Sudan University of Science and Technology

College of Graduate Studies

Detection of Genetic Variant of Ribosomal Protein S20 RPS20 among Sudanese Patients with Stroke

الكشف عن المتغير الجيني للبروتين الريبوسومي S20 لدى المرضى السودانين المصابين بالسكتة الدماغية

A thesis submitted for partial fulfillment of the requirements for the degree of M.Sc. Degree in Medical Laboratory Science (Hematology and Immunohematology)

Submitted by

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قال تعالى:

أعوذ بالله من الشيطان الرجيم

﴿وَلَقَدْ خَلَقْنَا الْإِنْسانَ مِنْ سُلالَةٍ مِنْ طِينٍ (12) ثُمَّ جَعَلْناهُ نُطْفَةً فِي قَرارٍ مَكِينٍ (13) ثُمَّ خَلَقْنَا النُّطْفَةَ عَلَقَةً فَخَلَقْنَا الْعَلَقَةَ مُضْغَةً فَخَلَقْنَا الْمُضْغَةَ عِظامًا فَكَسَوْنَا الْعِظامَ لَحْمًا ثُمَّ أَنْشَأْناهُ خَلْقًا آخَرَ فَتَبارَكَ اللَّهُ أَحْسَنُ الْخالِقِينَ (14)﴾

صدق الله العظيم

(سورة المؤمنون الآية 12ـــــ 14)

Dedication

To my lovely parents....

To my sisters and brother....

To my husband and my son...

For their endless love, encouragement and support

To my friends....

And you ....

I dedicate this work with love.

Acknowledgement

Our grateful thanks firstly to **Allah** who guided us to the straightway in our life and to provide us with all things to complete our research. Then many thanks to our kindly supervisor **Prof. Fathelrahman Mahdi Hassan** for his valuable advices follow up and endless efforts to make this work come into reality. The help and facilities provided by **Dr. Salah Jomaa** are indeed appreciated, thanks to my teacher **Dr. Abdallah Musa** for his endless advices. Our grateful appreciation should be extended to my lovely families who supported me. The help and support provided by my friends in college of medical science and laboratory are gratefully acknowledged.

ABSTRACT

Stroke, the second biggest cause of mortality worldwide, has a direct impact on quality of life. It has been established that the ribosomal protein S20 (RPS20) gene is linked to both colorectal cancer (CRC) and Diamond Black fan anemia (DBA).

In this descriptive cross sectional study; 50 Sudanese patients who had been diagnosed with stroke were included (36 men, or 72% of the total, and 14 women, or 28% of the total), which was done in Khartoum State between July and December 2022. The mean of patients' age was 64.7 years, with a range of 40 to 82. 34% from patients have both DM and HTN, 28% have HTN, 24% have diabetes mellitus, and 14% are disease-free. They experienced previous strokes on average once in 40 (80%), twice in 5 (10%), and three or more times in 5 (10%). 34 (68%) patients lack a family history of stroke, while 16 (32%) have family history.

Blood samples were collected from stroke patients in EDTA containers after filling the questionnaire. Salting out method was used to extract the DNA, and PCR-restriction fragment length polymorphism was used to determine the frequency of gene polymorphism in patients. The digested fragment was run on a 1.5% agarose gel which stained with ethidium promide, and visualized using a UV light source. Two bands were produced, one representing the wild-type gene AA (290 pb) and the other the mutant type gene AT (250 + 50 pb). The statistical package for social science (SPSS) version 21 computer application was used to analyze the results.

The results revealed that the frequency of the normal RPS20 gene AA was 46 (92%) and the frequency of the mutant RPS20 gene AT was 4 (8%). There was no significant difference in the frequency of mutation between males and females ($p = 0.88$), between the frequency of mutation and various age groups ($p = 0.29$), or between the frequency of mutation and associated chronic diseases ($p = 0.69$). There was a statistically significant difference between gene mutation and the number of prior strokes (p.value = 0.019).

In conclusion ribosomal protein S20 gene mutation was detected in 8% of stroke patients, further studies should be done to determine whether RPS20 mutation can be a potential risk for stroke as well as be a target for diagnosis and treatment.

المستخلص

السكتة الدماغية ثاني اكبر سبب للوفيات قي جميع انحاء العالم, لها تأثير مباشر على الحياة. ثبت أن جين البروتين الريبوسومي S20 مرتبط ب سرطان القولون والمستقيم وانيميا بلاك فان دايموند.

أقيمت هذه الدراسة الوصفية المستعرضة في الخرطوم في الفترة بين يوليو وديسمبر 2022 , حيث تم إختيار 50 مريضا سودانيا تم تشخيصهم بالسكتة الدماغية (36 من الذكور 72٪ و 14 من الإناث 28٪). وكان متوسط الأعمار 64.7 عاما، حيث تتراوح الأعمار بين 40 و 82 عاما. 34٪ من المرضى يعانون من مرض السكر والضغط, 28٪ يعانون من الضغط, 28٪ لديهم السكر و 14٪ ليس لديهم أي مرض مزمن. كان معدل تكرار السكتة الدماغية السابقة كالأتي: مرة واحدة عند 40 (80٪)، مرتين عند 5 (10٪)، أو 3 مرات و أكثر عند 5 (10٪). 34 (68٪) من المصابين ليس لديهم تاريخ عائلي بالسكتة الدماغية و 16 (32٪) لديهم تاريخ عائلي بالمرض.

بعد تعبئة الاستبيان جمعت عينات الدم من المرضى في وعاء يحتوي على مانع للتخثر إثيلين ثنائي الأمين ثلاثي حمض الخليك. أستخدمت طريقة التمليح لاستخراج الحمض النووي و سلسة التفاعل المبلمر (تعدد أطوال جزء الحصر) التحديد معدل الطفرة الجينية في المرضى. تم فصل الجزئية المقطوعة في 1.5٪ هلام مصبوغ ببروميد الإيثديوم وظهرت من خلال استخدام مصدر للأشعة فوق البنفسجية. تم التعرف على قطعتين واحدة تمثل الجين السائد AA عند طول 290 والأخرى تمثل الجين المتحور AT عند طول 250+40. تم تحليل النتائج باستخدام برنامج الحزم الإحصائية للعلوم المجتمعية النسخة 21.

أظهرت النتائج أن معدل الجين الطبيعي كان 46 (92٪) والجين المتحور كان 4 (8٪). لايوجد اختلاف معنوي في معدل الطفرة بين الرجال والنساء (القيمة المحتملة = 0.88) أو بين الطفرة والفئات العمرية المختلفة (القيمة المحتملة = 0.29) أو بين الطفرة والأمراض المزمنة المصاحبة (القيمة المحتملة = 0.69). أظهرت النتائج زيادة معنوية واضحة بين الطفرة وعدد مرات الإصابة بالسكتة الدماغية (القيمة المحتملة = 0.019).

ختاما تم اكتشاف الطفرة الجينية للبروتين الريبوسومي S20 في 8٪ من مرضى السكتة الدماغية, الدراسات القادمة يجب أن تختبر ما إذا كان هذا المتحور يمكن أن يكون عامل خطر للسكتة الدماغية اوان يكون كهدف للتشخيص والعلاج.

CONTENTS

List of Tables

List of figures

Abbreviations

aCL	anticardiolipin antibodies
ADP	Adenine diamine phosphate
APC	Activated protein C
APCR	Activated protein C resistance
Apl	Antiphospholipid antibodies
APS	Antiphospholipid syndrome
Arg	Arginine
AT	Anti-thrombin
β2GPI	β2 glycoprotein I
CNS	Central nervous system
CRC	Colon rectal cancer
CT	Computed tomography
CVAs	Cerebrovascular accident
DBA	Diamond-Blackfan anemia
DEG	Differentially expressed gene
DM	Diabetes mellitus
DVT	Deep venous thrombosis
GP	Glycoproteins
HC	Hperhomocysteinemia
HIT	Heparin induced thrombocytopenia
HMWK	High-molecular weight kininogen
HTN	Hypertension
IAT	Intra-arterial thrombolysis
ICH	Intracerebral hemorrhage

INR	International Normalization Ratio
IVT	Intravenous thrombolytic
LA	Lupus anticoagulants
LMWH	Low molecular weight heparin
MRI	Magnetic resonance imaging
MTHFR	Methylene tetrahydrofolate reductase
MW	Molecular weight
OC	Oral contraceptive
PC	Protein C
PCR	Polymerase chain reaction
PE	Pulmonary embolism
PoL I	Polymerase I
POL III	Polymerase III
PS	Protein S
PT	Prothrombin time
PTT	Activated partial thromboplastin time
RPG	Ribosomal protein gene
RPs	Ribosomal proteins
rRNA	Ribosomal RNA
TE	Tris EDTA buffer
TIA	Transient ischemic attack
VTE	Venous thromboembolism
vWF	Von Willebrand factor

CHAPTER ONE

INTRODUCTION

Chapter one

Introduction

1.1 Introduction

Hypercoagulability is a general hematologic concept that means an increased risk of thrombosis via enhanced levels of prothrombotic components in the bloodstream. This hypercoagulability is due to many alterations in the coagulation and hemostatic system, which can result from inflammatory factors, variations in the viscosity of blood and blood components, increased cytokines, and prothrombotic proteins in circulation, or deficiencies of natural or endogenous anticoagulant factors. (Ashorobi *et al*., 2022).

Thrombosis is a blood clot within blood vessels that limits the flow of blood. Acute venous and arterial thromboses are the most common cause of death in developed countries. The mortality rate varies with the location and acuity of thrombosis. Myocardial infarction and cerebrovascular accident (CVAs) account for the highest proportion of thrombosis associated death in the United States. The cause of thrombosis is multifactorial. Thrombosis occurs when there is an imbalance in endogenous anticoagulation and hemostasis through a complex pathophysiologic mechanism. (Ashorobi *et al.*, 2022).

Stroke is the second leading cause of death and a major contributor to disability worldwide. The prevalence of stroke is highest in developing countries, with ischemic stroke being the most common type. Considerable progress has been made in our understanding of the pathophysiology of stroke and the underlying mechanisms leading to ischemic insult. (Kuriakose and Xiao, 2020).

The incidence and prevalence of ischemic stroke has evolved over time. In 2016, the global incidence of ischemic stroke events was 9.5 million. In 2017, there were 2.7 million deaths due to ischemic stroke. (Campbell *et al.*, 2019).

Genetic risk is proportional to the age, sex and race of the individual but a multitude of genetic mechanisms can increase the risk of stroke. Firstly, a parental or family history of stroke increases the chance of an individual developing this neurological disorder.

Secondly, a rare single gene mutation can contribute to pathophysiology in which stroke is the primary clinical manifestation. Thirdly, stroke can be one of many after-effects of multiple syndromes caused by genetic mutation. Fourthly, some common genetic variants are associated with increased stroke risk. (Kuriakose and Xiao, 2020).

Therefore, this study will look for ribosomal protein S20 genetic variants among Sudanese stroke patients. Then, additional research would be conducted to determine whether or not this mutation contributes to the genetic predisposition to stroke or can be used as a target for diagnosis or treatment.

1.2 Rationale

Stroke is the second leading cause of death globally. It affects roughly 13.7 million people and kills around 5.5 million annually. The major risk factors for stroke are hypertension, diabetes, lack of physical exercise, alcohol and drug abuse, cholesterol, diet management and genetics. (Kuriakose and Xiao, 2020). Thrombosis has directly affected the quality of life with an increased rate of mortality. In the USA, more than 500,000 patients were diagnosed with deep venous thrombosis (DVT) each year. (Hassan *et al*., 2021). This study therefore looks for ribosomal protein-related genetic issues, which have been linked to human disease in numerous studies. In Sudan, to my best of knowledge we did not found any previous researches to study the association between RPS20 polymorphism and stroke. Therefore, this study would serve as an important key point for subsequent research into the possibility that this polymorphism contributes to the genetic susceptibility to stroke.

1.3 Objective

1.3.1 General objective

To detect genetic variant of ribosomal protein S20 among Sudanese patients diagnosed with stroke.

1.3.2 Specific objectives

- To detect genetic variant of ribosomal protein S20 among Sudanese patients diagnosed with stroke in both male and female with different age groups.

- To correlate between ribosomal protein S20 mutation and family history of the stroke patient.

- To identify ribosomal protein S20 mutation and some chronic disease associated with stroke.

- To compare between ribosomal protein S20 mutation and frequency of previous stroke.

CHAPTER TWO

LITRATURE REVIEW

Chapter two

Literature review

2.1 Hemostasis

The maintenance of circulatory hemostasis is depends on the process of balancing bleeding (hemorrhage) and clotting (thrombosis). Hemostasis depends on several components; the four major components are the vascular system, platelets (thrombocytes), blood coagulation factors and fibrinolysis (Turgeon, 2012).

2.1.1 The hemostatic functions

To maintain blood in a fluid state, to arrest bleeding at the site of injury by formation of a hemostatic plug, to limit this process at the site of the damage and to ensure the removal of the plug when healing is complete (Laffan, 2011)

2.1.2 Platelet production

Platelets are produced by fragmentation of the cytoplasm of megakarycytes, one of the largest cells in the body. (Hoffbrand and Moss, 2016).

Megakarycytes are up to 160 mm in size, the nuclear-cytoplasmic (N: C) is 1:12 and nucleoli are not visible (Turgeon, 2012).

The megakaryoblast arises by differentiation from the hemopoietic stem cell, the megakaryocyte matures by endomitotic synchronous replication (in the absence of nuclear or cytoplasmic division) and the cytoplasm becomes granular at the eight nucleus stage, each megakaryocyte give rise to 1000-5000 platelets taking 10 days from differentiation of stem cell to the production of platelets. (Hoffbrand and Moss, 2016).

2.1.3 Platelet structure

Platelets are a nucleate fragments derived from bone marrow megakaryocytes, they are 1.5 – 3.0 μm in diameter have a volume of 7 fl, by electron microscope show a fuzzy coat (glycocalix) on the surface; composed of membrane glycoproteins (GP), glycolipids, mucopolysaccharides and

plasma protein, Platelets have a channel system called open canalicular system which is composed of invaginations of the plasma membrane, also they have a dense tubular system which act as the major site of platelet thromboxane synthesis (Shmaier and Lazarus, 2012).

2.1.4 Platelet functions

Following the damage to the endothelium of a blood vessel, a series of events occur, including adhesion to the injured vessel, shape change, aggregation, and secretion (Turgeon, 2012).

2.1.4.1 Adhesion

When injury occur in the blood vessel, platelets adhere to exposed collagen (adhesion) through interactions of von Willebrand factor (vWF), and a specific glycoprotein complex on the platelet surface glycoprotein (GP) (Ib–IX), this interaction is important for platelet adhesion (Shmaier and Lazarus, 2012).

2.1.4.2 Aggregation

Following the adhesion the platelet form clumps this process called aggregation, that involve binding of fibrinogen to platelet receptors (IIb and IIIa) progressing the aggregation (Shmaier and Lazarus, 2012).

2.1.4.3 Secretion (release reaction)

Activated platelets release contents of their granules from dense granule, alpha granules and the lysosomal vesicles, ADP and serotonin released from the dense granules enhance the platelet activation after binding to specific receptor on platelet (Shmaier and Lazarus, 2012).

2.1.4.4 Platelet procoagulant activities

Several key enzymatic reactions of blood coagulation occur on the platelet membrane surface during platelet activation negatively charged phospholipids of the plasma membrane have role in accelerating specific coagulation reactions that occur on the platelet surface and leading to thrombin generation (Shmaier and Lazarus, 2012).

2.1.5 Hemostatic response

2.1.5.1 Vasoconstriction

In the injured vessel constriction of adjacent small arteries and arterioles is responsible for an initial slowing of blood flow to the area of injury, when there is widespread damage this vascular reaction prevents exsanguinations and allow activation of platelets and coagulation factors. (Hoffbrand and Moss, 2016).

2.1.5.2 Platelet reactions and primary hemostatic plug formation

Following the break in the endothelial lining, platelets adhere to the connective tissue potentiated by vWF. The exposure of collagen and production of thrombin at the site of injury stimulate the platelets to release their granule contents and activation of platelet for prostaglandin synthesis leading to the formation of thromboxane A2, then release ADP causes platelets to swell and aggregate, additional platelets are drawn to the area of injury. The continuing platelet aggregation promotes the growth of the hemostatic plug that soon covers the exposed connective tissue; this primary hemostatic plug provides temporary control of bleeding. (Hoffbrand and Moss, 2016).

2.1.6 The Mechanism of Coagulation

The initiation of the coagulation process may occur via one of two pathways: the extrinsic pathway and the intrinsic pathway, the outcome of this process is the conversion of circulating insoluble coagulation factors into a gelatinous fibrin clot with entrapped blood cells (Turgeon, 2012).

2.1.6.1 Coagulation factors

Coagulation factors can be classified into three groups:

1. The fibrinogen group (factors I, V, VIII, and XIII) is consumed during coagulation, factors V and VIII are labile and increase during pregnancy and inflammation (Ciesla, 2012).

2. The prothrombin group (factors II, VII, IX,and X) is dependent on vitamin K that play an important role in carboxylation of glutamic acid that need for calcium binding (Ciesla, 2012).

3. The contact group (factor XI, factor XII, prekallikrein, and high-molecular-weight kininogen [HMWK]) participates in the intrinsic pathway (Ciesla, 2012).

The (tissue factor, factor V, factor VIII, Fitzgerald factor and factor XIII) are cofactors which accelerate the activities of enzymes that participate in the coagulation cascade (Ciesla, 2012).

2.1.6.2 Extrinsic pathway

Is initiated with the release of tissue thromboplastin that form complex with factor VII this complex activate factor X to Xa that convert prothrombin to thrombin then thrombin converts fibrinogen to fibrin, this process done in 10-15 seconds. The reaction then enters the common pathway (Ciesla, 2012).

2.1.6.3 Intrinsic Pathway

Vascular trauma induces factor XII activation to factor XIIa (simultaneously) in the presence of the prekallikrein following by activation of factor XI to factor XIa in the presence of HMWK then Factor XIa activates factor IX to factor IXa which converts factor X to factor Xa in the presence of factors (VIIIa and PF3) and calcium, the reaction then enters the common pathway (Ciesla, 2012).

2.1.6.4 Common Pathway

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

2.1.7 Fibrinolysis

Fibrin clots are temporary structures that seal off a damaged area until healing can take place. Fibrinolysis is the physiological process that removes insoluble fi brin deposits by enzymatic digestion of the stabilized fi brin polymers. As healing occurs, the clots themselves are dissolved by plasmin. Plasmin digests fi brin and fi brinogen by hydrolysis to produce progressively

smaller fragments. This slow-acting process gradually dissolves away the clot as tissue repair is taking place, with the particulate matter being phagocytized by the mononuclear phagocytic system. (Turgeon, 2012).

2.1.8 Thrombophilia

The most common cause of death in the United State is thrombosis; more than 2million people die from arterial or venous thrombosis every year. Thrombophilia is any inherited or acquired disorder associated with increased tendency to thrombosis. (Mckenzi and Williams, 2010).

Thrombi are solid masses or plugs formed in the circulation, their clinical significance results from ischaemia from local vascular obstruction or distant embolization. Thrombi are involved in the pathogenesis of myocardial infarction, cerebrovascular disease, peripheral arterial disease and deep vein occlusion. (Hoffbrand and Moss, 2016).

2.1.8.1 Arterial thrombosis

Atherosclerosis of the arterial wall, plaque rupture and endothelial injury expose blood to subendothelial collagen and tissue factor. This initiates the formation of a platelet nidus on which platelets adhere and aggregate. The intrinsic pathway of fibrin formation is involved in pathological thrombosis in vivo by contact activation on damaged blood vessels. (Hoffbrand and Moss, 2016).

As blocking arteries locally also an emboli of platelet and fibrin may break from the primary thrombus and move to distal arteries such as, carotid artery thrombi leading to cerebral thrombosis and transient ischemic attacks, and heart valve and chamber thrombi leading to systemic emboli and infarcts. (Hoffbrand and Moss, 2016).

The risk factors for arterial thrombosis are related to the development of atherosclerosis which based on gender, age, elevated blood pressure, high levels of serum cholesterol, glucose intolerance, cigarette smoking and electrocardiogram abnormalities. (Hoffbrand and Moss, 2016).

2.1.8.2 Venous thrombosis

Virchow's triad suggests that there are three components that are important in thrombus formation; slowing down of blood flow, hypercoagulability of the blood and vessel wall damage. (Hoffbrand and Moss, 2016).

Patients with sepsis, in-dwelling catheters and sites of damage to veins by previous thrombosis are highly risk for venous thrombosis. (Hoffbrand and Moss, 2016).

2.1.8.3 Hereditary thrombophilia

Inheried thrombophilia refers to individuals with predisposing genetic defects resulting in tendency to thrombosis. (Mckenzi and Williams, 2010).

Patient diagnosed with hereditary thrombophilia usually present with:

1. Venous thromboembolism at young age.

2. Recurrent venous thromboembolism.

3. Family history of venous thromboembolism.

4. Thrombosis at unusual site. (Mckenzi and Williams, 2010).

2.1.8.3.1 Factor V Leiden gene mutation

Factor V is one of the essential clotting factors in the coagulation cascade. Its active form, factor Va, acts as a cofactor allowing factor X to stimulate the conversion of prothrombin to thrombin. Thrombin is then able to cleave fibrinogen to fibrin and a fibrin clot is formed. Activated protein C is a natural anticoagulant it limits the extent of clotting by destroying factor V and reducing further thrombin formation. (Van and Veeger, 2011).

Factor V is susceptible to inactivation by activated protein C (APC) by cleaved of factor Va at Arg506, Arg306 and Arg679 by APC. Factor V Leiden is not susceptible to inactivation by (APC) at Arg506 due to transition of guanine to adenine at nucleotide 1691(G1691) result in replacement of arginine by glutamine result in activated protein C resistance (APCR). (Khan and Dickerman, 2006).

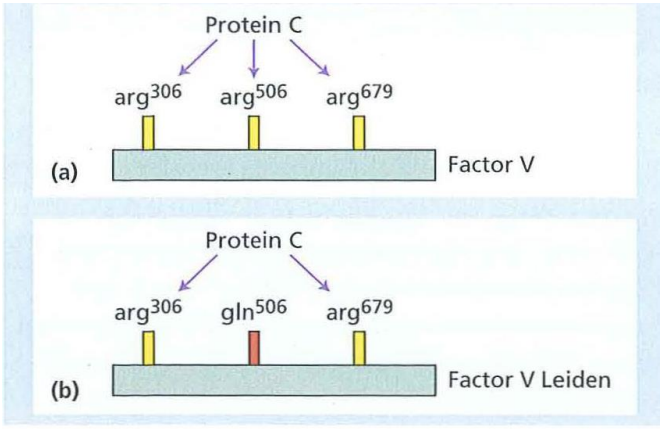


Figure 2.1 The genetic basis of factor V Leiden **(a)** Activated protein C (APC) inactivates factor Va by proteolytic cleavage at three sites in the Va heavy chain. **(b)** In the factor V Leiden mutation the Arg506Gln polymorphism leads to glutamine at position 506 with less efficient inactivation of factor V by APC and increased risk of thrombosis. (Hoffbrand and Moss, 2016).

Factor V (Leiden) poses a lifelong risk of deep venous thrombosis (DVT) with a greater frequency of occurrence of thrombi in the lower limbs than in the chest. (Turgeon, 2012).

2.1.8.3.2 Prothrombin gene mutation G20210A

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 mutation. (Poudel, 2020).

Prothrombin gene (G20210A) mutation is associated with an increased risk of thrombosis and it is the most identifiable risk factor for venous thrombosis and is in fact the second most common genetic defect for inherited thrombosis. (Khan and Dickerman, 2006).

Results of G→A substitution in the 3' untranslated region of prothrombin gene (nucleotide20210) is associated with elevation of prothrombin level, which contribute to an increased thrombotic risk by increased thrombin generation. (Mckenzi and Williams, 2010).

2.1.3.8.3 Protein C deficiency

PC is a vitamin K dependent inhibitor of coagulation, reduction of PC level predispose to venous thrombosis due to inability to inhibit thrombin production lead to fibrin generation. There are two major types of congenital PC deficiency: type I the most common and characterized by decreased of both antigenic and functional activity of PC but in type II there are normal PC antigenic level but reduced functional level. (Mckenzi and Williams, 2010).

Over 160 different mutations in the PC gene, which encodes protein C have been linked to PC deficiency. (Roshal**,** 2013).

PC deficiency is increased risk of DVT and warfarin- induced skin necrosis. (Khan and Dickerman, 2006).

2.1.3.8.4 Protein S deficiency

PS is a cofactor for APC and inherited in an autosomal dominant manner, there are three type of PS deficiency; type I characterized by low total antigen and functional activity, type II characterized by normal concentration of both total and free PS but decreased of functional activity and type III characterized by normal total PS but a decreased free and functional PS concentration. (Mckenzi and Williams, 2010).

Sixty percent of protein S circulates in a protein bound form, and only the remaining 40% free form is biologically active. Certain conditions, such as pregnancy, inflammation, and surgical stress, lead to increased levels of the complement 4b-binding protein, which binds to protein S, and thereby decrease protein S activity (Namee *et al*., 2012).

PS deficiency associated with DVT and tendency to skin necrosis with warfarin therapy. (Hoffbrand and Moss, 2016).

2.1.3.8.5 Antithrombin deficiency

AT is the major inhibitor of the serine proteases involved in coagulation, reduction of AT level predispose to venous thrombosis. (Mckenzi and Williams, 2010).

Most AT deficiencies are a result of an inheritance of a single defective allele of the SERPIN1 gene located on chromosome 1. (Roshal, 2013).

Deficiency of AT is transmitted as an autosomal dominant disorder, it is two types; type I which is deficient of quantity and type II is a defect of a qualitative nature which characterized by decreased heparin cofactor activity. (Turgeon, 2012).

2.1.3.8.6 Hperhomocysteinemia

Homocysteine is an amino acid that metabolized either by transsulfuration to cystathionine or by remethylation to methionine. (Mckenzi and Williams, 2010).

HC occurs when amino acid accumulate in the blood due to impaired metabolism of homocysteine. Genetic HC results from production of variant of methylene tetrahydrofolate reductase (MTHFR) with reduced enzymatic activity. The gene contains an alanine to valine substitution at amino acid 677(C677T). (Khan and Dickerman, 2006).

HC is associated with increased risk for both venous and arterial thrombosis. (Hoffbrand and Moss, 2016).

However, lowering homocysteine levels with vitamin therapy has not resulted in improved outcomes in vascular disease and thrombosis. (Greer *et al.*, 2014).

2.1.3.8.7 Dysfibrinogenemia

Dysfibrinogenemia is defined by a defective fibrinogen molecule that forms clots which are difficult to degrade by fibrinolytic agents. Dysfibrinogenemia can be associated with both venous and arterial thrombosis. (Greer *et al.*, 2014).

The abnormal fibrinogen usually exhibits an abnormal thrombin-mediated conversion to fibrin. Patients with dysfibrinogenemia are clinically asymptomatic some are present with bleeding diathesis. (Khan and Dickerman, 2006).

2.1.8.4 Acquired disorder

Acquired thrombophilia s range from rare disorders such as Behçet's disease to the very common initial presentation of malignancy. Acquired thrombophilia may present at any age. (Deloughrey, 2019).

2.1.8.4.1 Venous stasis and immobility

These factors are responsible for the postoperative venous thrombosis and for venous thrombosis associated with congestive cardiac failure, myocardial infarction and varicose veins. In atrial fibrillation, thrombin generation from accumulation of activated clotting factors leads to a high risk of systemic embolization. The use of muscle relaxants during anaesthesia may also contribute to venous stasis. Venous thrombosis also has a higher frequency after prolonged aeroplane journeys. (Hoffbrand and Moss, 2016).

2.1.8.4.2 Inflammatory Bowel Disease

Patients with inflammatory bowel disease are at higher risk for thrombosis. Autopsy series show that 33% of patients had thrombi present at the time of death. Patients with inflammatory bowel disease have been shown to have reduced levels of free protein S. (Deloughrey, 2019).

2.1.8.4.3 Surgery

The surgical thrombophilia is complex. Venous stasis due to immobility during surgery and the recovery process certainly plays a role. The inflammatory response with the release of inflammatory cytokines is also important. (Deloughrey, 2019).

2.1.8.4.4 Heparin induced thrombocytopenia

Heparin is used to prevent or treat thrombosis. Its use can cause thrombocytopenia in some patients associated with platelet activation. HIT is due to an autoantibody directed against heparin complexed with platelet factor 4.The most serious complication of HIT is activation of clotting system and thrombosis, which can be life threatening. The most common venous thrombotic complications are DVT, PE and arterial thrombosis. (Mckenzi and Williams, 2010).

2.1.8.4.5 Nephrotic Syndrome and Other Renal Disease

Nephrotic syndrome has long been associated with a thrombophilia. Patients with nephrotic syndrome have an increased incidence of renal vein and other thrombosis. Thrombophilia in nephrotic syndrome is due to urinary loss of natural anticoagulants also low levels of both antithrombin and protein S. (Deloughrey, 2019).

2.1.8.4.6 Hemolytic disease

Patients with a broad spectrum of acquired and congenital hemolytic diseases appear to be at a higher risk of thrombosis. The thrombosis associated with hemolysis is due to damaged red cells. One constituent of the red cell membrane, phosphatidylserine, is very effective at promoting coagulation. (Deloughrey, 2019).

2.1.8.4.7 Pregnancy

The condition of hypercoagulability in pregnancy is most likely evolved to protect women against the bleeding challenges of childbirth or miscarriage. Pregnant women are at a four- to five fold increased risk of thromboembolism during pregnancy and the postpartum period compared to nonpregnant women. Normal pregnancy beginning is associated with increased concentrations of coagulation factors VII, VIII, and X and von Willebrand factor. In addition, a significant change in fibrinogen is noted and PS is decreased. (Turgeon, 2012).

2.1.8.4.8 Oral contraceptive

High dose of OC have been associated with increased venous and arterial thrombosis related to the estrogen dose. The mechanism by which OC induce a prothrombotic state are unclear its use is associated with changes in the level of maney coagulation proteins including increases of fibrinogen, F-VII, and F- VIII abd decreases of AT and PS. (Mckenzi and Williams, 2010).

2.1.8.4.9 Malignancy

The association between thrombosis and malignancy has been recognized for more than 100 years. The causes of thrombosis in cancer patient are complex, the three component play a significant role in thrombogenesis; stasis, activation of blood coagulation, and vascular injury are present in patient with malignant disease. (Mckenzi and Williams, 2010).

2.1.8.4.10 Antiphospholipid syndrome

Antiphospholipid syndrome (APS) is an acquired prothrombotic disorder characterized by the presence of antiphospholipid antibodies (aPL). Antiphospholipid antibodies are autoantibodies that target phospholipid-binding proteins, and may cause prolongation of phospholipid-dependent coagulation assays such as the activated partial thromboplastin time (PTT). The aPL that are most strongly correlated with pathologic thrombosis are lupus anticoagulants (LA), anticardiolipin antibodies (aCL) and anti-β2 glycoprotein I (anti-β2GPI) antibodies. The target for most aPL appears to be β2-glycoprotein I (β2GPI). (Lim, 2013).

Antiphospholipid antibodies (APLAs) are associated with venous thrombosis, arterial thrombosis, neurological disease, frequent miscarriages, and thrombocytopenia. (Deloughrey, 2019).

2.1.8.5 Investigation of thrombophilia

Tests that may be abnormal in patients with a tendency to thrombosis include:

- Blood count, Blood film examination and.erythrocyte sedimentation rate.

- Prothrombin time (PT), APTT, thrombin time (and reptilase time) and fibrinogen assay

- Anticardiolipin and anti-β2-GPI antibodies.

- DNA analysis for factor V Leiden and Prothrombin gene analysis for the G20210A variant.

- Antithrombin, protein C and protein S – immunological and functional assays.

- Plasma homocysteine estimation. (Hoffbrand and Moss, 2016).

2.1.8.6 Diagnosis of thrombosis

Using these methods; clinical suspicion, plasma D-dimer concentration, compression ultrasound, computed tomography (CT) and magnetic resonance imaging (MRI). (Hoffbrand and Moss, 2016).

2.1.8.7 Treatment of thrombophilia

Anticoagulant drugs are used widely in the treatment of venous thromboembolic disease. Their value in the treatment of arterial thrombosis is less well established. An orally or parenterally active drug is available acting either directly or indirectly at a specific site or at multiple sites in the coagulation cascade. (Hoffbrand and Moss, 2016).

2.1.8.7.1 Parenteral drugs

2.1.8.7.1.1 Heparin

This acidic unfractionated mucopolysaccharide of average molecular weight (MW) 15 000–18 000, the effective biological half-life is approximately 1 hour. Heparin potentiates the formation of complexes between antithrombin, thrombin (IIa) and factors IXa, Xa and Xia.This complex formation inactivates these factors irreversibly. Also heparin impairs platelet function . Itis monitored by maintaining the APTT at 1.5–2.5 times the ULN (upper limit of the normal) value. (Hoffbrand and Moss, 2016).

Low molecular weight heparin (LMWH) preparations (MW 2000–10 000) have a greater ability to inhibit factor Xa than to inhibit thrombin and interact less with platelets than standard heparin. Is used routinely for treatment of deep vein thrombosis (DVT) and pulmonary embolus (PE), is also used as prophylaxis of venous thrombosis, pregnant women; because heparin does not cross the placenta. Heparin is also used during cardiopulmonary bypass surgery. (Hoffbrand and Moss, 2016).

2.1.8.7.1.2 Other drugs

Fondaparinux, a synthetic analogue of the antithrombin-binding pentasaccharide of heparin, is an indirect factor Xa inhibitor, bivalirudin is used as an alternative to heparin in patients undergoing percutaneous coronary interventions and argatroban is a small molecule direct inhibitor of thrombin given by continuous intravenous infusion. (Hoffbrand and Moss, 2016).

2.1.8.7.2 Oral anticoagulants

2.1.8.7.2.1 Warfarin

Warfarin, a coumarin which is vitamin K antagonists, used to decrease biological activity of the vitamin K-dependent factors II, VII, IX and X. Used for recurrent venous thrombosis, for embolic complications of rheumatic heart disease or atrial fibrillation, and with prosthetic valves and arterial grafts and in selected patients with the APS. The dose is monitored by PT and INR, warfarin can cross placenta so avoided during pregnancy. (Hoffbrand and Moss, 2016).

2.1.8.7.2.2 Fibrinolytic agents

Two fibrinolytic agents, streptokinase and tissue plasminogen activator, are most frequently used to lyse fresh thrombi. These drugs may be used systemically for patients with acute myocardial infarction, major PE and locally in patients with acute peripheral arterial occlusion. (Hoffbrand and Moss, 2016).

2.1.8.7.2.3 Antiplatelet drugs

Aspirin is an antiplatelet agent that inhibits platelet cyclo-oxygenase irreversibly, thus reducing the production of platelet thromboxane A2. It is commonly used in patients who have a history of coronary artery or cerebrovascular disease. (Hoffbrand and Moss, 2016).

2.1.8.7.2.4 Other drugs

Dabigatran is an oral thrombin inhibitor, used for prevention of cerebral and systemic embolism also rivaroxaban and apixaban, these are factor Xa inhibitors, they are used for prevention of cerebral and systemic embolism and treatment and prevention of DVT and PE. (Hoffbrand and Moss, 2016).

2.2 Stroke

Stroke is a leading cause of death and disability worldwide and can be broadly classified into ischemic stroke and hemorrhagic stroke. (Campbell *et al.*, 2019).

Stroke is the second leading cause of death globally. It affects roughly 13.7 million people and kills around 5.5 million annually. Approximately 87% of strokes are ischemic infarctions. (Kuriakose and Xiao, 2020).

2.2.1 Pathophysiology of Stroke

Stroke is defined as an abrupt neurological outburst caused by impaired perfusion through the blood vessels to the brain posteriorly (the circle of Willis). (Kuriakose and Xiao, 2020)

2.2.1.1 Ischemic stroke

Ischemic stroke is caused by deficient blood and oxygen supply to the brain; ischemic occlusions contribute to around 85% of casualties in stroke patients. Ischemic occlusion generates thrombotic and embolic condition in the brain. In thrombosis blood flow is affected by narrowing of vessels due to atherosclerosis. The build-up of plaque will eventually constrict the vascular chamber and form clots, causing thrombotic stroke. In an embolic stroke, decreased blood flow to the brain region causes an embolism; the blood flow to the brain reduces, causing severe stress and untimely cell death (necrosis). (Kuriakose and Xiao, 2020).

Other key events contributing to stroke pathology are inflammation, energy failure, loss of homeostasis, acidosis, increased intracellular calcium levels, acidosis, increased intracellular calcium levels, free radical-mediated toxicity, cytokine-mediated cytotoxicity, complement activation, impairment of the blood–brain barrier and activation of glial cells. (Kuriakose and Xiao, 2020).

2.2.1.2 Hemorrhagic stroke

Hemorrhagic stroke accounts for approximately 10–15% of all strokes and has a high mortality rate.in this condition the stress and the brain tissue and internal injury cause blood vessels to rupture. It produces toxic effects in the vascular system, resulting in infarction .It is classified into intracerebral and subarachnoid hemorrhage. In ICH, blood vessels rupture and cause abnormal accumulation of blood within the brain. The main reasons for ICH are hypertension, disrupted vasculature, excessive use of anticoagulants and thrombolytic agents. In subarachnoid hemorrhage, blood accumulates in the subarachnoid space of the brain due to a head injury or cerebral aneurysm. (Kuriakose and Xiao, 2020).

2.2.2 Risk Factors for Stroke

2.2.2.1 Non modifiable risk factor

These include age, sex, ethnicity, Transient ischemic attack (TIA) and hereditary characteristics. The average age of incidence of stroke was 69.2 years. Recent research has indicated that people aged 20–54 years are at increasing risk of stroke. Women are at equal or greater risk of stroke than men. US research shows that Hispanic and black populations are at higher risk of stroke than white populations. (Kuriakose and Xiao, 2020).

Transient ischemic attack is classified as a mini stroke; In TIA, the blood supply to part of the brain is blocked temporarily. It acts as a warning sign before the actual event, providing an opportunity to change lifestyle and commence medications to reduce the chance of stroke. (Kuriakose and Xiao, 2020).

Genetic risk is proportional to the age and sex of the individual but a multitude of genetic mechanisms can increase the risk of stroke; a parental or family history of stroke increases the chance of an individual developing this neurological disorder, also a rare single gene mutation can contribute to pathophysiology in which stroke is the primary clinical manifestation. (Kuriakose and Xiao, 2020).

2.2.2.2 Modifiable Risk Factors

In these factors timely and appropriate medical intervention can reduce the risk of stroke in susceptible individuals. The major modifiable risk factors for stroke are hypertension, diabetes, lack of physical exercise, alcohol and drug abuse, cholesterol, diet management and genetics. (Kuriakose and Xiao, 2020).

2.2.3 Diagnosis

The clinical presentation of stroke involves the sudden onset of a focal clinical deficit, referable to a specific site in the CNS. Symptoms can include muscular weakness, hemianaesthesia (numbness on one side of the body), aphasia (language disorder) and homonymous hemianopia (loss of the same half of the visual field in each eye). The diagnosis of stroke requires differentiation from common mimics including migraine, seizures and functional disorders, and

is assisted by neuroimaging. In addition, ischemic stroke needs to be differentiated from intracerebral hemorrhage. The brain imaging is the key to diagnosis. Globally, imaging usually involves CT, but MRI is the first-line imaging. (Campbell *et al.*, 2019).

2.2.4 Prevention and Treatment Strategies for Stroke

Stroke prevention involves modifying risk factors within a population or individuals, while stroke management depends on treating its pathophysiology. Stroke acute care: By managing blood pressure, diabetes, alcohol and drugs and hyperlipidemia. Reperfusion: using the intravenous thrombolytic (IVT) and Intra-arterial thrombolysis (IAT). Rehabilitation: including physical therapy, occupational therapy, speech therapy and neurorehabilitation. Cognitive decline: Drug development, robotics, cortical stimulation and stem cell therapy. Neuroprotection and repair: Anti-excitability, anti-inflammatory and anti-apoptosis. (Kuriakose and Xiao, 2020).

2.3 Ribosomal proteins

Ribosomes are organelles located in the cytoplasm that help facilitate the production of proteins composed of small subunits of the 40S as well as 60S formed of 4 RNAs, in which given or taken different 80 proteins structures**.** (Doherty *et al*., 2010).

Ribosome formation occurs mainly in the nucleolus, being later completed in the nucleoplasm and in the cytoplasm. In the nucleolus, ribosomal genes are transcribed by RNA polymerase I (Pol I) to generate the 47S rRNA precursor, which undergoes to site-specific methylation and pseudo uridylation, and processing to give rise to the mature 18S, 5.8S, and 28S rRNA. The fourth type of rRNA, the 5S rRNA, is synthesized in the nucleoplasm by RNA polymerase III (Pol III) and then imported in the nucleolus together with the ribosomal proteins (RPs), whose mRNA is transcribed by RNA polymerase II (Pol II). The assembling of rRNA molecules with the RPs constitutes the two subunits of the mature ribosome, the large 60S and the small 40S subunit. The large 60S subunit is constituted by one each of the 28S, 5.8S, and 5S RNA molecules, together with 47 ribosomal proteins (RPLs); the small 40S subunit contains only one 18S RNA molecule and 33 ribosomal proteins (RPSs). Both subunits migrate from the nucleolus to the cytoplasm where they form the 80S ribosome particle. In the process of ribosome

biogenesis, more than 150 non-ribosomal proteins and around 70 small nucleolar RNAs are involved. (Derenzini *et al*., 2018).

2.3.1 Ribosomal proteins function

R-proteins were the actual enzymatic components in the ribosome. The role of rRNA provides a structural scaffold for the r-proteins enzymatic activity. The primary role of eukaryotic r-proteins is to stabilize the interactions between different do-mains in rRNA. The r-proteins can be divided into, early and late rRNA binders. Early r-proteins bind to the 5' region of rRNA and have a large proportion of their surface area buried in the rRNA, late rRNA binding r-proteins that burry a small surface area with rRNA, bind the 3' end of rRNA and participate in extensive protein-protein surface interactions. (Simoff, 2009).

They participate in balancing the synthesis of the RNA and protein components of the ribosome itself and are sentinels for the self-evaluation of cellular health. The disturbance of ribosome synthesis frees ribosomal proteins to interface with the p53 system, leading to cell cycle arrest or to apoptosis. (Warner and McIntosh, 2009).

2.3.2 Ribosomal protein gene

Eukaryotic organisms often contain multiple copies of the genes coding for r-proteins, single gene copy codes for every r-protein. In contrast to rDNA genes that are located on one single chromosome, the RPGs are scattered throughout the genome. (Simoff, 2009).

2.3.3 Ribosomal proteins and pathology

The ribosomal protein gene family comprises 80 genes,8 at least 11 of which are known to be mutated in Diamond-Blackfan anemia (DBA), a dominantly inherited form of pure red cell aplasia, growth retardation, and congenital anomalies. RPS20 mutation associated with colorectal cancer susceptibility, whereas mutations in 11 other ribosomal protein genes cause predisposition to DBA.RPS19 or RPS20 shown to stabilize p53, which in turn had different effects in different cell types. Effects of RPS20 insufficiency might play a role in RPS20-associated colon tumorigenesis in humans, with disturbed ribosome biogenesis, and altered p53 dosage. The constant activation of p53 consecutive to ribosomal stress induced by RPS20

mutation could favor, on the long run, the selection of cells that escape regulation by p53. (Nieminen, 2014).

Adriana say in her study that both under- and overexpression of RPs have also been implicated in several malignancies. Acquired RPS14 haploinsufficiency has been found to be causative of the bone marrow failure found in 5q– myelodysplastic syndromes. (Vlachos, 2017).

Guiming Wang study show that ribosomal protein family genes, such as RPL9, RPL5, RPS20, and RPL23, and DEG of TP53 may have the potential to be used as targets for diagnosis and treatment of VTE. (Wang *et al*., 2018).

Fathelrahman M. Hassan found a strong association between ribosomal protein genes (RPL5 and RPL9) with the thrombosis incidence and development in the Saudi population, which may have helped to enhance the medical diagnosis of thrombosis. (Hassan *et al.,* 2021).

CHAPTER THREE

MATERIAL AND METHODS

Chapter three

Material and Methods

3.1 Study design

This study is descriptive cross sectional study.

3.2 Study area and duration

The study was conducted in Khartoum state in period between July and December 2022.

3.3 Study population

Fifty Sudanese patients who has been diagnosed with stroke.

3.3.1 Sampling

The sample size in this study was calculated for each category (on average) to give a minimum of error (0.05) with a propability of (a = 0.05). The formula below was used

$$n = \frac{z^2 x\ (pxq)}{d^2}$$

n = sample size

p = prevalence of the stroke

q = 1-p

d = acceptable error

z = 1.96 (standard normal deviate)

$$n = \frac{(1.96)2\times(0.5\times0.5)}{(0.05)2} = 385$$

In this study 50 patients were included difficult to obtain sample, limited period and high cost of research.

3.4 Inclusion criteria

Sudanese patients diagnosed with stroke of both gender.

3.5 Exclusion criteria

Patient with cancer or any other thrombophilic conditions were excluded.

3.6 Ethical consideration

Verbal consent of selected individuals study was taken after being informed with all detailed objective of study.

3.7 Data collection

Non self-administered questionnaire was designed to obtain information.

3.8 Methodology

3.8.1 Sample collection

Venous blood 2.5 ml was dispensed in EDTA container under septic condition.

3.11.2 DNA extraction

1. Whole blood 300 µl was added to1.5 ml tube containing 900 µl RBCs lysis solution, mixed and incubated for 5min at room temperature.
2. Then it was centrifuged at 10000 RPM for 1 min and supernatant was removed, this step was repeated until clear supernatant obtained.
3. Cell lysis solution300 µl was added to the remaining cell and pipetting up and down was done to lyse the cells.
4. PPT buffer 100 µl was added to cell lysate and vortexed roughly for 20 sec, then incubated on ice for 5min.
5. Then it was centrifuged at 13000 RPM for 3 min, and 300 µl of supernatant was transferred to 1.5 ml tube and 300 µl of 100% isopropanol was added and mixed by inverting gently several times.
6. Centrifuged at 13000 RPM for 1 min, then supernatant was poured off and tube drained briefly on clean absorbent paper.
7. 70% Ethanol 1ml was added and tube inverted several times to wash the DNA pellet, then centrifuged at 13000 RPM for 1 min and carefully poured off the Ethanol.
8. Inverted and drained the tube on clean absorbent paper for 10-15min.
9. DNA rehydration buffer 150 µl was added then rehydrated DNA by incubating at 65ºC for 30-60 min.
10. The collected DNA was stored at -20 ºC.

3.8.3 Primers sequences (NCBI Reference Sequence)

Primers for PCR were designed using primer 3 software according to the reference assembly Homo sapiens (RPS20) NC 000008.11:56073198 in the NCBI.

Table (3.1) Primers sequence design for ribosomal protein S20 gene

Primer name	Primer sequence	Product size bp
Forward primer	5' TCGCTTGTGAATTCTCATCTGG-3'	290 bp
Reverse primer	5'-AACAGGCGCAAGCTCTAAGG-3'	

3.8.4 Polymerase chain reaction (PCR)

PCR mixture of 20 µl was prepared using premix master mix tube (FIREPol) for each sample (5 µl), in Ependorf tube 4 µl of master mix was added to 14 µl of distilled water, then 1 µl of forward primer and 1µl of reverse primer were added.

3.8.5 PCR protocol

Table (3.2) shows the number of cycles used for polymerase chain reaction:

Steps	Temperature	Time	Number of cycles
Initial Denaturation	95ºC	5min	1 cycle
Denaturation	94ºC	30sec	35 cycles
Annealing	52ºC	30sec	
Extension	72ºC	30sec	
Final Extension	72ºC	5min	1 cycle

3.8.6 Detection of PCR product

- Detection of the product done by gel electrophoresis.

- Agarose gel 1.5% which stained by ethidium bromide was used.

- 1X Tris EDTA buffer (TE) used as running buffer.

- The product 5µl of was applied into the gel; the voltage was 100 volt for 30 minutes.

- DNA ladder (100bp) was used as molecular weight marker.

3.8.7 RPS20 Digestion

- Restriction enzyme (SfaNI) 0.5µl was added to 7.5 µl of distilled water.

- Enzyme buffer 2 µl and 10 µl of PCR product was added and Incubated at 37 ºC 16 hours.

- The reaction was Stopped by applying 4 ºC.

- Then 10 µl digested products was loaded into 1.5% agarose after mixing with loading dye.

3.8.8 Result interpretation

After gel electrophoresis the wild type DNA (homozygous AA) yields a band of 290 pb, the mutant DNA (homozygous AT) yields two bands of (250 and 40) bp and (hetrozygous AA/AT) yields three bands of (290, 250 and 40) bp.

3.9 Data analysis

The data were analyzed with SPSS version (21) to calculate the mean, frequency, and P.value, which was determined by the chi-square test at 0.05, which was regarded statistically significant.

CHAPTER FOUR

RESULTS

Chapter four

Results

4.1 Results

This study aimed to detect the RPS20 mutation in stroke patient. A Total of 50 individuals diagnosed with stroke are involved in the study, 36 male (72%) and 14 women (28%). Their age range from 40 to 82 years divided into 3 groups: (40-60), (61-80) and (>80). (figure 4.1). 12 (24%) of patient have DM, 14(28%) have HTN, 17(34%) have both DM and HTN and 7(14%) without any chronic disease. (Table 4.1). According to number of stroke they had we divided them to once 40 (80%), twice 5 (10%) and 3 times and more 5 (10%). 16 (32%) have family history with stroke and 34(68%) with no family history. (Figure 4.2).

The RPS20 mutation was found in 4 (8%) of the patients, while 46 (92%) of patients were unaffected. (Table 4.2). The prevalence is higher in males 3 (6%) than females 1 (2%) with no significant relation (Table 4.3). The age range of all the mutant samples is (61-80). (Table 4.4). These chronic diseases are linked to the mutation as: One (2%) has both DM and HTN, two (4%) have HTN, and one (2%) has DM with no significant correlation (Table 4.5). According to the number of previous strokes there was significant correlation, the distribution of mutations was (4%) for once, (4%) for three strokes and more. (Table 4.6). According to family history, it is distributed as 2% in patients with family history and 6% in patients without family history (Table 4.7).

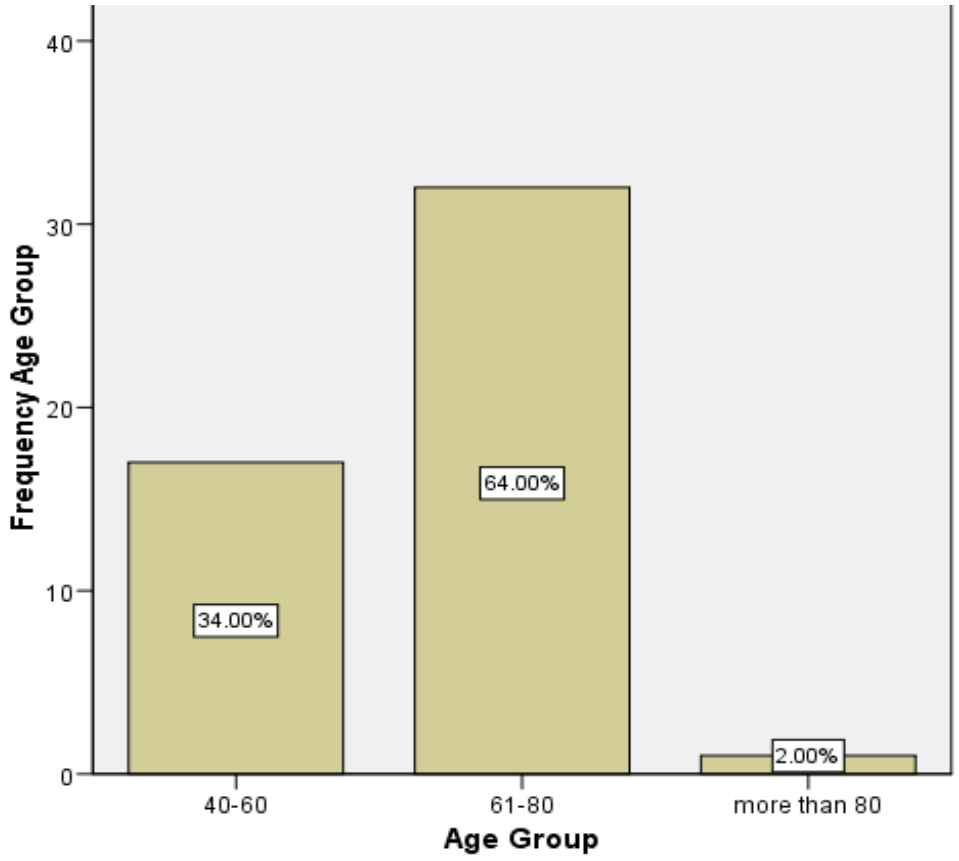


Figure 4.1 Frequency distribution of age groups among stroke patients, most stroke patients (64% of them) fell into the 61–80 year age range, followed by the 40–60 year age range (34%) and those beyond 80 years of age (2%).

Table (4.1) Frequency distribution of some chronic disease among stroke patients.

Chronic disease	Frequency(n)	Percent
Diabetes mellitus	12	24%
Hypertension	14	28%
Both DM and HTN	17	34%
Without any chronic disease	7	14%
Total	50	100

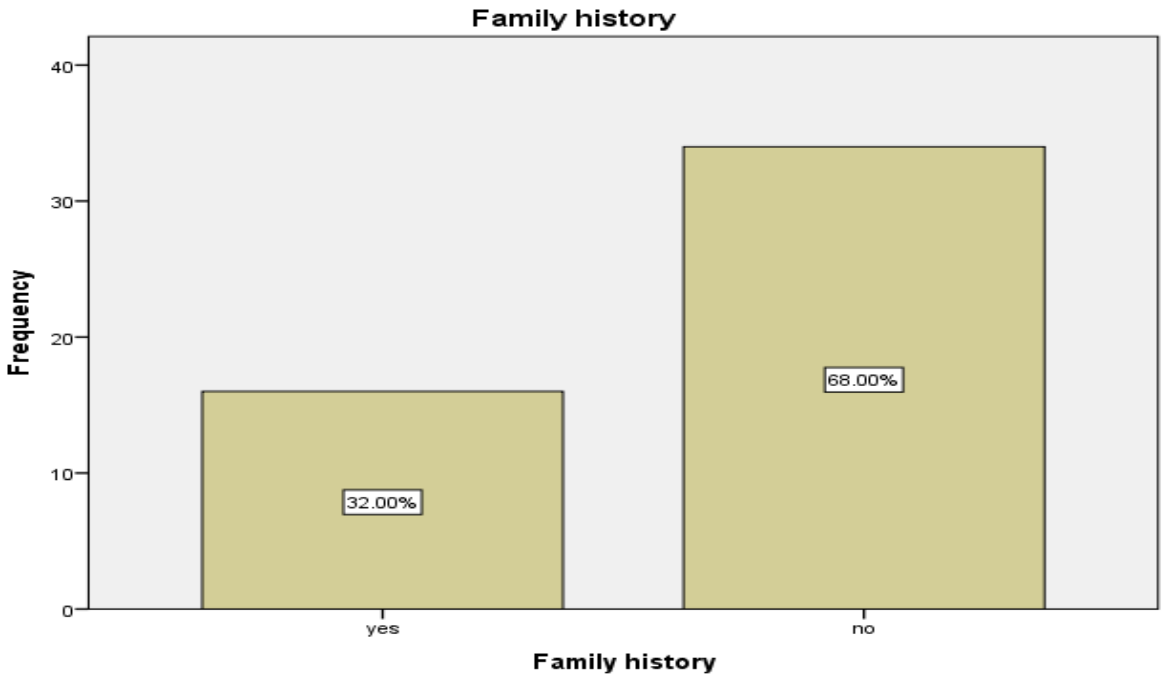


Figure 4.2 Frequency distribution of family history among stroke patients, 32% of patients with stroke had a family history, while 68% did not have.

Table (4.2) Distribution of mutation among stroke patients

Mutation	Frequency(n)	Percent
Mutant gene (AT)	4	8%
Wild type gene (AA)	46	92%
Heterozygous gene (AA/AT)	0	0
Total	50	100

Table (4.3) Relation between mutation and gender among stroke patients

Gender	Mutation	Normal	Total	p.value
Male	3 (6%)	33 (66%)	36	0.889
Female	1 (2%)	13 (26%)	14	
Total	4	46	50	

p.value ≤ 0.05 is considered as significant

Table (4.4) Relation between mutation and age groups among stroke patients

Age group	Mutation	Normal	Total	P value
40-60 (years)	0	17 (34%)	17	0.294
61-80 (years)	4 (8%)	28 (56%)	32	
>82 (years)	0	1 (2%)	1	
Total	4	46	50	

p.value ≤ 0.05 is considered as significant

Table (4.5) Relation between mutation and some chronic disease among stroke patients

Chronic disease	Mutation	Normal	Total	P value
Diabetes mellitus	1 (2%)	11 (22%)	12	0.690
Hypertension	2 (4%)	12 (24%)	14	
Both DM and HTN	1 (2%)	16 (32%)	17	
Without any chronic disease	0	7 (14%)	7	
Total	4	46	50	

p.value ≤ 0.05 is considered as significant

Table (4.6) Relation between mutation and number of previous strokes

Stroke numbers	Mutation	Normal	Total	P value
Once	2 (4%)	38 (76%)	40	0.019
Twice	0	5 (10%)	5	
3 times or more	2 (4%)	3 (6%)	5	
Total	4	46	50	

p.value ≤ 0.05 is considered as significant

Table (4.7) Relation between mutation and family history among stroke patients

Family history	Mutation	Normal	Total	P value
Yes	1 (2%)	15 (30%)	16	0.754
No	3 (6%)	31 (62%)	34	
Total	4	46	50	

p.value ≤ 0.05 is considered as significant

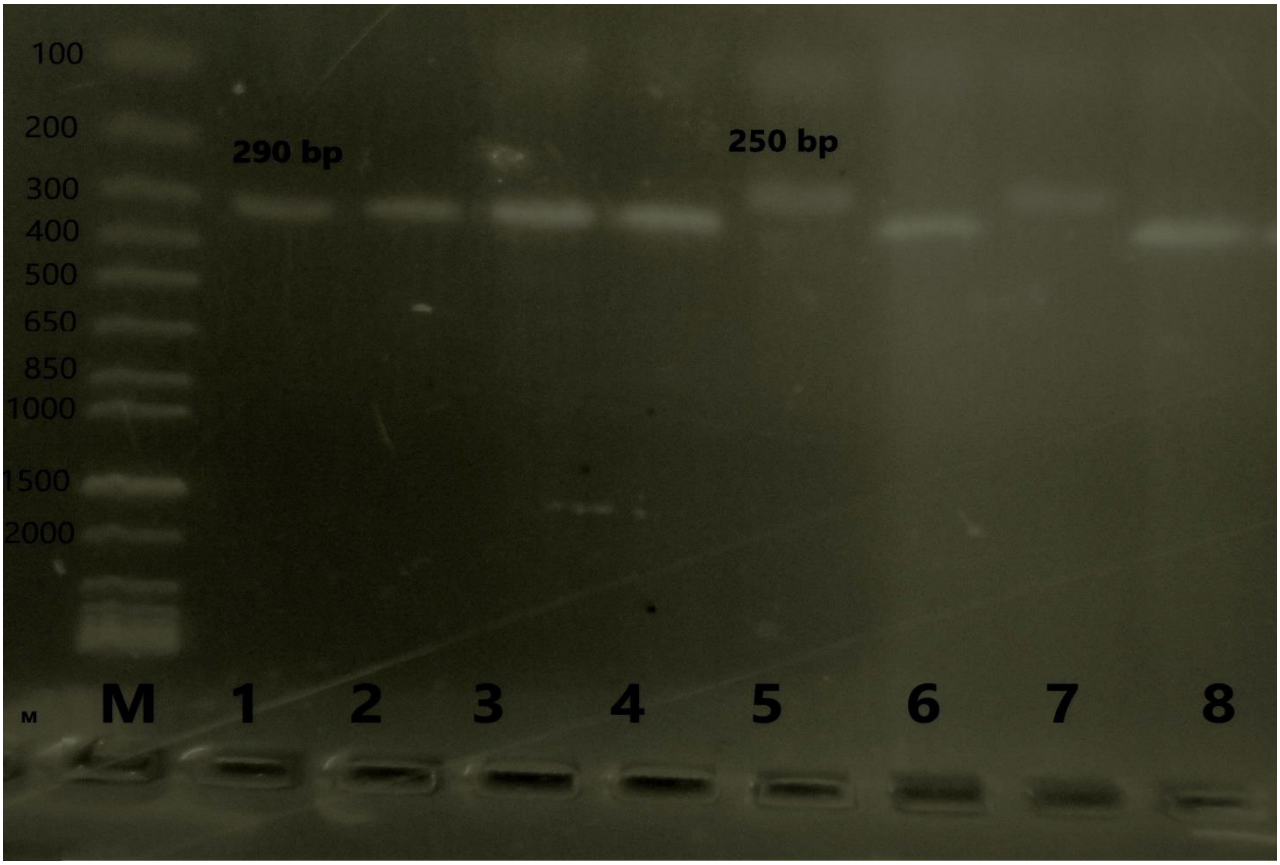


Figure 4.3 PCR amplification of RPS20 gene mutation

Lane M: molecular weight marker 1Kp plus. Lane 5 and 7 mutant (250 and 40) bp, lane 1,2,3,4,6 and 8 wild type (undigested and remains at 290 bp).

CHAPTER FIVE

DISSCSION, CONCLUSION AND RECOMMENDATIONS

Chapter five

Discussion, Conclusion and Recommendations

5.1 Discussion

Stroke has a high mortality rate that is caused by a variety of environmental and genetic factors; in Sudan, no prior researches have been conducted to identify ribosomal protein gene mutations linked to stroke. In order to detect RPS20 mutation in stroke patients, 50 samples from people who have been diagnosed with stroke were gathered for this descriptive cross-sectional study, which has been carried out in Khartoum state between July and December 2022.

The average age of the group study was (64.7) years, which indicated that older age may increase the risk of stroke which consistent with a study of Hassan who found that the mean age was (61.2) years. All stroke patients with the mutant RPS20 gene (AT) are in between 61-80 years age category with no correlation (p.value = 0.29), and there was no correlation between mutation and gender of patients (*p. value* = 0.88). There was a statistically significant relation between gene mutation and the number of prior strokes (p.value = 0.019).

Also consistent with (Hassan, 2021) study, there was no significant correlation (*p.value* = 0.75) between gene mutation and family history of stroke, therefor stroke patients without family history were (68%) more prevalent than those with family history (32%). In this study, all stroke patients with the RPS20 gene mutation (AT) had some associated chronic disease with significance as (p.value = 0.69), this new correlation could be considered for more clinical studies to found out the causative agent compared to the mutant gene.

About 8% of the stroke patients in this study had the mutant RPS20 gene (AT), while 92% had the normal RPS20 gene (AA), this is in line with a research done by Wang et al. (2021) that found RPS20 was one of the genes that were up-regulated in VTE patients.

In agreement with the study done by Taina T. Nieminen et al which showed that the germline mutation of RPS20 is associated with dominant predisposition to colorectal cancer. This was the first report linking germline mutation of RPS20 to human disease. (Nieminen *et al*., 2014). The present study also detects the mutation of RPS20 in humans diagnosed with stroke. The study done by Saleh Bahar et al provided the evidence that variant of RPS20 they found were causative of with Diamond-Blackfan anemia (DBA), this variant reduce the RPS20 protein level (Bahar *et al*., 2020). In this study the gene level wasn't measured, so further studies should measure gene level. The results of Bryony A. Thompson et al study who found the RPS20 as a CRC predisposition gene, and expand RPS20 mutation to include polyposis. (Thompson *et al.*, 2020). The present study has some limitations, including the small sample size, the restriction of the sample to Khartoum State mean that the results may not be fully generalizable, the lack of an examination of the impact of RPS20 as a risk factor for stroke or not, the fact that it is cross sectional in nature and assessed respondent perception of the obligation at a specific time, the quick turnaround time, and the lack of published studies to our study. According to published studies the RPS20 is associated with Diamond Blackfan Anemia (DBA) (Bahar *et al*., 2020 and Vlachos, 2017) and colorectal cancer (CRC) (Thompson *et al*., 2020 and Nieminen *et al*., 2014). The platelet in the DBA is impacted, which is similar to our studies on stroke patients who are thrombotic and have defective platelets.

5.2 Conclusion

In summary, the study concluded that the ribosomal protein S20 (RPS20) mutation was detected in 8% of stroke patients. There was no correlation between the RPS20 mutation and stroke patient age, some concomitant chronic diseases, or family history of stroke. RPS20 mutation and the frequency of prior strokes were found to be significantly correlated.

5.3 Recommendations

Present study recommended:

1. Additional researches can be done to determine whether the RPS20 genetic variant is a risk factor for stroke or can use as target for diagnosis and treatment.

2. Increase sample size and include stroke patients from other states to produce accurate and precise results.

3. Other hemostatic tests that should be carried out to evaluate the stroke patients include the prothrombin test, the activated partial thromboplastin test, the D-dimer test, and the fibrinogen level.

4. Measuring the RPS20 level to check for protein level regulation, either up or down.

5. Use gene sequencing to validate the mutation and reveal whether stroke patients have any other variants.

REFERENCES

References

Ashorobi, D., Ameer, MA. and Fernandez, R. (2022). Thrombosis. [online] Avaliable at:http://creativecommons.org/licenses/by-nc-nd/4.0/ (Accessed: 20 July. 2022).

Bacigaluppi, M., Semerano, A., Gullotta, GS. and Strambo, D. (2019). Insights from thrombi retrieved in stroke due to large vessel occlusion. *Journal of Cerebral Blood Flow & Metabolism*, **39**(8):1433-1451.

Bhar, S., Zhou, F., Reineke, L.C., Morris, DK., Khincha, PP., Giri, N., Mirabello, L., Bergstrom, K., Lemon, LD., Williams, CL. and Toh, Y. (2020). Expansion of germline RPS20 mutation phenotype to include Diamond–Blackfan anemia. *Human Mutation*, **41**(11):1918-1930.

Campbell, BC., De Silva, DA., Macleod, MR., Coutts, SB., Schwamm, LH., Davis, SM. and Donnan, GA. (2019). Ischaemic stroke. *Nature Reviews Disease Primers*, **5**(1):1-22.

Castellone, D. Overview of hemostsasis and platelet physiology, in: Ciesla, B. (2012). Second edition, Hematology in Practice, Philadelphia: F.A. Davis Company, P 239, 241.

Doherty, L., Sheen, MR., Vlachos, A., Choesmel, VO., ’Donohue, MF., Clinton, C., Schneider, HE., Sieff, CA., Newburger, PE., Ball, SE., Niewiadomska, E., Matysiak, M., Glader, B., Arceci, RJ., Farrar, JE., Atsidaftos, E., Lipton, JM., Gleizes, PE. and Gazda, HT**.** Ribosomal protein genes RPS10 and RPS26 are commonly mutated in Diamond-Blackfan anemia. *Am J Hum Genet*. 2010 Feb 12; **86**(2):222-228.

Deloughrey, TG. (2019), Hemostsasis and thrombosis. Fourth edition. USA. Landes Bioscience, P 121, 124, 124, 127.

Derenzini, E., Rossi, A. and Treré, D., 2018. Treating hematological malignancies with drugs inhibiting ribosome biogenesis: when and why. *Journal of Hematology & Oncology*, **11**(1) :1-13.

Hassan, FM., Alsultan, A., Alzehrani, F., Albuali, W., Bubshait, D., Abass, E., Elbasheer, M. and Alkhanbashi, A**.** (2021). Genetic Variants of RPL5 and RPL9 Genes among Saudi Patients Diagnosed with Thrombosis. *Medical Archives*, **75**(3):188-193.

Hoffbrand, AV., Moss, PAH. and Pettit, JE. (2016). Essential Haematology. 7th ed., London USA: Blackwell, P 265, 275, 287.

Khan, S and Dickerman, J.D. (2006).Hereditary thrombophilia. *Thrombosis journal.* **4**(1):1-17.

Kumar, V., Abbas, A., Fausto, N. and Aster, J. (2010). Robbins and Cotran Pathologic Basis of Disease. 8th ed. Philadelphia: PA: Saunders Elsevier, P119.

Kuriakose, D. and Xiao, Z. (2020). Pathophysiology and treatment of stroke: present status and future perspectives. *International journal of molecular sciences*, **21**(20):7609.

Laffan, M. and Manning, R. Investigation of haemostasis, in: Bain, B J., Bates, I., Laffan, MA. and Lewis, SM. (2011). 11th ed, Dacie and Lewis Practical Hematology. London: Elsevier Churchill Livingstone, P 394, 395.

Lim, W. Immune Medicated Coagulopathies in; Shaz, B.H., Christopher, D.H., Mikhail, R., Charles, S.A. (2013). Second edition, Transfusion medicine and hemostasis. United States of America: Elsevier Churchill Livingstone, P 894, 900.

McKenzie, S.B. and Williams, J.L. (2010). Clinical laboratory hematology. second ed. New Jersey.P 960-963, 966, 967, 969, 970, 977, 980, 981.

Namee, K., Dawood, F. and Farquharson, R**.** (2012).Recurrent miscarriage and thrombophilia: an update.*Curr Opin Obstet Gynecol*. ***24***(4):229-234.

Nieminen, TT., O'Donohue, MF., Wu, Y., Lohi, H., Scherer, SW., Paterson, AD., Ellonen, P., Abdel-Rahman, WM., Valo, S., Mecklin, JP. and Järvinen, HJ. (2014). Germline mutation of RPS20, encoding a ribosomal protein, causes predisposition to hereditary nonpolyposis colerectal carcinoma without DNA mismatch repair deficiency. *Gastroenterology*, **147**(3):595-598.

Poudel, S., Zeb, M., Kondapaneni, V., Gutlapalli, S.D., Choudhari, J., Sodiya, O.T., Toulassi, I.A. and Cancarevic, I. (2020). Association of G20210A Prothrombin Gene Mutation and Cerebral Ischemic Stroke in Young Patients. *Cureus*, **12**(12).

Rao, AK. And Essex, DW. Platelet function in hemostsasis and inherited disorders of platelet number and function, in: Schmaier, AH. And Lazarus, HM. (2012). First edition, Concise Guide to Hematology. UK: Blackwell, P 140-141.

Robert CP. And George MR. Thrombosis and antithrombotic therapy, in: Greer, JP *et al*. (2014). 13th ed, Wintrobe's Clinical Hematology. Philadelphia: Lippincott Williams & Wilkins, P 230.

Roshal, M. Tests for hypercoagulable state in; Shaz, B.H., Christopher, D.H., Mikhail, R., Charles, S.A. (2013). Second edition, Transfusion medicine and hemostasis. United States of America: Elsevier Churchill Livingstone, P 681.

Simoff, I. (2009). Ribosomal proteins L5 and L15: Functional characterisation of important features, in vivo (Doctoral dissertation, The Wenner-Green Institute, Stockholm University). P 17, 18.

Thompson, BA., Snow, AK., Koptiuch, C., Kohlmann, WK., Mooney, R., Johnson, S., Huff, C., Yu, Y., Teerlink, CC., Feng, BJ. and Neklason, DW. (2020). A novel ribosomal protein S20 variant in a family with unexplained colorectal cancer and polyposis. *bioRxiv*, pp.2019-12.

Turgeon, M. 2012. Clinical Hematology Theory & Procedures. 5th ed. Philadelphia: Lippincott Williams & Wilkins, P 400, 403, 404, 406, 413, 414, 450-452, 456.

Van Vlijmen, EF., Veeger, NJ., Middeldorp, S., Hamulyák, K., Prins, MH., Büller, HR. and Meijer, K. (2011). Thrombotic risk during oral contraceptive use and pregnancy in women with factor V Leiden or prothrombin mutation: a rational approach to contraception. *Blood, The Journal of the American Society of Hematology*, **118**(8):2055-2061.

Vlachos, A., (2017). Acquired ribosomopathies in leukemia and solid tumors. Hematology, the American Society of Hematology Education Program Book, 2017(1), pp.716-719.

Wang, G., Zhao, W., Yang, Y., Yang, G., Wei, Z. and Guo, J. (2018). Identification of biomarkers of venous thromboembolism by bioinformatics analyses. *Journal of Vascular Surgery: Venous and Lymphatic Disorders*, **6**(5):674-675.

Wang, W., Nag, S., Zhang, X., Wang, M.H., Wang, H., Zhou, J. and Zhang, R. (2015). Ribosomal proteins and human diseases: pathogenesis, molecular mechanisms, and therapeutic implications. *Medicinal research reviews*, **35**(2):225-285.

Warner, J.R. and McIntosh, K.B., 2009. How common are extraribosomal functions of ribosomal proteins?. *Molecular cell*, **34**(1): 3-11.

APPENDICES

Appendix (1)

بِسْمِ ٱللَّهِ ٱلرَّحْمَٰنِ ٱلرَّحِيمِ

SUDAN UNIVERSITY OF SCIENCE AND TECHNOLOGY

Collage of Post Graduate Studies

Department of Hematology and Immunohematology

Detection of genetic variant of Ribosomal protein S20 among Sudanese patients diagnosed with stroke

-Date: / /2022

-Name:……

-Age:……years

-Gender:……

-Tel No:……

-History of disease;

When did you have stroke?.....

Did you have stroke once or more?.....

If more how many?.....

Did strock common in your family?.....

What symptoms did you have?

Weakness in arms or legs yes { } No { }

Lost sensation yes { } No { }

Facial paralysis yes { } No { }

Didficulty with speech yes { } No { }

Lost vision yes { } No { }

Numbness yes { } No { }

Diziness yes { } No { }

Treatment used ……..

Other diseases……...

Laboratory results:

PCR:

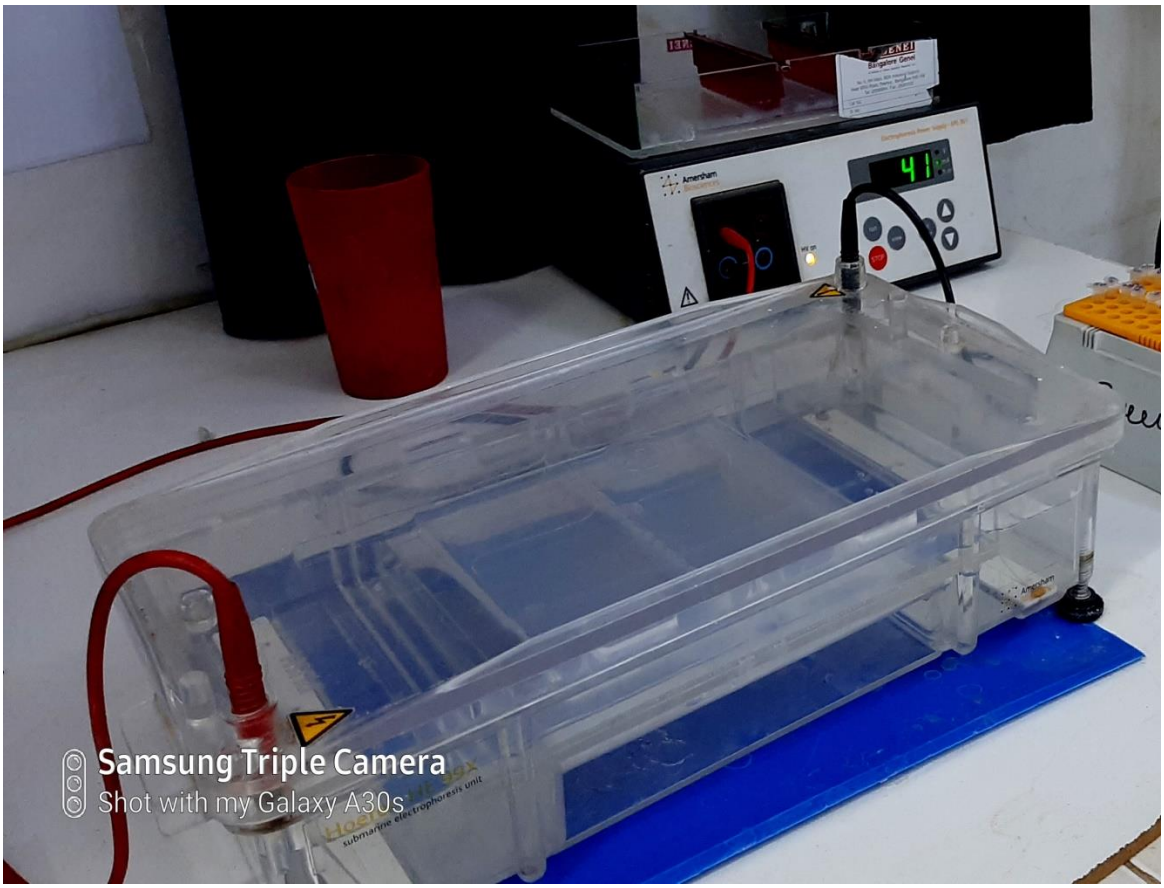
RPS20……..

Signature……

Appendix (2)

PCR Machine

Appendix (3)



Gel Electrophoresis