorder which is increase tendency to form abnormal blood clots that can block a blood vessel. People with factor V Leiden thrombophilia have a higher than average risk of developing a type of blood clot called a deep venous thrombosis (DVT) and it is the most common inherited form of thrombophilia. (Bethesda,2020). particular mutation in the FV(G1691A) gene causes factor V Leiden thrombophilia. (Hoffbrand V, et al,2016)

The FV gene provides instructions for making a protein called coagulation factor V. This protein plays a critical role in the coagulation system, which is a series of chemical reactions blood clots in response to injury. The coagulation system is controlled by several proteins, including a protein called activated protein C (APC). APC normally inactivates coagulation factor V, which slows down the clotting process and prevents clots from growing too large. However, in people

with factor V Leiden thrombophilia, coagulation factor V cannot be inactivated normally by APC. As a result, the clotting process remains active longer than usual, increasing the chance of developing abnormal blood clots. (Bethesda,2020).

#### 2.3.1.2. Prothrombin G20210A mutation

Human prothrombin is a vitamin K-dependent glycoprotein synthesized by the liver. It is changed to thrombin by activated factor X, which has a vital role in forming the fibrin clot to stop bleeding at the injured site. A prothrombin gene mutation is the second most common mutation after factor V. In patients with prothrombin gene mutation, the guanine is substituted by adenine base pair in nucleotide position 20210 at the 3' untranslated region of the prothrombin gene. This mutation results in unchecked thrombin formation predisposing to the thromboembolic phenomenon. Homozygous and heterozygous are the two different genotypes associated with the prothrombin gene mutation. When the mutation is inherited from both parents, it is said to be homozygous for the gene mutation. If either of the paternal or maternal copies of the prothrombin gene is mutated, it is heterozygous for the gene mutation. (Puodel S, et al, 2020)

Geographical and ethnic differences in carrier frequencies of the prothrombin G20210A mutation appeared between healthy populations with a trend towards lower frequencies from south to north of Europe (range approximately from 0 to 4%). On the other hand, prothrombin G20210A mutation was found to be very rare or even absent in Asian and African populations and native populations of America (Amerindians) and Australia. In North America, the prevalence ranged from 1.6 to 2.4%, whereas in South America (Brazil), a prevalence of 0.7% was found. The prothrombin mutation seems to increase the risk of arterial thrombosis combined with other established risk factors (e.g. smoking, metabolic risk factors) at a young age. (Puodel S, et al,2020).

### 2.3.1.3. Protein C deficiency

Protein C is a vitamin K-dependent protease circulating in plasma at low concentrations and serves a critical role in the regulation of thrombin Levels. protein C maturation is later than many other coagulation proteins, with levels increasing from birth until 6 months and into puberty. Protein C becomes activated to form activated protein C (APC) by interactions with thrombin. APC acts to downregulate coagulation by cleaving and inactivating clotting factors V and VIII. A deficiency of protein C, and thus APC activity, leads to an inability to inactivate clotting factors and control thrombin production.

Protein C deficiency is a rare disorder, characterized by a reduction in the activity of protein C, a plasma serine protease involved in the regulation of blood coagulation. The active form of protein C, activated protein C (APC), exerts potent anticoagulant activity. A deficiency in protein C is characterized by the inability to control coagulation, resulting in the excessive formation of blood clots (thrombophilia) .it may be acquired or congenital. Congenital protein C deficiency results from mutations in the PROC gene. More than 160 PROC mutations have been described and may result in reduced levels of protein C (Type I) or the production of an altered protein C molecule with decreasing levels of activity (Type II). Protein C deficiency is an autosomal dominant condition. Mutations in a single copy in heterozygous individuals cause mild protein C deficiency, whereas individuals with homozygous mutations present with severe protein C deficiency. ( Gupta A,and Patipandla S 2021).

### 2.3.1.4. Protein S deficiency]

Protein S is an anticoagulant protein that facilitates the action of activated protein C (APC) on activated factor v (F5a) and activated factor viii (F8a). A deficiency in protein S characteristically demonstrates the inability to control coagulation, resulting in the excessive formation of blood clots (thrombophilia) and venous thromboembolism (VTE). Protein S deficiency can be hereditary or acquired. The acquired deficiency is usually due to hepatic disease, nephrotic syndrome, or vitamin K deficiency. Hereditary Protein S deficiency is an autosomal dominant pathology. Mutations in a single copy in heterozygous individuals cause mild protein S deficiency, whereas individuals with homozygous mutations present with severe protein S Deficiency Thrombosis is observed in both heterozygous and homozygous genetic deficiencies of protein S.

Congenital protein S deficiency caused due to mutation I the PROS1 gene, Most PROS mutations are point mutations, such as transversion mutations that produce a premature stop codon and thus result in a truncated protein S molecule. More than 200 PROS mutations have been described and may result in three different forms of protein S deficiency:

Type 1was quantitative defect presenting with low levels of total protein S (TPS) and free protein S (FPS), with reduced levels of protein S activity. while Type 2 (also known as type 2b) Decreased protein S activity, with normal levels of TPS and FPS antigens and Type 3 (also known as Type 2a): quantitative defect presenting with normal levels of TPS, but reduced levels of FPS and protein S activity. (Gupta A, Tun AM et al,2022).

**2.3.1.5. Antithrombin deficiency**

Antithrombin (previously termed antithrombin III) is a glycoprotein act as serpin (serine protease inhibitor) and its primary targets are thrombin, factor FXa and FIXa. Its concentration in the circulation is higher than that of prothrombin, and as only a fraction of prothrombin is converted to thrombin during coagulation, antithrombin is potentially present in vast excess over the protease, although the site will determine the local concentration of the enzyme and inhibitor. The plasma half-life of antithrombin is about 90 hours. .(Hoff brand V,et al,2016). Inherited AT deficiencies are uncommon, with prevalence in the general population between 1 in 500 and 1 in 5000. AT deficiency is associated with an increased risk for venous thromboembolism (VTE) and pregnancy loss. The association with arterial thrombosis is only weak. (Patnaik MM ,2008). The heritable antithrombin deficiency Type 1 is characterized by a quantitative reduction of antithrombin, with a parallel reduction in function (measured as inhibitory activity) and level of protein in the plasma (measured immunologically as the antigenic level). Type 2 deficiency is due to the production of a qualitatively abnormal antithrombin protein with dysfunction due to disturbance of the complex mechanism of protease inhibition by a mutation in SERPINC1 gene that cause abnormality in heparin binding side. The functional activity is discrepantly low compared with the antigenic level. Type 2 deficiency is sub classified according to the nature of the functional deficit. .(Hoffbrand V, et al,2016).

### 2.3.1.6. Hyperhomocystenemia

Homocysteine is a sulfhydryl-containing amino acid produced from the metabolism of the essential amino acid methionine. Homocysteine can undergo auto-oxidation, resulting in the formation of key, biologically reactive products that participate in signaling pathways associated with increased cell toxicity. It follows that homocysteine has been identified as a contributor to four fundamental mechanisms of disease: thrombosis oxidant stress, apoptosis, and cellular proliferation. Normal homocysteine metabolism is dependent upon adequate stores of three dietary vitamins: folic acid, vitamin B12 (cobalamin), and vitamin B6 (pyridoxal phosphate). Inherited hyperhomocystenemia is autosomal recessive disorder inborn error of metabolism (1:200,000 live U.S. births). (Bradley A, Maron M and LocalzoJ, 2009)

Hyperhomocystenemia may be caused by genetic abnormalities, but only the severe inherited abnormalities of homocysteine metabolism (homozygous cystathionine β-synthase deficiency and homozygous deficiency of methylenetetrahydrofolate reductase) result in congenital homocystinuria associated with an increased risk of both arterial and venous thrombosis, as well as premature atherosclerosis and mental retardation, epilepsy, and skeletal and eye problems. Half of patients present with venous or arterial thrombosis before the age of 30 years. .(Hoffbrand V,et al,2016).

### 2.3.2. Acquired thrombophilia

Acquired thrombophilia is associated with an increased risk of venous thromboembolism (VTE). Antiphospholipid syndrome (APS) is the most prevalent acquired thrombophilia and is associated with both venous and arterial thromboses. Human immunodeficiency virus (HIV) is another form of acquired thrombophilia. Risk factors associated with VTE in this population include those related to the disease itself, host factors, and the pharmacotherapy for HIV. A significant proportion of VTE events occur in patients with malignancies. There is an increase in mortality associated with patients having cancer who experience VTE when compared to patients having cancer without VTE. Combination oral contraceptive (COC) use infers risk of thromboembolic events. The risk is dependent upon the presence of an underlying inherited thrombophilia, the estrogen dose, and generation of progestin. Patients at highest risk of VTE include those receiving high-dose estrogen and fourth-generation, progesterone-containing

contraceptives. With the exception of APS, thrombophilia status does not alter the acute treatment of an initial VTE in non-pregnant patients (Armstrong E, et al 2014)

### 2.4. Myocardial infraction

#### 2.4.1. Over view of myocardial infraction

Myocardial infarction (MI), colloquially known as "heart attack," is caused by decreased or complete cessation of blood flow to a portion of the myocardium. Myocardial infarction may be" silent," and go undetected, or it could be a catastrophic event leading to hemodynamic deterioration and sudden death. Most myocardial infarctions are due to underlying coronary artery disease, the leading cause of death in the United States. With coronary artery occlusion, the myocardium is deprived of oxygen. Prolonged deprivation of oxygen supply to the myocardium can lead to myocardial cell death and necrosis. Patients can present with chest discomfort or pressure that can radiate to the neck, jaw, shoulder, or arm. In addition to the history and physical exam, myocardial ischemia may be associated with ECG changes and elevated biochemical markers such as cardiac troponins. (OjhaN, Dhamoon,2022) Troponin is the gold-standard for diagnostic evaluation of patients with suspected myocardial infarction (MI) (Neumann et al., 2019).

Myocardial infarction (MI), also commonly referred to as "heart attack," is necrosis of the heart muscle resulting from ischemia. The major underlying cause of IHD is atherosclerosis; while MIs can occur at virtually any age, the frequency rises progressively with aging and with increasing risk factors for atherosclerosis (Kuma Abbas and Aster ,2018).

#### 2.4.2. myocardial infarcts may take one of the following patterns

-Transmural infarctions: thrombus formation is a dynamic 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).

- Sub endocardial infarctions are MIs limited to the inner third of the myocardium; these infarcts typically do not exhibit ST segment elevations or Q waves on the ECG tracing (so-called "non–

ST-segment elevated MIs" or "NSTEMIs"), although they can have ST-segment depressions or T wave abnormalities. As mentioned earlier, the sub endocardial region is most vulnerable to hypo perfusion and hypoxia. Heart severe coronary artery disease, transient decreases in oxygen delivery (as from hypotension, anemia, or pneumonia) or increases in oxygen demand (as with tachycardia or hypertension) can cause sub endocardial ischemic injury. This pattern also can occur when an occlusive thrombus lyses before a full-thickness infarction can develop. • (Kumar, Abbas and Aster, 2018).

-Microscopic infarcts occur in the setting of small-vessel occlusions and may not show any diagnostic ECG changes. These can occur in the setting of vasculitis, embolization of valve vegetation or mural thrombi. (Kumar, Abbas and Aster, 2018).

### 2.4.3. Classification of myocardial infraction

MI also can be classified in to five types based on etiology and circumstance

Type 1: spontaneous MI caused by ischemia due to primary coronary event e.g(plaque rupture erosion or fissure coronary dissection

Type2: ischemic due to increase d oxygen demand e.g. (hyper tension or decreased supply, coronary artery spasm or embolism)

Type3: related to sudden un expected cardiac arrest

Type 4a: associated with percutaneous coronary intervention in which sign and symptoms MI with cTn value<5 ×$99^{th}$ percentage URL

Type 4b: associated with documented stent thrombosis

Type 5: associated with coronary artery by-pass grafting sign and symptoms of MI with values<10 × $99^{th}$ percentile URL (Sweis R, and Jivan A,, 2022)

### 2.4.4. Symptoms of myocardial infraction

The symptoms of myocardial infraction include chest pain with travel from left arm to neck , short ness of breath, sweating, nausea, vomiting ,abnormal heart beating, anxiety ,fatigue weakness ,stress depression .(LeiluM and RongR,2015).

### 2.4.5. Risk factor of myocardial infraction

Etiology of myocardial infarction is closely associated with coronary artery disease a risk factors for coronary artery disease include Smoking, Abnormal lipid profile/blood apolipoprotein (raised ApoB/ApoA1) Hypertension, Diabetes mellitus, Abdominal obesity (waist/hip ratio) (greater than 0.90 for males and greater than 0.85 for females).

Psychosocial factors such as depression, loss of the locus of control, global stress, financial stress, and life events including marital separation, job loss, and family conflicts.

Lack of daily consumption of fruits or vegetables, Lack of physical activity

Alcohol consumption (weaker association, protective)

The risk factors were significantly associated with acute myocardial infarction except for alcohol consumption, which showed a weaker association. Smoking and abnormal apolipoprotein ratio showed the strongest association with acute myocardial infarction. The increased risk associated with diabetes and hypertension were found to be higher in women, and the protective effect of exercise and alcohol was also found to be higher in women.

Other risk factors include a moderately high level of plasma homocysteine, which is an independent risk factor of MI. Elevated plasma homocysteine is potentially modifiable and can be treated with folic acid, vitamin B6, and vitamin B12. (OjhaN, Dhamoon ,2022). Genetics risk factor of MI, particularly if one has a male first degree relative (father, brother) who had a myocardial infarction before age55 years, or a female first relative (mother, sister) Genome wide association studies have found 27 genetic variants that are associated with an increased risk of myocardial infarction. (Steptoe and Kimivaki ,2012). The strongest association of MI has been found in chromosome 9 on short arm p at locus 21, which contain genes CDKN2A and 2B, although the single nucleotide polymorphisms that are implicated are within a non- coding region (Hooper, Martin and Davey smith ,2015).

### 2.4.6. Pathophysiology of myocardial infraction

The pathogenesis of acute myocardial infarction (AMI) and risky angina is the rupture of the coronary artery plaque resulting in acute thrombotic occlusion of a coronary artery. Consequently, the thrombus paperwork an essential part of the atherosclerotic coronary plaques.

There is a consensus that intracoronary thrombus makes up a difficult target for revascularization due to its precise traits. These interest opinions the cause and presentation of coronary artery thrombus (Adnan G, Singh DP, MahajanK, 2021). ischemia differs barely from myocardial hypoxia in that ischemia Myocardial infarction is the give up end result of both acute or chronic myocardial infraction. Myocardial effects in a stasis of waste merchandise of cellular metabolism in addition to a lack of oxygen transport, main to cell damage above and beyond that from hypoxemia. Myocardial infarction is a pathologic prognosis and depending on whether it's miles acute or persistent, is characterized by loss of ordinary cardiac myocyte shape (i.e., myocytolysis, coagulative necrosis, inflammatory cell infiltration, and fibrosis). Myocardial infarction has a bunch of reasons and is a main reason of cardiovascular disorder and loss of life in people. (Mark A. Oyama ,2009).

### 2.4.7. Diagnosis of Myocardial infarction

#### 2.4.7.1. Electrocardiogram (ECG)

Heart attack records electrical signals as they travel through the heart. Sticky patches (electrodes) are attached to the chest and sometimes the arms and legs. Signals are recorded as waves displayed on a monitor or printed on paper. An ECG can show if you are having or have had a heart attack (BarretK, Bell M, Belloli F, 2022)

**2.4.7.2. Blood tests:** Certain heart proteins slowly leak into the blood after heart damage from a heart attack. Blood tests can be done to check for these proteins (cardiac markers). (BarretK, Bell M, Belloli F, 2022) but cardiac troponin and creatine kinase-MB can be used as biomarkers for the diagnosis, treatment and prognosis of MI (Shi N and Shen B, 2019) 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 ( Ruseva, 2005)

**2.4.7.3. Chest X-ray:** A chest X-ray shows the condition and size of the heart and lung

**2.4.7.4. Echocardiogram:**. Sound waves (ultrasound) create images of the moving heart. This test can show how blood moves through the heart and heart valves. An echocardiogram can help identify whether an area of damaged heart.

**2.4.7.5. Coronary catheterization (angiogram):** A long, thin tube (catheter) is inserted into an artery, usually in the leg, and guided to the heart. Dye flows through the catheter to help the arteries show up more clearly on images made during the **test**

**2.4.7.6. Cardiac CT or MRI:** These tests create images of the heart and chest. Cardiac CT scans use X-rays. Cardiac MRI uses a magnetic field and radio waves to create images of your heart. For both tests, you usually lie on a table that slides inside a long tubelike machine. Each test can be used to diagnose heart problems. They can help show the severity of heart damage. (BarretK, Bell M, Belloli F… et al,2022).

### 2.4.8. Treatment of myocardial infarction

A number of different medication can also be used to treat heart attack

- Aspirin to break up the blood clot
- Antiplatelet to prevent new clot formation and existing clots from growing such as clopidogrel
- Nitroglycerin used as vasodilator
- Diuretic help to decrease fluid build up (Ballingit,2021)

### 2.4.9. Total white blood cell and myocardial infarction

Although an elevated white blood cell counts an indicator of inflammation, has been accepted as part of healing response of myocardial infarction and associated with cardiovascular disease (NunezJ2005).

## 2.5. Ribosomal protein s20

Ribosomes are essential components of the protein synthesis machinery. The process of ribosome biogenesis is well organized and tightly regulated. (Wang W, Nag S, Zhang X…et al, 2015) Ribosomes are organelles that catalyze protein synthesis consists of small 40S subunit and large 60S sub unit together these sub units are compose of 4RNA species and mainly 80 structurally distance protein, (Hassan F et al, 2021). Ribosomal proteins (RPs) have extra ribosomal functions that are involved in cell proliferation, differentiation, apoptosis, DNA repair, and other cellular processes. The dysfunction of RPs has been linked to the development and progression of hematological, metabolic, cardiovascular diseases and cancer. (Wang W et al, 2015).

Ribosomal protein S20 gen encodes ribosomal protein that is a component of the 40Ssubunit.the protein consist of 119 amino acids and its molecular mass is 13373 Da, belong to S10P family of ribosomal protein. Its located in the cytoplasm. Ribosomal protein s20 gen is co transcript with the small nuclear RNA gen U54, (which located in chromosome 8P12.1in the second intron. As is typical for genes encoding ribosomal proteins. Two transcript variant encoding different isoform have been identified for this gen (Leider B et al, 2022) (Saftan M et al, 2022)

Ribosomes are vital to life and generate all proteins needed to grow cells and sustain them. A mature ribosome consist of consists of small 40S subunit and large 60S sub unit together these sub units are composing of 4RNA (Hassan F et al,2021) ribosome protein S20 belong to S10Ufamily and lay in chromosome 8 P13 the variant of ribosome protein S20 gen A>88T rs (56073198) polymorphism was detected in Diamond black fan anemia also associated with anoval colorectal cancer but in another position A145+177G>A.Diamond black fan anemia and colorectal cancer are both inherited disorders with platelet disturbance. BFA is a ribosomopathy disorder that includes macrocytic anemia, erythroid hypoplasia, and megakaryocytic dysplasia with thrombocytosis. This condition is also linked to 5q Showman syndrome, (Bahr S et al, 2020) which RPS20 considers a risk factor for it, and colorectal cancer, which was described by MCGwan K et al in 2011. Who suggested that platelets are a fundamental component of tumor microenvironments, All BFA, 5qShowman Syndrome, and CRC are dependent on P53, and RPS20 is one of the activators of P53. (Wang P,et al, 2022). This study was based was found by (Bahr S et al, 2020), who discovered the RPS20 A88T polymorphism in diamond black fan

anemia patients. And based on study of (Nieminen T et al) at2014 also detected a novel RPS20 gen polymorphism in CRC. ((Nieminen T et al.2014).

### 2.5.1. Extra ribosomal function of ribosomal protein S20 gen

The main function of the RPs is ribosome assembly and the maintenance of the efficiency and accuracy of translation. Defects in the RPs themselves will lead to impaired protein synthesis, and as such, the level of critical tumor suppressors may be decreased below a threshold level. This may lead to the cell not being protected from genotoxic and other insults, which can ultimately cause malignant transformation. Mutations or loss of certain RPs may dramatically affect the level or function of their binding partners (either tumor suppressors or oncogenes).

Ribosomal proteinS20 act as activator to **protein 53** (ZhouX, 2015)

P53 is essential for maintaining the genomic stability during cell growth and division the predominant negative regulator of p53 is MDM2. MDM2 and p53 form a negative feedback loop, in which p53 activates MDM2 transcription and MDM2, in turn, inactivates p53 by targeting it for ubiquitination and proteasomes in normal cells, the p53 protein is maintained at low levels through this MDM2-p53 negative feedback loop. In response to various stress signals, the inhibitory effect of MDM2 on p53 can be circumvented by multiple cellular mechanisms. (Wang W, Nag S& Zhang X, 2016). Ribosomal protein S20 act as activator to P53 by binding to MDM2 to inhibit MDM2 E3 ligase activity, leading to p53 stabilization and cell cycle arrest (Daftuar L, Zhu Y& Jacq X, 2013).

### 2.5.2. Hematological disease associated with ribosomal protein S20 gen polymorphism

Diamond black fan anemia is one of ribosomopathyis disorder associated with RPL5, L10, L13, S5&S20, In Diamond Black Fan anemia, the induction of p53 causes p21 accumulation and subsequent cell cycle arrest in erythroid progenitor cells and megakaryocytic dysplasia leading to hypo proliferative anemia. Clinical evidence also suggests that mutations in the large ribosomal unit proteins are linked to specific physical abnormalities observed in DBA patients. L5 is associated with higher incidences of cleft lip/palate and cardiac anomalies associated with DBA. (Wang W, Nag S& Zhang X, 2016)(Wang P. DBA patients show increased apoptosis in erythroid cells of the bone marrow and p53-mediated apoptosis is the cause of the developmental phenotypes observed in mice with mutations in S19 and S20, (Warner J and McIntosh K, 2009)

Low expression of RPS20 cause Shwachman diamond syndrome (SDS) is a rare autosomal recessive disorder manifested as bone marrow failure, skeletal deformities, and exocrine pancreatic hypoplasia and leukemia's (.Wang W, Nag S& Zhang X, 2016)

# Chapter III

# Materials and Methods

# Materials and methods

## 3.1. Study design

Descriptive cross section hospital based study was conducted in Khartoum state

## 3.2. Study area

The study was conducted at Alshaab hospital, in Khartoum state

## 3.3. Study period

This study was being achieved in the period from (March,2022to decemberr2022 in Khartoum state

## 3.4. Study population

Fifty Sudanese patients diagnosed with myocardial infarction by ECG and cardiac enzyme in emergency department in Al-shaab hospital.but three of sample is lossed due to fault in storage of this sample.

## .3.5. Inclusion criteria

Sudanese patient diagnosed with myocardial infarction was been included in the study either STEMI or NSTEMI in all age groups.

## 3.6. Exclusion criteria

Patients with leukemia's, DIC, cancers, inflammations and other heart disease were excluded from this study.

## 3.7. Ethical considerations

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

## 3.8. Data collection method and tools

Clinical data were collected by non-self-questionnaire and recording forms.

### 3.8.1. Sampling

The sample size in this study was calculated for each category (on average) to give a maximum of error (0.05) with a probability of ($\alpha = 0.05$). The formula bellow was used

$$n = \frac{z^2 . p . q}{d^2}$$

$$N = \frac{(1.96)^2 \text{ x } (0.23) \text{ x } (0.77)}{(0.05)^2} = 272$$

z = the value in normal curve corresponding to level of confidence 95% = 1.96

p = probability prevalence in the community is (average prevalence reported in Khartoum 23% (Omer M et al 2016)

q = (1-p) = 1-0.23= 0.77

d = margin of error = 0.05

But in this study the study population about 50 patients due to limited time and difficult to obtain the sample from myocardial infarctions patients, beside the financials issues.

### 3.8.2. Sample collection:

Two ml of venous blood was collected from each patient with myocardial infarction in EDTA tube.

### 3.9. DNA extraction method

Three hundred microliter(0.3 ml) was taken from whole blood and added to 0.9ml from red cell lysis buffer then centrifuged for 2minuts at10000 round and rejected the supernatant , this step was repeated until got the clear supernatant then add 0.3ml from cell lysis buffer to the re-suspended cell and up to down to lyse this cell, after that 0.1ml precipitate the protein by added protein precipitate buffer to the cell lysate, and vortex vigorously at high speed for 20seconds and put the sample in 20- C for 5-minuts then centrifuged3minuts in 30000 round and transfered0.3-0.4ml from the supernatant to other tube and 0.3ml 100% Isopropanol was added next mix it by inverting gently and centrifuge at 30000rpm(DNA was visible as small white pallet)pour of the supernatant and add 70%alcohol ,centrifuge 30000rpm for1minuts and drain

the tube in clean absorbent paper and allow to air dry for 15minutes then add 0.1ml from DNA hydration buffer to dissolve the DNA pallet and incubate it at 65C for 30mnt finally it's ready to use and store DNA at20-C.

### 3.10. DNA storage:

DNA was preserved at -20°C until PCR was performed.

### 3.11. Primer design

Primers for PCR was based on the references assembly Homo sapiens (RPS20) NC 000008.11: r s (56073198) in the NCBI by primer 3soft war program

**Table 3.1. Primer sequence for S20 gen**

| Primer name | Primer sequence | Product size bp |
|---|---|---|
| Forward primer | TCGCTTGTGAATTCTCATCTGG | 290bp |
| Reverse primer | AACAGGCGCAAGCTCTAAGG | |

**Table 3. 2. PCR mixture by using master mix tube**

| Reagent | volume |
|---|---|
| Distilled water | 14 |
| Master mix | 4 |
| Forward primer | 1 |
| Reverse primer | 1 |
| DNA | 5 |
| total | 25 |

**Table 3. 3. PCR protocol and cycles**

| Profile | temperature | Time duration | Number of cycles |
|---|---|---|---|
| Initiation denaturation | 95 | 5minutes | 1 |
| denaturation | 94 | 30 second | 35 |
| annealing | 55 | 30 second | 35 |
| extension | 72 | 30 second | 35 |
| Final extension | 72 | 5 minutes | 1 |

### 3. 12. Detection of PCR product

Detection of the product done by gel electrophoresis by using 1.5% agarose gel which stained by ethidium bromide, and 1X Tris EDTA buffer (TE) used as running buffer, 5µl of the product was applied into the gel, the voltage was 100 volt for 30 minutes and DNA ladder (100bp) was used as molecular weight marker.

### 3.13. RPS20 digestion

Half ul of restriction enzyme (SfaNI) was added to 7.5ul of distil water, 2ul of enzyme buffer and 10ul of PCR product a quick spinning is needed, incubated at 37 ºC 16 hours, and the reaction was automatically Stopped then 18 µl digested products was loaded into 1.5% agarose.

### 3.14. Data analysis

Data were entered and analyzed by SPSS version 26 program to calculate independent T test and chi square test with mean and Standerd deviation

# Chapter IV
# Results

# Results

## 4. Results

### 4.1. Demographic data

This study aimed to investigate the genetic variant of ribosomal protein S20gen among Sudanese patient diagnosed with myocardial infarction. A total of 47patient with myocardial infarction represent more than 76% population were males conducted in this study the age respondent ranged from39 – 82. The mean of age was 58±10years and more than 76% of patient from Khartoum state, represent in **(table 4.1)**

### 4.2. Clinical data

The percentage of previous history thrombophilia and myocardial infraction was 17%, 44% **(figur4.1)**. The most clinical symptoms explanted as Chest pain 87.2% shortness of breathing 55.3%, anxiety 44%. that represent in table **4. 2** In chronic disease the hypertension represents 31% and diabetes mellitus 27% both 21%. and risk factors under study included: obesity29% and smoking42%. that show in (table **4. 3**). The laboratory finds test the mean of total White blood cell, was 8.4±2.24×109\Lto show significant increase of TWBCs in patient with chronic disease(P:0.00) and insignificant for risk factors( (P: 0.5)that represent in **table 4.4**

### 4.3. Genotyping

**Figure (4.2)** represent All the patient confirmed to have myocardial infarction had the wild gene and hence were negative for ribosome proteinS20 gene mutation .Digestion of RPS20 gene with SfaNI on 1.5% agarose gel dissolved in 1X TBE buffer, stained with ethidium bromide, Lane M molecular weight marker 100bp , Lane 2 undigested (290bp), Lane ,3and 4 were wild type (AA).

**Table 4.1.1. Demographic data in myocardial infarction patient**

| | | Frequency (%) | P.value |
|---|---|---|---|
| **Age** | <50years | 11 (23.4%) | 0.04 |
| | >50year | 36 (76.6%) | |
| **Gender** | Male | 36 (76.6%) | 0.04 |
| | Female | 11 (23.4%) | |
| **Residence** | Khartoum | 36 (76.6%) | 0.00 |
| | Jazeera | 6 (12.8%) | |
| | Kasala | 1 (2.1%) | |
| | Dongola | 1 (2.1%) | |
| | Gadarif | 1 (2.1%) | |
| | South kordufan | 1(2.1%) | |
| | Port Sudan | 1(2.1%) | |
| | | | |
| **History of thrombophilia** | No | 39 (83.0%) | 0.03 |
| | Yes | 8 (17.0%) | |

*Made by using chi-square test; (P.value set to level < 0.05).

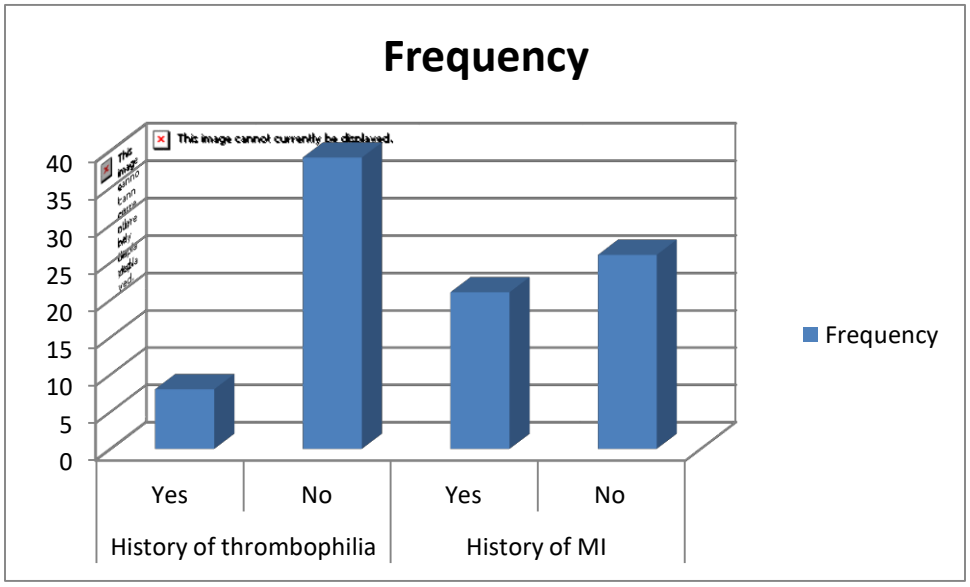



**Figure 4.1.1 Distribution of history of thrombophilia and previous myocardial infraction Related to RPS20 among patient with myocardial infarction.**

**Table 4.1.2 Frequency of clinical symptoms in myocardial infarction patient**

| | | n Frequency | Percent% |
|---|---|---|---|
| **Chest pain** | Yes | 41 | 87.2 |
| | No | 6 | 12.8 |
| **Shortness of breath** | Yes | 26 | 55.3 |
| | No | 21 | 44.7 |
| **Cold sweating** | Yes | 8 | 17.0 |
| | No | 39 | 83.0 |
| **Vomiting** | Yes | 8 | 17.0 |
| | No | 39 | 83.0 |

**Table 4.1.3 Frequency of risk factor and chronic disease among patient with myocardial infarction**

| | **(n) frequency** | **Percent%** |
|---|---|---|
| **smoker** | 19 | 42 |
| **Obese** | 13 | 29 |
| **Obese &smoker** | 8 | 17 |
| **HTN** | 14 | 31 |
| **DM** | 12 | 27 |
| **DM&HtN** | 9 | 21 |

**Table (4.1.4) Correlation of TWBCs to risk factor and chronic disease**

| | | **Mean± Std. Deviation** | **P.Value** |
|---|---|---|---|
| Risk factor | Smoking | 7.9412±2. | 0.2 |
| | Obesity | 7.7727±2 | |
| | Smoke/obesity | 6.6333±1 | |
| Chronic disease | DM | 7.8231±2 | 0.008 |
| | HTN | 7.4462±2 | |
| | DM/HTN | 9.0444±1 | |
| | SLE | 12.7000 | |

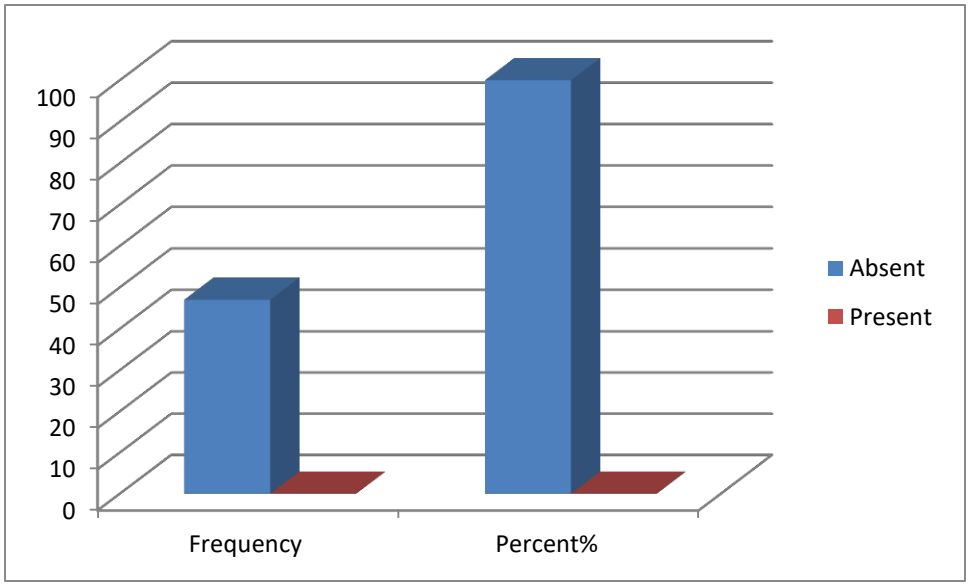



**Figure 4.1.2: Distribution of ribosomal s20 polymorphism among MI patients**

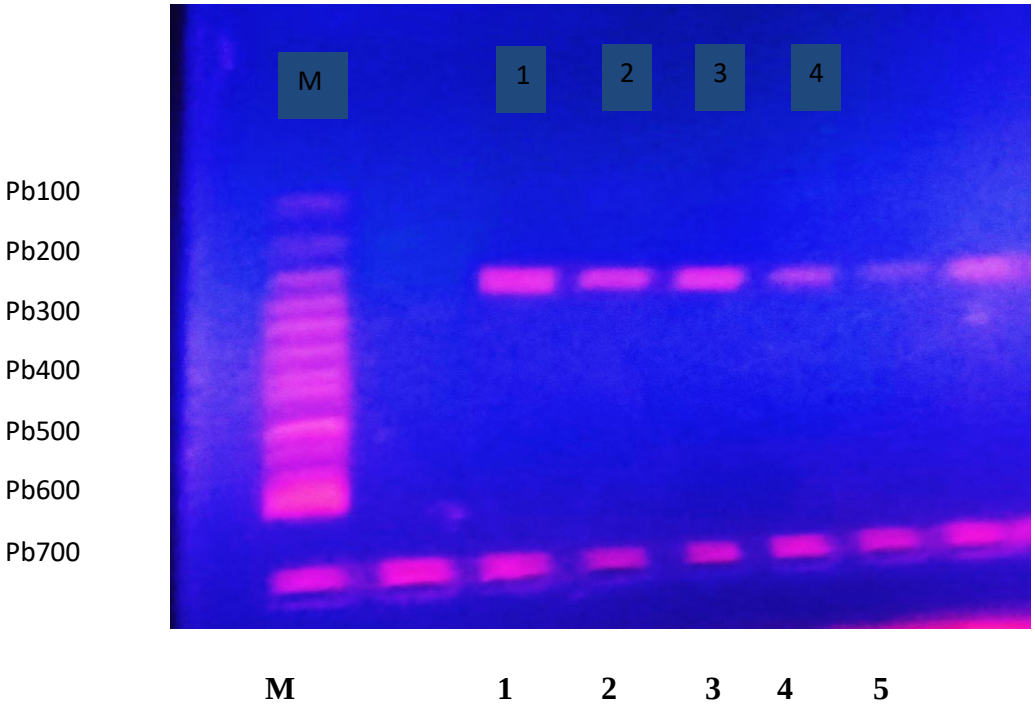



**Figure (4.1.3): PCR amplification of ribosome protein S20 gene mutation** Digestion of RPS20 gene with SfaNI on 1.5% agarose gel dissolved in 1X TBE buffer, stained with ethidium bromide, Lane M molecular weight marker 100bp , Lane 2 undigested (290bp),Lane ,3and 4 were wild type (AA).

# Chapter V

# Discussion, Conclusion &Recommendation

## 5.1 Discussion

In this hospital based descriptive cross sectional study, about 50 patients with myocardial infarction involved to determine the presence of ribosome protein S20 polymorphism. Patients were mostly men, and as age rise, increasing the incidence of myocardial infarction. The study agrees with Alkheder B study done in 2016 in MI Sudanese patient and represent the high incidence of myocardial infraction associated elder patients. the majority of patients come to Khartoum due to the city's big migrant population. The main clinical symptoms of myocardial infarction were chest pain and difficult breathing and this result agree with LeI LuM and rong R (2015) the but his study show chest pain and anxiety represent in all myocardial infarction patient.

This research represented the obesity and smoking were the common risk factors for myocardial infarction this study supported the study of Neeland I. et al in 2017.'who found the obesity was linked to older myocardial infarction. Additionally, agreed with (Dicker D et al, 2016.) who Suggested that, the smoking and obesity both increase the incidence of myocardial infarction as while disagree with Ahmed M In 2017, who presented the overweight and increased BMI body max index were not associated with myocardial infarction. In this study the diabetes mellitus and hypertension as were the major chronic disease in myocardial infraction patient, this result was consistent to (Ahmed, M,2017) Who represented the strong association of myocardial infarction with Diabetes mellitus' and hypertension in Sudanese patient (AhmedM,2017).

In this study History thrombophilia was not associated with MI, whereas recurrent MI was common among MI Sudanese patients with myocardial infarction.

Therefore, this study showed that the total white blood cells (TWBCs) were significantly increased in Sudanese patients with myocardial infarction who had a chronic disease. This result is consistent with ( Nunez J,2005) , who confirmed that WBCs were elevated might used as a marker for morbidity in myocardial infarction patients. this study agreed with study of (shanker A et al 2004) who represented the elevated WBCs count was associated with incidence of hypertension in cardiovascular disease.

The risk factor with TWBCs in the present research found No correlation of risk factors with TWBCs which in contrasted to with study of Jamshidi Z and Seif A in Iran since 2017 whom represented a relationship between WBCs count and obesity as a risk factor.

All 47 myocardial infarction patients have a wild allele for ribosome protein S20 and thus are negative for the RPS20gen A88T polymorphism. There was no statistically significant correlation between ribosome protein S 20 gen polymorphism and acute myocardial infarction. due to the low prevalence of RPS20 polymorphism in African population .(Musumeci, et al 2010).This study was limited by Collected the sample from one area leading to decrease the diversity in study population and Detection of polymorphism in one position is not enough to understand potential mechanistic contribution of this gene.

## 5.2. Conclusion

Age above 50 years consider as risk factor of myocardial infarction, most patient were male (67%) chest pain (87%)& shortness of breath (55%) were the main symptoms of myocardial infraction, recurrent myocardial infarction is wide distributed in Sudan, smoking and obesity both are highly risk factor of myocardial infarction and myocardial infarction is highly related with hypertension and diabetes mullets. All 47 patients whom diagnosed with myocardial infarction represent the wild allele(AA) from ribosomal protein S20 gen and the mutant allele (TT) is not detected, that indicate for no association between myocardial infarction and ribosome proteinS20 poly morphism and elevated white blood cell count had a strong relation with chronic disease in myocardial infarction patient and not association found for risk factor.

## 5.3. Recommendation

**At the present study recommended the following**

-The sample size should be increased collect the study group from different Sudanese state to increase the diversity of population.

-increase the Auditee of result by using the northern blotte technique, which detects the mRNA of ribosome protein gene.

-identify a novel alleles of Ribosomal protein S20 in MI by using DNA sequencing

# References

# References


**Adnan G,** Singh DP and Mahajan K**,** 2022 coronary artery thrombus in statpearl. Treasure island publishing 2022jan

**Ahmed M,** Awadalla H, ALmobarak,A, 2017,prevelance and risk factor for acute coronary syndrome among Sudanese individuals with diabetes: apopulation based study, pol J Radiol;81, 138-140

**Armstrong E ,**Bellone J ,hornsbyL **et al** 2014 Acquired thrombophilia, pharmacy practical vol 27(3) page234 – 242

**Ashorobi, D, Ameer, M**., Fernandez, R. (2021) thrombosis, Statpearls Publishing, Treasure Island puplishing January 2022

**Bahr S,**Morries D,Bergestrom K, et al 2021,expansion of germline RPS20 mutation phenotype to include Diamond black fan anemia Hum mutat 41(11) 1918-1930

**Balingit A** 2021 acute myocardial infarction( heart attack),health line

**BarretK,** Bell M& Belloli F, 2022 heart attack diagnosis and treatment, mayoclinnic

**Bethesda R.** (2020). Factor v Leiden thrombophilia, heart attack MedlinePlus Genetics. MedlinePlus 48:5.

**Bochenek M** BaurT, NadirY et al 2018 typeI and type I Imyocrdial infarction, blood Adv. 2(11): 1300 – 1314

**Boekholdt S** and Kramer M. (2007) Arterial thrombosis and the role of thrombophilia. Semin Thromb Hemost; 33(6):588-96

**Bradley M and** Localzo J, 2009 the treatment of hyper homocysteinemia , annual review of medicine ,vol 60 page39- 50.

**Daftuar L,** Yan Zhu Y , Jacq X, et al 2013 Ribosomal Proteins RPL37, RPS15 and RPS20 Regulate the Mdm2-p53-MdmX Network. Journal pone vol 8 issue 7

**DickerD,** Feldman B, leventer M et al 2016, factor contributes more to the incidence of myocardial infarction, European journal of internal medicine ,( 32) 43-46

**Elkhader B,** Abdulla A and Omer M ,2016, correlation of smoking and Sudanese male patient above 40 years age, pol J Radioj 81:138-140

**Furie B**. (2009) Pathogenesis of thrombosis. Hematology Am Soc Hematol Educ Program. 2009(1): p 255–258

**Gale A** and Andrew J2011 "Continuing Education Course #2: Current Understanding of Hemostasis." Toxicological Pathology, volume. 39, no. 1, pp. 273–280.,

**Gupta A** and PatipandlaS 2022 protein S deficiency. Stat Pearls . Treasure Island (FL):2022 Jan.

**Gupta A,** Tun AM, Gupta K, et al 2022. Protein S Deficiency. Stat Pearls . Treasure Island (FL):2022 Jan.

**Hall J**. (2010) Guyton and Hall Textbook of Medical Physiology, Hemostasis and blood coagulation: Enhanced E-Book. 11th ed. Philadelphia: Elsevier Health Sciences: pp. 457–459

**Hassan F,** AL sultan A , Al-Zahrani F et al 2021 Genetic Variants of RPL5 and RPL9 Genes among Saudi Patients Diagnosed with Thrombosis MED ARCH. ; 75(3): 188-193

**Hillard A,** Wincheter D, Russell T et al 2020, myocardial infraction classificationand it, s implications on measure of cardiovascular out comes, quality, and racial/ethic disparities clin cardiol(43) 1076 – 1083

**Hoffbrand V,** Higgs D, Keeling et al, Post graduate hematology 7th edition London UK pp 44page 796 - 797

**Hooper L,** Abdelhamid A and Davey Smith G. (2015) "Reduction in saturated fat intake for cardiovascular disease". The Cochrane Database of Systematic Reviews.6(6)

**Jamshidi L** and Seif A,2021,association between obesity, white blood cell and platelet count, Zehedan J Res Med Sci 19(2)

**KaushansK,** Lichman M, Prchal J etal 2015,Williams Hematology. 8th ed. New York,

**Kumar,** Abbas and Aster, ,2018 Robbins basic Pathology. 12th ed. Philadelphia: , PA: Saunders Elsevier, chapter11.pp. 415–420.

**Leider B** 2022 ribosomal protein S20 Homosapenies NCBI

**Leilu M** and Rong R, 2015.Myocardial Infarction: Symptoms and Treatments. Cell Biochem Biophys. 72(3): p 865-7

**luscher TF**.(2015) FESC mi mechanisms, diagnosis and complications. European heart journal. 36(16): p 947-949
.

**Mcgowan A,**PangW,BashG, et al,2011 reduced ribosomal protein gen dosag and P53activation in low risk myelodysplastic syndrome, blood, 118(13) p 3622-3633

**Meier C** and Oyama M 2009 myocardial infarction ,molecular biology and transient data science direct

**Monroe DM,** Hoffman M, Roberts HR .(2010) Molecular biology and biochemistry of the coagulation factors and pathways of hemostasis McGraw-Hill : pp 614–630

**Mustafa A,** fraser G,El Omer M et al,2015, transdermal in STelevation myocardial infarction, current cardiology reports vol17(30)

**Musu L** ,Arther J ,Lippman S et al, 2022, single nucleotide differences in the dbSNP database may lead to error in genotyping and haplotyping Studies, Hum Mutat.31(1)67-73

**Neeland I,** Das S, Simon D et al 2017 the obesity pardox,extreme obesity and long term outcome in older adults with ST-segment elevation myocardial infarction :result from the NCOR, EuropeanHeart jounral of quality of care and clinical outcome (3), 183-191

**Neumann JT,** Sorensen NA, Rubsamen N, …et al. (2019) Evaluation of a new ultra-sensitivity troponin I assay in patients with suspected myocardial infarction. Int J Cardiol. 283(1): p 35-40.

**NieminenT,** Abdel-rehman W ,peltomaki P 2014, germline mutation of RP S20, encoding ribosomal protein, causes predisposition to hereditary non polyposis colorectal carcinoma without DNA mismatch repair deficiency Gstroentermology;147:595-598

**Nunez j,** facila L,sanchis J et al 2005, prognosis value of white blood cell count in acute myocardial infarction Rev Esp cardio 58(6):631-9

**Ojha N** and Dhamoon **A,** 2022S. Myocardial Infarction, stat Pearls Treasure Island (FL): Stat Pearls Publishing; 2022 January

**Palta S,** Saroa R, and Palta A. (2014) Overview of the coagulation system. Indian J Anaesth; 58(5): pp 515–523.

**Patnaik MM** ,2008 inherited antithrombophin deficiency: areview haemophilia, 14(6) 1229 – 3.

**Puodel S,** KondapaneniV, cancarevic I et al,2020 association of G20210A prothrombin gene mutation and cerebral ischemic strocke in young patient. Cureus12(12)

**Ruseva A.** (2005) Laboratory diagnosis of acute myocardial infarction. Trakia J Sci. 3(1): p 8–14.

**Saftan M** et al2022 ribosome protein S20 Homosapenies Gen Card

**ShankerA,** Kelein B andKlein B 2004 relationship between white blood cell count and incidence of hypertension, American journal f hypertension, Vol17(3)233-239

**Shaz, B,** Hillyer, C, Reyes M…et al, (2019). Transfusion medicine and hemostasis: Clinical and laboratory aspects third edition, Elsevier

**SteptoA** and Kivimaki M 2012 stress and cardiovascular disease. Nature review cardiology, 9(6) 360-370.

**Sweis R** and Jivan A, 2022 acute myocardial infraction(MI)cardiovascular disorder MSD manual professional edition

**Theisler, C.** (2022) "Myocardial infarction/heart attack," *Adjuvant Medical Care*, pp. 231–233.

**Thompson B, Snow A,Kuptiuch C, et al,** 2020, a novel ribosome protein S20 variant in family with unexplained colorectal cancer and polyposis, bioRxiv inter national license

**Wahed A,** Quesada A and disgupta R.**,** 2019 hematology and coagulation second edition USA page237 - 276

**Wang W,** Nag S, and Zhang X, 2016 Ribosomal Proteins and Human Diseases: Pathogenesis, Molecular Mechanisms, and Therapeutic Implications HHS Public Access35(2): 225–285
.

**Wang B**, ZoaW, and cao H, 2022, development of platelet-related prognosis model for colorectal cancer, Front genet, Jan2022

**York M**. (2013) Compressive guid to toxicology in preclinical drug development: Clinical pathology, 1st ed. United states. Academic press, Elsevier: p 167-211

**Zhan, C,** Shi M., Wu, R. et al(2019). MIRKB: A myocardial infarction risk knowledge base. Oxford

**Zhou X,** Liao W, Liao J et al,2015, Ribosomal proteins: functions beyond the ribosome: Journal of Molecular Cell Biology, 7(2), 92–104

# Appendices

Sudan University of science and Technology College of graduate studies

Hematology and immunohematology department

**Genetic Variant of ribosomal proteinS20 gene among Sudanese patient diagnosed with myocardial infarction**

**NO ( )**

Name ..... Sample number .....

Age .... Gender ☐ Male ☐ Female

Residence ..... telephone number.....

History of thrombophilia ☐ yes ☐ No

Treatment use .....

history of myocardial infraction ☐ yes ☐ No

Treatment use ……

Chronic disease

☐DM ☐ hypertension

……

Risk factor :( ) Obesity ( ) smoking

Sign and Symptoms

pressure or tightness in the chest ☐ yes ☐No

shortness of breath. ☐ yes ☐No

Cold sweating. ☐ yes ☐No

vomiting. ☐ yes ☐No

anxiety. ☐ yes ☐No

other Laboratory finding

……
…….....

2- polymerase chain reaction
resultl……
……

**Molecular reagents:**

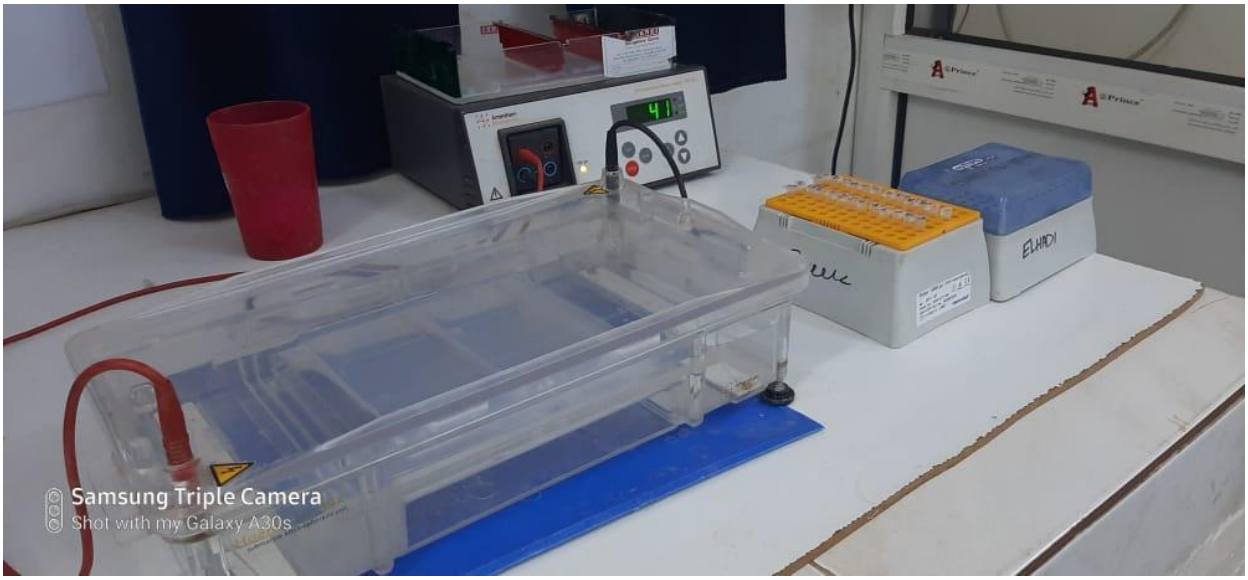
RBCs lysis buffer consist of 8.3 g NH4CL, 1.19 g of NaHCO3 or KHCO3, 1.8 g of 5% EDTA dissolved in 1 litter of D.W.

-WBCs lysis buffer contain 7.88 g of Tris HCL, 5.44 g of EDTA, 0.146 g of NaCL and 10g of TBS dissolved in 500 ml of D.W.

-Agarose gel 1.5% prepared by dissolving 6 g of agarose powder in 100 ml TBE buffer 1xdilluted by 900 ml of distelled water to prepare 10XTBE and this solution must be heated in microwave for 9 minutes until the powder dissolve completely. -1x TBE buffer contain 27 g of Tris base powder ,14 g of boric acid and 1.9 g of EDTA dissolved in 2500 ml of D.W.

-Master mix solution containing 0.25U/ul Taq DNA polymerase, 0.4 Mm dNTPs, 3.2 mM Mg+2, 0.02% bromophenol blue and 2x reaction buffer at optimal concentrations for efficient amplification of DNA template by PCR.


**Appendices 2 PCR machine**




**Appendices3 Gel electrophoresis machine**