



**Sudan University For Science and Technology**



**College of Graduate Studies**

**Determination of ABO Blood Groups and Rhesus Factor in  
Sudanese Patients with Cardiovascular Diseases**

**تحديد الزمر الوظيفية للفصائل الدموية والعامل الريصي لدي السودانيين المصابين  
بأمراض القلب والأوعية الدموية**

**A dissertation Submitted in Partial Fulfillment of  
Requirements For M.Sc Degree in Medical Laboratory  
Science**

**(Hematology and Immunohematology)**

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**2016**

# الآية

قال تعالى:

﴿ اقْرَأْ بِاسْمِ رَبِّكَ الَّذِي خَلَقَ ﴾ 1 ﴿ خَلَقَ الْإِنْسَانَ مِنْ عَلَقٍ ﴾ 2 ﴿ اقْرَأْ وَرَبُّكَ  
الْأَكْرَمُ ﴾ 3 ﴿ الَّذِي عَلَّمَ بِالْقَلَمِ ﴾ 4 ﴿ عَلَّمَ الْإِنْسَانَ مَا لَمْ  
يَعْلَمُ ﴾ 5 { .

صدق الله العظيم

سورة العلق الآيات ﴿ 5-1 ﴾

# Dedication

- ❖ To ...My beloved and blessed mother who gives meaning and light to my life.
- ❖ To ...My wonderful beautiful half (my husband) for his support and encouragement.
- ❖ To ...My wonderful supervisor Dr.Khalda Meirghani, who helped whenever I needed her.
- ❖ To ...our teacher's very special friends and colleagues who were integral part of our support group.
- ❖ Special dedication to all patients from whom samples were collected.

## **Acknowledgment**

First of all my thanks to Allah for giving me assistance, health and patience to complete this work.

I would like to express my deep thanks to my supervisor Dr.Khalda Meirghani for her expertise, support and endless valuable advice.

Thanks for all staff of the hematology department in sudan university for their valuable assistance and encouragement throughout there search.

Thanks so much to you all

Hadeel

## **Abstract**

This is analytical case control study aimed to determine the frequency of blood group and Rhesus factor of patients with cardiovascular disease attended Sudan heart center during the period from February to August 2016.

Two hundred patients with different types of cardiovascular disease attend to Sudan Heart Center were enrolled in this study as a case and one hundred subject without cardiovascular disease were taken as control.

A questionnaire comprising gender, Type of cardiovascular diseases, Blood group, Rh factor, age was not comprising in this study.

ABO and Rh factors were determined by slide techniques and du method techniques using specific anti-sera after blood sample collection the data were analyzed by SPSS computer program.

The results showed that most common blood group in patients with cardiovascular diseases was O (49%) vs (57%) control, followed by A (30.5%) case vs (21%) control, B (16.5%) case vs (11%) control and least frequent was AB (4%) case vs (11%) control .

Majority of case were Rh positive (91.5%) vs (88%) control.

Most common cardiovascular diseases were found in males (61%) vs (39%) females.

Most common blood group is O then A then B and least AB

Majority of patient is Rh positive.

The percentage of cardiovascular disease in males is more than females.

Most common type of cardiovascular disease is vascular disease.

The results of the present showed there is no association of ABO blood group and Rh factor with cardiovascular disease all though the frequent of cardiovascular disease was higher in group O, this difference statistically insignificant.

## المستخلص

هذه دراسة حاله ضابطة للمتريدين علي المركز السوداني للقلب هدفت لتحديد تكرار زمر الدم في المرضي المصابين بأمراض القلب و الاوعية الدموية في الفترة من فبراير الى أغسطس 2016. شملت مئتان من المصابين بمختلف أنواع امراض القلب و الاوعية الدموية ومائة حاله ضابطة لاتعاني من امراض القلب.

شمل الاستبيان كل من الجنس، انواع امراض القلب و الاوعية الدموية، فصيلة الدم والعامل الريصي ولم يشمل العمر.

اختبارات زمر الدم والعامل الريصي أجريت باستخدام طريقة الشرائح والامصال المضادة وطريقة دي يو، ثم حلت البيانات باستخدام نظام الحزم الإحصائية للعلوم الاجتماعية.

وجد أن اكثر زمر الدم انتشارا في مرضى امراض القلب هو و (49%) مقابل (57%) حاله ضابطة، ثم أ (30.5%) من مرضى امراض القلب مقابل (21%) حاله ضابطة ، ب (16.5%) من مرضى امراض القلب مقابل (11%) حاله ضابطة واقلها تكراراً أ ب (4%) من مرضى امراض القلب مقابل (11%) حاله ضابطة .

تكرار العامل الريصي في المصابين بأمراض القلب والأوعية الدموية الموجب (91.5%) مقابل (88%) حاله ضابطة .

نسبة انتشار أمراض القلب و الاوعية الدموية في الرجال (61%) و النساء (39%) .  
وجد ان اكثر زمر الدم انتشاراً هو و ثم أ ثم ب واقلها أ ب .

اكثر المصابين بامراض القلب و الاوعية الدموية هم موجبي العامل الريصي .

نسبة انتشار امراض القلب و الاوعية الدموية في الرجال اعلي من النساء .

كما وجد أن أكثر أنواع أمراض القلب و الاوعية الدموية انتشارا هو امراض الاوعية.

النتائج الحالية لهذه الدراسة اظهرت عدم وجود علاقة بين فصائل الدم و العامل الريصي و امراض القلب

و الاوعية الدموية، بالرغم من تكرار حدوث امراض القلب و الاوعية الدموية اعلي في فصيلة الدم ( و ) الا ان الاختلاف احصائياً ليس له دلالة.

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## List of abbreviation

<b>CAD</b>	Coronary Artery Disease
<b>CDC</b>	Centers of Disease Control and Prevention
<b>CHD</b>	Congestive Heart Disease
<b>CHF</b>	Congestive Heart Failure
<b>CVD</b>	Cardiovascular Disease
<b>DCM</b>	Dilated Cardiomyopathy
<b>HF</b>	Heart Failure
<b>IgG</b>	Imunoglobulin G
<b>IgM</b>	Imunoglobulin M
<b>IHD</b>	Ischemic Heart Disease
<b>ISBT</b>	International Society of Blood Transfusion
<b>K,EDTA</b>	Potassium Ethylene Diamine tetra Acetic Acid
<b>MI</b>	Myocardial Infarction
<b>PPM</b>	Permanent Pacemaker
<b>Rh</b>	Rhesus
<b>VHD</b>	Valvular Heart Disease
<b>SPss</b>	Statistical Package of Social Science
<b>vWF</b>	Von will brand Factor
<b>DcE</b>	Allelic genes
<b>LISS</b>	Low ionic strength saline

# **Chapter One**

## **Introduction and Literature Review**

## Chapter One

### Introduction and literature review

#### 1.1 Introduction

In 1901, Landsteiner identified ABO blood groups as the first recognized human blood group system. The clinical significance of ABO blood type extends beyond transfusion medicine and solid organ/hematopoietic transplantation. To numerous reports have suggested important associations between ABO blood groups and various diseases, for example, gastric cancer (Elhag *et al.*, 2007). Periodontal diseases and cardiometabolic disease. Retrieved from Studies on the associations between cardiovascular disease and ABO blood groups have a long history. In 1955, Woolf proposed an odds ratio as a measure to quantify the disease risk conferred by blood group type (Woolf, 1995). Reported a deficit of patients with blood group O among those who received anticoagulants for venous thromboembolism (Jick *et al.*, 1969). Prior to mutation detection in haemophilia carriership analysis, likelihood ratios of carriership of hemophilia A were based on Factor VIII levels conditional on blood group (Green *et al.*, 1986). A number of later studies elucidated that ABO blood groups, particularly non-O blood groups, are associated with major cardiovascular risk factors and/or increased rate of cardiovascular events. However, there is limited consensus regarding the magnitude and significance of the ABO effects at the population level, and whether it relates to all disorders equally or predominantly modulates thrombotic pathways individuals with ABO blood group O increases the susceptibility to and rate of proteolysis by (ADAMTS13), although data available so far are not in support of the role of proteolysis by (ADAMTS13), in vWF clearance from the circulation (Badirou *et al.*, 2010). Due to their physical association in blood, lower circulating vWF results in lower levels of FVIII. Whether vWF in platelets (a relatively

abundant source) undergoes any modification by ABO remains controversial; such modification could alter platelet production and subsequent turnover of vWF, particularly locally during platelet-driven arterial thrombosis, although this remains to be established. Indeed, current knowledge suggests that ABO does not modify platelet vWF but the role of ABO in regulating platelet vWF and platelet function in thrombosis requires greater study (Grath *et al.*, 2010).

## **1.2 Literature Review**

### **1.2.1 Blood group system**

Although all blood is made of the same basic elements. In fact, there are eight different common blood types, which are determined by the presence or absence of certain antigens substances that can trigger an immune response if they are foreign to the body. Since some antigens can trigger a patient's immune system to attack the transfused blood, safe blood transfusions depend on careful blood typing and cross-matching. (Hoff *et al.*, 2000).

### **1.2.2 History of ABO discoveries**

The two most significant blood group systems were discovered by Karl Landsteiner during early experiments with blood transfusion: the ABO group in 1901. Development of the coombs test in 1945, (Coombs *et al.*, 1945). The advent of transfusion medicine, and the understanding of hemolytic disease. of the new born, led to discovery of more blood groups, and now 30 human blood group systems are recognized by the international society of blood transfusion (ISBT), (Lewis *et al.*, 1990). And across the 30 blood groups, over 600 different blood group antigens have been found (Lewis *et al.*, 1991). Many of these are very rare or are mainly found in certain ethnic groups. Landsteiner was in advertently the first individual to Perform forward and reserve grouping. The forward grouping is defined as using known sources of reagent anti-sera to detect antigens on an individual red cells Reverse grouping is defined as using reagent cells with known ABO antigens and testing the serum of patient for ABO group anti bodies (Denis and Hameening, 1998).



### **1.2.3 Importance of ABO blood group system**

The ABO blood group system is the most important blood type system in human blood transfusion. Found on platelets, epithelium, and cells other than erythrocytes, AB antigens (as with other serotypes) can also cause an adverse immune response to organ transplantation ( Muramatsu *et al.*, 2014).

The associated anti-A and anti-B antibodies are usually IgM antibodies, which are produced in the first years of life by sensitization to environmental substances, such as food, bacteria, and viruses. ABO blood types are also present in some other animals, for example rodents and apes, such as chimpanzees, bonobos, and gorillas (Maton *et al.*, 1993).

### **1.2.4 Classification of ABO blood group**

A blood type is a classification of blood based on the presence or absence of inherited antigenic substances on the surface of red blood cells. These antigens may be proteins, carbohydrates, glycoproteins, or glycolipids, depending on the blood group system. Some of these antigens are also present on the surface of other types of cells of various tissues. Several of these red blood cell surface antigens can stem from one allele (or an alternative version of a gene) and collectively form a blood group system (Maton *et al.*, 1993).

Blood types are inherited and represent contributions from both parents. A total of 35 human blood group systems are now recognized by the International Society of Blood Transfusion (ISBT). The two most important ones are ABO and the RhD antigen; they determine someone's blood type (A, B, AB and O, with +, - or Null denoting RhD status).

Many pregnant women carry a fetus with a blood type which is different from their own, which is not a problem. What can matter is whether the baby is RhD positive or negative. Mothers who are RhD - and carry a RhD+ baby can form antibodies

against fetal RBCs. Sometimes these maternal antibodies are IgG, a small immunoglobulin, which can cross the placenta and cause hemolysis of fetal RBCs, which in turn can lead to hemolytic disease of the newborn called erythroblastosis fetalis, an illness of low fetal blood counts that ranges from mild to severe. Sometimes this is lethal for the fetus; in these cases it is called hydrops fetalis ( Letsky *et al.*, 2000).

### **1.2.5 Antigens of ABO blood groups system**

The ABO blood group antigens remain of prime importance in transfusion medicine they are the most immunogenic of all the blood group antigens. The most common cause of death from a blood transfusion is a clerical error in which an incompatible type of ABO blood is transfused. The ABO blood group antigens also appear to have been important throughout our evolution because the frequencies of different ABO blood types vary among different populations, suggesting that a particular blood type conferred a selection advantage (e.g., resistance against an infectious disease.)

However, despite their obvious clinical importance, the physiological functions of ABO blood group antigens remain a mystery. People with the common blood type O express neither the A nor B antigen, and they are perfectly healthy. Numerous associations have been made between particular ABO phenotypes and an increased susceptibility to disease. For example, the ABO phenotype has been linked with stomach ulcers (more common in group O individuals) and gastric cancer (more common in group A individuals). Another observation is that individuals with blood type O tend to have lower levels of the von Willebrand Factor (vWF), (Laura., 2005).

### **1.2.6 ABO sub groups**

The A blood type contains about 20 subgroups, of which A1 and A2 are the most common (over 99%). A1 makes up about 80% of all A-type blood, with A2 making up almost all of the rest. These two subgroups are not always interchangeable as far as transfusion is concerned, as some A2 individuals produce antibodies against the A1 antigen. Complications can sometimes arise in rare cases when typing the blood ( Seltsam *et al.*, 2003).

With the development of DNA sequencing, it has been possible to identify a much larger number of alleles at the ABO locus, each of which can be categorized as A, B, or O in terms of the reaction to transfusion, but which can be distinguished by variations in the DNA sequence. There are six common alleles in white individuals of the ABO gene that produce one's blood type (Ogasawara *et al.*, 1996).

### **1.2.7 ABH Secretors status**

About 80% of UK populations are ABH secretors as they have H antigen plus A or B according to their ABO genotype, in a water – soluble form in their body secretion. The remaining 20% are non – secretors and have no secreted ABH antigens, regardless of ABO phenotype (Hoff *et al.*,2000).

#### **1.2.2.1 The Rhesus system**

The Rh blood group system is one of the most polymorphic and immunogenic systems known in humans. In the past decade, intense investigation has yielded considerable knowledge of the molecular background of this system. The genes encoding 2 distinct Rh proteins that carry C or c together with either E or e antigens, and the D antigen, have been cloned, and the molecular bases of many of the antigens and of the phenotypes have been determined. A related protein, the Rh glycoprotein is essential for assembly of the Rh protein complex in the erythrocyte membrane and for expression of Rh antigens (Denis *et al.*, 1998).

### **1.2.2.2 Discoveries Rh system**

The rhesus blood type named after the rhesus monkey was first discovered in 1937 by Karl Landsteiner and Alexander S. Wiener. The significance of the discovery was not immediately apparent and was only realized in 1940, after subsequent findings by Philip Levine and Rufus Stetson. This serum that led to the discovery was produced by immunizing rabbits with red blood cells from a rhesus macaque. The antigen that induced this immunization was designated by them as Rh factor to indicate that rhesus blood had been used for the production of the serum (Landsteiner *et al.*, 1940).

In 1939, Phillip Levine and Rufus Stetson published in a first case report the clinical consequences of non-recognized Rh factor, hemolytic transfusion reaction and hemolytic disease of the newborn in its most severe form (Levine, 1939).

It was recognized that the serum of the reported woman agglutinated with red blood cells of about 80% of the people although the then known blood groups, in particular ABO were matched. No name was given to this agglutinin until now. In 1940, Karl Landsteiner and Alexander S. Wiener made the connection to their earlier discovery, reporting a serum that also reacted with about 85% of different human red blood cells ( Landsteiner *et al.*, 1940).

### **1.2.2.3 Rh antibodies**

Rh antibodies are IgG antibodies which are acquired through exposure to Rh-positive blood (generally either through pregnancy or transfusion of blood products). The D antigen is the most immunogenic of all the non-ABO antigens. Approximately 80% of individuals who are D-negative and exposed to a single D-positive unit will produce an anti-D antibody. The percentage of alloimmunization is significantly reduced in patients who are actively exsanguinating.

All Rh antibodies except D display dosage antibody reacts more strongly with red cells homozygous for an antigen than cells heterozygous for the antigen (EE stronger reaction vs Ee).

If anti-E is detected, the presence of anti-c should be strongly suspected (due to combined genetic inheritance). It is therefore common to select c-negative and E-negative blood for transfusion patients who have an anti-E. Anti-c is a common cause of delayed hemolytic transfusion reactions (Mais, 2009).

#### **1.2.2.4 Rh nomenclature**

The Rh blood group system has two sets of nomenclatures one developed by Ronald Fisher and R.R. Race, the other by Wiener. Both systems reflected alternative theories of inheritance. The Fisher-Race system, which is more commonly in use today, uses the CDE nomenclature. This system was based on the theory that a separate gene controls the product of each corresponding antigen (e.g., a "D gene" produces D antigen.). However, the d gene was hypothetical, not actual.

The Wiener system used the Rh–Hr nomenclature. This system was based on the theory that there was one gene at a single locus on each chromosome, each contributing to production of multiple antigens. In this theory, a gene R1 is supposed to give rise to the “blood factors” Rh<sub>0</sub>, rh', and rh'' (corresponding to modern nomenclature of the D, C and E antigens) and the gene r to produce hr' and hr'' (corresponding to modern nomenclature of the c and e antigens).

Notations of the two theories are used interchangeably in blood banking (e.g., Rho(D) meaning RhD positive). Wiener's notation is more complex and cumbersome for routine use. Because it is simpler to explain, the Fisher-Race theory has become more widely used.

DNA testing has shown that both are partially correct. There are in fact two linked genes, the RHD gene which produces a single immune specificity (anti-D) and the RHCE gene with multiple specificities (anti-C, anti-c, anti-E, anti-e). Thus, Wiener's postulate that a gene could have multiple specificities (something many did not give credence to originally) has been proven correct. On the other hand, Wiener's theory that there is only one gene has proven incorrect, as has the Fischer-Race theory that there are three genes, rather than the 2. The CDE notation used in the Fisher-Race nomenclature is sometimes rearranged to DCE to more accurately represent the co-location of the C and E encoding on the RhCE gene, and to make interpretation easier (Weiner *et al.*, 1949).

### **1.2.2.5 Rh phenotypes**

The completeness with which the Rh phenotype can be determined depends on the anti sera available; if anti- c is available but not anti- C, samples can be classified as c positive (i.e. cc or Cc) and c negative (i.e. CC). If anti- C is also available, Cc can be distinguished from cc. If a sample is tested with anti- D, anti- C, anti-c and anti- E and gives positive reactions with all for anti sera: the phenotype is written DCcE. Red cells that fail to react with a anti-D are described as dd. Mountran'notation is occasionally misleading for example, although a negative reaction with anti-E usually implies that the cells are ee (Mollison, 1997).

### **1.2.2.6 Clinical significance of Rh system**

On the basis of structural homology it has been proposed that the product of RHD gene, the RhD protein, is a membrane transport protein of uncertain specificity (CO<sub>2</sub> NH<sub>3</sub>) and unknown physiological role. The three-dimensional structure of the related RHCG protein and biochemical analysis of the RhD protein complex indicates that the RhD protein is one of three subunits of an ammonia transporter. Three studies have reported a protective effect of the RhD-positive phenotype,

especially RhD heterozygosity, against the negative effect of latent toxoplasmosis on psychomotor performance in infected subjects. RhD-negative compared to RhD-positive subjects without anamnestic titres of anti-Toxoplasma antibodies have shorter reaction times in tests of simple reaction times. And conversely, RhD-negative subjects with anamnestic titres (i.e. with latent toxoplasmosis) exhibited much longer reaction times than their RhD-positive counterparts. The published data suggested that only the protection of RhD-positive heterozygotes was long term in nature; the protection of RhD-positive homozygotes decreased with duration of the infection while the performance of RhD-negative homozygotes decreased immediately after the infection (Biver *et al.*, 2006).

### **1.2.3.1 Cardiovascular disease**

Cardiovascular diseases are the leading cause of death globally. This is true in all areas of the world except Africa (Shanthi *et al.*, 2011). Together they resulted in 17.3 million deaths (31.5%) in 2013 up from 12.3 million (25.8%) in 1990. Deaths at a given age from CVD are more common and have been increasing in much of the developing world, while rates have declined in most of the developed world since the 1970s (Fuster *et al.*, 2010). Coronary artery disease and stroke account for 80% of CVD deaths in males and 75% of CVD deaths in females. (Shanthi *et al.*, 2011) Most cardiovascular disease affects older adults. In the United States 11% of people between 20 and 40 have CVD, while 37% between 40 and 60, 71% of people between 60 and 80, and 85% of people over 80 have CVD (Mozaffarian *et al.*, 2013). The average age of death from coronary artery disease in the developed world is around 80 while it is around 68 in the developing world. Disease onset is typically seven to ten years earlier in men as compared to women (Shanthi *et al.*, 2011).

### **1.2.3.2 Types of cardiovascular disease**

There are many cardiovascular diseases involving the blood vessels. They are known as vascular disease, coronary artery disease and ischemic heart disease, Peripheral arterial disease, Renal artery stenosis, Aortic aneurysm, There are also many cardiovascular diseases that involve the heart, Cardiomyopathy – diseases of cardiac muscle, Hypertensive heart disease (Bridget,2010).

### **1.2.3.3 Artificial heart valve**

An artificial heart valve is a device implanted in the heart of a patient with valvular heart disease (Bertazzo *et al.*,2013). When one of the four heart valves malfunctions, the medical choice may be to replace the natural valve with an artificial valve. This requires open-heart surgery.

Valves are integral to the normal physiological functioning of the human heart. Natural heart valves are evolved to forms that perform the functional requirement of inducing unidirectional blood flow through the valve structure from one chamber of the heart to another. Natural heart valves become dysfunctional for a variety of pathological causes. Some pathologies may require complete surgical replacement of the natural heart valve with a heart valve prosthesis (Miller and Jordan, 2013).

### **1.2.3.4 Heart failure**

Heart failure is a condition in which the heart can't pump enough blood to meet the body's needs. In some cases, the heart can't fill with enough blood. In other cases, the heart can't pump blood to the rest of the body with enough force. Some people have both problems.

**The leading causes of heart failure are diseases that damage the heart. Examples include coronary heart disease (CHD), high blood pressure, and diabetes (Lung, 2014).**



### **1.2.3.5 Myocardial infarction**

Myocardial infarction (MI) (*i.e.*, heart attack) is the irreversible necrosis of heart muscle secondary to prolonged ischemia. Acute myocardial infarction, reperfusion type. In this case, the infarct is diffusely hemorrhagic. There is a rupture track through the center of this posterior left ventricular trans mural infarct. The mechanism of death was hemopericardium ( AMaziar *et al.*,2015).

### **1.2.3.6 Congenital heart defect**

Congenital heart defect (CHD), is a problem in the structure of the heart that is present at birth signs and symptoms depend on the specific type of problem symptoms can vary from none to life-threatening. When present they may include rapid breathing, bluish skin, poor weight gain, and feeling tired it does not cause chest pain. Most congenital heart problems do not occur with other diseases. Complications that can result from heart defects include heart failure (brideget *et al.*,2010) .

## **1.3 Previous studies**

Many reports have appeared in recent years showing an association between blood groups and cardiovascular diseases.

Sheikh *et al.*,(2009), Found association between blood group B and myocardial infraction in Banglandesh. Biswas *et al.*,(2008), are showed the prevalence of coronary Artery Disease higher in blood group O than other blood groups, Hafeezullai, *et al.*, (2005) found the incidences of CHD in pakistani population the blood group A are associated with higher risk of CHD compared to group O, Dharmendra *et al*, (2103) found in Gujarati population there higher risk of CAD in group B.

## **1.4 Rationale**

Various studies have been done to determine the association of ABO blood groups with disease like (Diabetes mellitus, gastric cancer, carcinoma of stomach). But few studies in Sudan were conducted to associate ABO and cardiovascular disease.

The result of this study may add new results for early prevention of cardiovascular disease according to ABO and Rh blood group systems.

## **1.5 Objectives**

### **1.5.1 General Objective**

To Determine the frequency of ABO blood groups and Rhesus factor in Sudanese patient with cardiovascular disease.

### **1.5.2 Specific objective**

1. To determine frequency of ABO and Rh blood groups of patients with cardiovascular diseases.
2. To determine of ABO and Rh blood groups according to types of cardiovascular diseases.
3. To determine frequency of ABO and Rh blood groups according to gender.

# **Chapter Two**

## **Materials and Methods**

## **Chapter Two**

### **2. Materials and Methods**

#### **2.1 Study design**

This is analytical case control study conducted in Khartoum State during the period of February to August 2016 in Sudanese patients with cardiovascular diseases to determine frequency of ABO and Rh blood groups in Sudanese patients and to associate between ABO and Rh blood groups with cardiovascular diseases .

#### **2.2 Study population**

Two hundred patients with cardiovascular diseases and one hundred control both males and females were included .

#### **2.3 Inclusion criteria**

Patients who were diagnosed with cardio vascular diseases attended Sudan Heart Center in the present study both gender were included .

#### **2.4 Exclusion criteria**

Healthy individuals were excluded from this study .

#### **2.5 Data collection**

Data were collected using questionnaires. The questionnaires were specifically designed to collect demographic data information about sex, type of cardiovascular disease .

#### **2.6 Ethical consideration**

Participant was informed about the research and its benefits, sample collection, and the approval consent was taken after their agreement

## **2.7 Materials**

General equipment and reagents :

- Syringe
- Cotton and gloves
- 70 % alcohol
- EDT A containers
- Slides
- Antibody A
- Antibody B
- Antibody AB
- Antibody D
- Applicator sticks
- Pipettes

## **2.8 Methods**

### **2.8.1 Blood sample collection**

Two and half ml venous blood was drawn after make sterilization by 70% alcohol used 20 or 21 G needle with limited occlusion of the arm by the tourniquet. The blood was collected in K2 EDTA (anti coagulant) and mixed gently.

## **2.8.2 ABO slide agglutination test**

### **2.8.2.1 Principle**

When red cells were mixed with various reagents of antisera (soluble antibody), agglutination occurred on the slides containing cells positive (possessing the antigen) for the corresponding antigen. No agglutination occurred in the red cells did not contain the corresponding antigen (Walker *et al.*, 1999) .

### **2.8.2.2 Procedure**

The a slide divided to two section:

In section one of slide labeled anti- A one drop of antibody A was placed and section two of slide labeled anti- B one drop of antibody B was placed and One drop of cells was placed in each antibody containing circle, Mentioned solution was mixed carefully with a separate applicator stick, The slide was slowly tilted for one minute, then agglutination was observed, Results were recorded .

### **2.8.2.3 Interpretation of result**

Agglutination (clumping) of the red blood cells is positive. No agglutination is negative .

### **2.8.2.4 Quality controls**

Known positive (+ve) and negative (-ve) (RBCs for A, B antigen) were included in accordance with the relevant guide lines of quality assurance .

## **2.8.3 Rh (D) red blood cell typing**

### **2.8.3.1 Principle**

Rh (D) typing is based on the principle of agglutination. Normal human red blood cells processing antigen will clump in the presence of antibody directed toward the antigens .

Agglutination of patient or control red blood cells with anti- D serum and no agglutination with the control reagent is a positive test result, which indicates the presence of the D antigen on the red blood cells. Absence of agglutination is a negative test result, which indicates the D antigen is not demonstrable .

If Rh typing is negative, Du typing is automatically performed .

## **2.8.4 Du Method (The indirect anti globulin)**

### **2.8.4.1 Principle**

The indirect antiglobulin test is used for the detection of antibodies that may cause red cell sensitization in vitro. If both IgG antibodies and the corresponding antigens are present in serum, red cell mixture incubation will cause the antibody to attach antigenic receptor on red cell .

### **2.8.4.2 The technique of Du method**

1-Two drop of mixture (IgG and IgM) anti- D was placed in 10x75mm test tube .

2- One drop of washed 5% suspension of the test cell was added .

3- Mixed well, and the tube was incubated at 37c for 15 minutes in LISS.

4- After incubation, the mixture was centrifuged and then he result was Read and recorded .

5- The mixture was washed 3-4 times in large volume of saline, and then each wash was decanted completely .

6- Two drops of anti globulin reagent was added, mixed well and incubated for 4-5 minutes at room temperature .

7- The mixture was centrifuged at 3400rpm for 15 seconds .

8- The final results were read and recorded.



### **2.8.4.3 Requirements**

- Test tubes
- Water bath at 37c
- Anti- D sera
- Coomb's sera
- Pasteur pipette
- Microscope
- Bench centrifuge

### **2.8.4.4 Interpretation**

Agglutination in test sample mean a positive test and the sample are labeled Rh (d) positive .

# **Chapter Three**

## **Results**

## Chapter Three

### 3. Results

This is case control study aimed to determine frequency of ABO and Rh blood groups in Sudanese patients with cardiovascular disease, this study done on two hundred patient's 61% males, 39% females and one hundred as control 60% males and 40% females .

Most common type of cardiovascular was vascular disease 24%, valvular disease 24% and myocardial fraction 17% as shown in table (3-1).

According to table (3-2) the most frequent blood group in Case group was O 49%, followed by A 31%, B 33% and least frequent was AB 8%, comparing to control most common blood group was O 57%, A 21%.

The majority Rh in the study group were Rh positive (91.5%). as shown in table (3-3).

The results of this study shown that cardiovascular diseases have been found in males more than females. and control group have same result male more than female table (3-4).

Most common type of disease frequent in female was valvular 12%, vascular 11% and myocardial fraction 6% in male most common valvular disease 12% vascular disease 13% and myocardial fraction 11% as shown in table (3-5).

**Table (3.1) Frequency of different types of cardiovascular disease**

Types of cardiovascular Diseases	Frequency	Percent %
Coronary arterial disease	29	14.5
Valvular disease	48	24
Vascular disease	48	24
Congenital heart failure	22	11
Pulmonary artery disease	15	7.5
Aortic disease	1	.5
Myocardial infraction	34	17
Venous thrombosis	3	1.5
Total	200	100

**Table (3.2) Distribution of blood group among study group**

Blood groups	Case		control		P. value
	No	%	No	%	
A	61	30.5	21	21	0.545
B	33	16.5	11	11	
O	98	49	57	57	
AB	8	4	11	11	
Total	200	100	100	100	

**Table (3.3) Frequency of Rh in study group**

Rh	case		control		P. value
	No	%	No	%	
Positive	171	91.5	88	88	0.514
Negative	29	8.5	12	12	
Total	200	100	100	100	

**Table (3.4) Distribution of patients according to gender**

Blood groups	Case		control	
	No	%	No	%
Male	122	61	60	60
Female	78	39	40	40
Total	200	100	100	100

**Table (3.5) Distribution of different types of cardiovascular disease according to gender**

Types of cardiovascular disease		Gender	
		Male	Female
Coronary arterial disease	No	24	5
	%	12	2.5
Valvular disease	No	24	24
	%	12	12
Vascular disease	No	26	22
	%	13	11
Congenital heart failure	No	16	6
	%	8	3
Pulmonary arterial disease	No	9	6
	%	4.5	3
Aortic disease	No	1	0
	%	.5	0
Myocardial infraction	No	22	12
	%	11	6
Venous thrombosis	No	0	3
	%	0	1.5
Total	Total	122	78
	%	61	39

# **Chapter Four**

**Discussion, Conclusion, and Recommendation**



## Chapter Four

### 4. Discussion, conclusion, and recommendation

#### 4.1 Discussion

Blood group system has been involving in many disease as pathogenesis such as (Diabetes mellitus, gastric cancer, carcinoma of stomach).

In the present study we want to relate it to cardiovascular disease Also the study aimed to determine most frequent type of cardiovascular disease, gender distribution of patients.

commonest blood group in patients was O followed by A, B and least frequent was AB, in the control group the same result.

majority of Rh blood group in the present study were the positive .

A similar result has been done by (Reela, 2014) obtained that the prevalence of Ischemic heart disease ( IHD) in blood group O is higher than all other ABO blood groups .

Dharmendra *et al*, 2013 found in Gujarati population there higher risk of CAD in group B and also this study disagree with result of the present study.

(Biswas, *et al* 2008) found the incidences of CHD in pakistani population the higher risk in blood group A compared to group O. This finding disagree with my result.

The majority of patients with cardiovascular diseases have been found in males more than females.

This is difference could be due to life style and number of samples or social habits of population.

Jeanine, 2002 found no association between cardiovascular diseases gender, this study disagree with result of the present study.

In the present study most common cardiovascular diseases among patient was vascular and valvular .

## 4.2 Conclusion

- There is no association between cardiovascular diseases and ABO and Rh blood group.
- Vascular disease were the major cardiovascular disease.
- Most common cardiovascular disease in both was vascular followed by valvular disease .

## **5. Recommendations**

- 1- Further study is required to give baseline data regarding distribution of ABO of patients with cardiovascular diseases.
- 2 -further investigation with much larger population must done.

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بسم الله الرحمن الرحيم  
جامعة السودان للعلوم والتكنولوجيا  
كلية الدراسات العليا - برنامج الماجستير - في علوم المختبرات الطبية  
تخصص علم الدم والمناعة الدموية

إقرار موافقة

الاسم: .....

سوف يتم أخذ عينة دم من الوريد بواسطة حقنة طعن وذلك بعد مسح مكان أخذ العينة بواسطة المطهر، كل الأدوات المستخدمة لأخذ العينة معقمة ومتبع فيها وسائل السلامة المعملية.

وأنا أقر بأن هذه العينات سوف يتم تحليلها فقط لغرض البحث في تحديد الزمر الوظيفية للفصائل الدموية والعامل الريصي لدي السودانيين المصابين بأمراض القلب والاعوية الدموية.

أوافق أنا المذكور أعلاه بأخذ عينة لإجراء الدراسة.

الاسم: .....

الإمضاء: .....

**Sudan University of Science and Technology**

**College of Graduate Studies**

**Hematology and Immunohematology**

**Questionnaire about ABO Blood Groups and Rhesus among  
Patients with Cardiovascular Diseases**

Sample No: .....

Gender:.....

Type of cardiovascular diseases:.....

**Laboratory investigation:**

Blood group:.....

Rh factor:.....

Date: / /

Signature: .....



