بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Association between ABO Blood Groups and Rhesus Factor with Cardiovascular Diseases in Sudanese Patients in Khartoum state

علاقة فصائل الدم والعامل الريصي بأمراض القلب و الأوعية الدموية لدى المرضى السودانيين في ولاية الخرطوم

A dissertation submitted in partial fulfillment of requirement of M.Sc degree in Medical Laboratory Science

(Hematology and Immunohematology)

By:

Hiba Abdalla Osman Mohamed

(B.Sc Medical Laboratory Science, UMST, 2001)

Supervisor:

Dr: Khalda Meirghani Hamza

2017

الآية

بسم الله الرحمن الرحيم

قال تعالى :

الرَّحْمَنُ (1) عَلَّمَ الْقُرْآنَ (2) خَلَقَ الْإِنْسَانَ (3) عَلَّمَهُ الْبَيَانَ(4)

سورة الرحمن الآية(4-1)

Dedication

To my lovely beautiful mother for her patience and support.

To my husband for his understanding and encouragement.

To my wonderful supervisor Dr.Khalda Meirghani, for her help and wishing happiness for her.

To Dr. Kawthar Abdelgaleil for her great help and support.

To everyone who help me to finish this work. Special thank to my sister Huda.

Hiba

ACKNOWLEGMENTS

First and foremost I'd like to thank all the members of the collage of graduated studies and doctors who have instilled in me the knowledge and confidence to properly write this dissertation.

Special thanks to Dr. Khalda Meirghani for her help throughout the period during which I wrote this dissertation. Thanks to my colleagues who helped me.

Thanks to all peoples who's approved to participate in this work.

Abstract

Many studies and researches was done to find the associations between ABO blood groups and Rhesus factors with various diseases like diabetes , renal problems and heart diseases.

This study was a prospective study aimed to associate between of ABO blood groups and Rhesus factors with different types of heart problems in Sudanese patients. The study involved 200 patients attended the Sudan Heart Centre. Samples collected from February to May 2016, after an informed consent obtained from every volunteers. Then, ABO and Rhesus factor slide agglutination test done. Du technique was done to Rhesus negative results. Data analyzed using SPSS computer program ,chi-square test and *P-Value* was determined.

The study evolved both sexes with 110/200 (55 %) males while 90/200 (45%) was females. O group was the common blood group type with 103/200(51.5 %). The majority of patients was Rhesus positive (+ve) with 181/200 (90.5 %).

The common cardiovascular disease type was valvular diseases with 66/200 (33%) followed by vascular heart disease with 62/200 (31%) and the least frequent was congenital heart diseases with 22/200 (11 %).

Finally, these results was compared with apparently healthy volunteers and showed no association between ABO blood groups and Rhesus factors with cardiovascular diseases.

المستخلص

أجريت عدة دراسات و أبحاث لإيجاد علاقة بين فصائل الدم والعديد من الأمراض مثل مرض السكر, مشاكل الكلى و أمراض القلب.

الدراسة تعتبر دراسة متقدمة باستخدام مائة عينة ضابطة لتحديد تكرار زمر الدم و العامل الريصي لدى المرضى السودانيين المصابين بأنواع مختلفة من أمراض القلب والأوعية الدموية. . شملت هذه الدراسة مائتان شخص حضر لمركز السودان للقلب . جمعت العينات من شهر فبراير إلى شهر مايو من عام 2016 م بعد موافقة المتطوعين.أجريت اختبارات زمر الدم والعامل الريصي باستخدام طريقة الشرائح و استخدام محاليل تحتوي على أمصال مضادة. وتم أجراء فحص تأكيدي لكل نتيجة فحص كان العامل الريصي فيها سالب. ثم حللت هذه البيانات في الحاسب الآلي باستخدام نظام الحزم الإحصائية للمجتمع.

شملت الدراسة الجنسين وكان معظمهم من الذكور بنسبة 200/110 (55%) بينما النساء بنسبة 200/90 (45%). وجد ان O أكثر زمر الدم انتشارا بنسبة200/103(51.5%).اغلب المرضى كانوا حاملين للعامل الريصي الموجب بنسبة بلغت 200/181(90.5%).

وجد أن أكثر أمراض القلب و الأوعية الدموية شيوعا هو مرض القلب الصمامي بنسبة بلغت 200/66(33%) و تلاه أمراض القلب الوعائية بنسبة 200/62 (31%) واقل نسبة كانت مرض نشوة الخلقي منذ الولادة للقلب بنسبة 200/22 (11%) .

أخيرا , هذه النتائج تمت مقارنتها مع نتائج المتطوعين الأصحاء و وجد انه لا يوجد علاقة بين فصائل الدم مع أمراض القلب و الأوعية الدموية.

List of Contents

List of Tables

List of Figures

List of Abbreviations

CAD	Coronary Artery Disease
CHD	Congestive Heart Disease
CVD	Cardiovascular Disease
HF	Heart Failure
GAL	D-galactose
GAL NAC	N-acetyle-galactosemine
GNAc	N-acetyle-glucoseamine
IgG	Immunoglobulin G
IgM	Immunoglobulin M
GWASs	Genome-wide association studies
K2EDTA	Potassium Ethylene Diamine Tetra Acetic Acid
LISS	Low Ionic Strength Saline
MI	Myocardial Infarction
Rh	Rhesus
VHD	Valvular Heart Disease
vWF	von Willebrand Factor
SPSS	Statistical Package of Social Science

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction:

Over time, the development of sciences introduced a relationship between blood grouping system and cardiovascular diseases. Many studies in recent years found some types of ABO blood group as a risk factor to varieties of diseases like diabetes ,renal failure and cardiovascular diseases. Pakistan, found low percentage of blood groups A and B while blood group AB had higher percentage in diabetic patients and positive association between Rh-ve blood group and diabetes. (Waseen *et al*., 2012)

There is a normal variation in distribution of ABO blood group types throughout the world.

Discovery of the ABO blood group, over 100 years ago, caused great excitement. Until then, all blood had been assumed to be the same, and the often tragic consequences of blood transfusions were not understood. As our understanding of ABO group grew, not only did the world of blood transfusion become a great deal safer, but scientists could now stay one of the first human characteristics proven to be inherited. A person's ABO blood type was used by lawyers in paternity suits, by police in forensic science, and by anthropologist in the study of different populations. 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 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). Numerous association have been made between particular ABO phenotypes 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

type O tend to have lower levels of von Willebrand Factor (vWF) , which is protein involved in blood clotting.(Dean, 2005)

1.2 Literature Review:

1.2.1.1 Composition of blood :

Blood is a complex fluid tissue responsible for supply of O_2 and food to other tissues of the body and for removal of CO_2 and waste products from them. Blood is made up of 3 elements, the cells floating (suspended) in protein and salts solution known as plasma. The cellular elements include erythrocytes (Red cells), leukocytes(white cells), and thrombocytes (platelets).Red cells (erythrocytes) in human are small, non-nucleated (possessing no nuclease), biconcave disc that contain Hb (an O_2-carrying pigments responsible for red color of fresh blood). The Red cell membrane (or outer cover) is composed 3 bimolecular leaflet or phospholipids (hydrophobic) cover internally by layers of protein (hydrophilic). The surface carry a negative charge and thus repels other red cells. An important component of red cell membrane is blood group antigens, which situated largely on external surface. (Bryant, 1994)

1.2.1.2 Importance of Blood Typing:

The importance of knowing blood is to prevent the risk of receiving an incompatible blood type at time of need, such as blood transfusion or during surgery, if two different blood types are mixed, it can lead to a clumping of blood cells that can be potentially fetal. Blood type is inherited from parents and determined by two factors, ABO grouping system and Rh factor. Starting with ABO system, there are 4 blood groups A, B, AB and O. Type of blood is based on the presence or absence of antigens and antibodies in blood. Antigens are proteins that stick to the surface of red cells, while antibodies are produced in the plasma or liquid portion of the blood. The blood type depend on type of antigens on red cell surface. If blood enters the system with antigens that's not found, the body create antibodies against it. However, some people can still safety receive blood that is not the same type of blood group, as long as the blood doesn't have any antigens that mark it as foreign, their antibodies won't attack it. (Krans, 2015)

1.2.1.3 Chemical Characteristics of Blood Group Antigens:

The precursor's substance is composed of 4 sugar molecules. Two are known as D-galactose (GAL), one is N-acetyl-glactosmine (GAL NAC) and the last is N-acetyl-glucoseamine (GNAc). Two types of precursors substance have been identified, known as type 1 and 2 chains. These chains differ in the linkage of the terminal galactose molecules to sub terminal N-acetylegalactosamine molecules. In type 1 chain the linkage is beta (1- 3), where's in type 2 chain the linkage is beta (1 – 4). (Bryant, 1994)

1.2.1.4 ABO Blood Group System:

The ABO blood groups are defined by the presence of two alternative antigens on red blood cells, determined by three alterative alleles at single locus. Two basic rules governing this system are as follows:

1-The blood type is defined by the presence of two red blood cells antigens,(A) and (B). RBCs of type A have the antigen A on their surface, those of type B have antigen B, type AB red cells bear both antigens, while type O cells bear neither antigen.

2- Natural antibodies called isoagglutinins exist in an individual's serum, directed against whichever of the A and B antigens is not presented on that person's red cells. (Gutman, 2011)

Table (1-1):Type of Antigens and Antibodies (Gutman, 2011)

BLOOD TYPE	RBC ANTIGENS	SERUM ANTIBODIES	FREQUENCY
A	A	anti-B	40%
B	B	anti-A	10%
AB	A *and* B	none	5%
O	none	anti-A *and* anti-B	45%

The success of blood transfusions depends on ensuring the compatibility of the blood types between donor and recipient. If the recipient has antibodies to the infused red cells, these red cells will be rapidly destroyed, resulting in a potentially lethal transfusion reaction. Type A blood given to type B recipient, for instance, can result in such a reaction, since the recipient's serum contain anti- A antibodies. (Gutman, 2011)

1.2.1.5 History of blood group discovery:

Karl Landsteiner at the University of Vienna, in year 1900, discovered why some blood transfusions were successful while others could be deadly. Landsteiner discovered the ABO blood group system by mixing the red cells and serum of each of his staff. He demonstrated that the serum of some agglutinated the red cells of other. From these early experiments, he identified three types, called A, B and C (C was later to be re-named O for the German "Ohne", meaning "without", or "Zero", "null" in English). The fourth less frequent blood group AB was discovered a year later. Gene that determined human ABO blood type is located on chromosome 9 (9q34.1) and is called ABO glycosyltransferase. The ABO locus has three main allelic forms: A, B and O, each of them is responsible for the production of it glycoprotein. It is therefore the combination of alleles that are inherited from parents that determines

which glycoprotein's (antigens) are found on person's blood cells and there by their ABO blood type. (Farhud and Yeganeh, 2013)

1.2.1.6 ABO blood system antibodies:

As Landsteiner recognized in early experiments, individuals possess the ABO antibodies in their serum directed against the ABO antigens absent from their red cells. Landsteiner's rule remains an important consideration in the selection of blood products given that ABO antibodies exist in healthy individuals. These ABO antibodies present in individuals with no known exposure to blood or blood products, were originally thought to be "naturally". (Blaney and Howard. 2013)

Antibodies of ABO system are mainly IgM class. Antibodies are named according to the antigens with which they react. An antibodies that react with A antigens (A red blood cells) is called Anti-A. An antibody that react with B antigens (B red blood cells) is called Anti-B. O cells are named because they have no A or B antigens, therefore is no anti O antibodies . ABO System occur naturally in serum, if an antigen is missing from an individual's cells, the antibody specific for the missing cell antigen will be present.(Estridge *et al.*, 2000)

1.2.1.7 Secretor and Non-Secretor:

ABH allo antigens are present on erythrocytes and also on other tissue cells such as the kidneys, liver and even sperm. About 80% of human population produce blood group alloantigen in a soluble form and these are found in saliva, sweat, and gastric juices, peoples belong to this majority called " Secretors ". The remaining 20% do not produce soluble blood group antigen and constitute the "Non secretor". Alleles of a single gene (Se/se) control this trait. It is thought that the presence of these secreted blood group mucins may influence the type of bacteria that take up residence in gut, some gut bacteria produce enzymes that allows them to degrade the terminal sugar of ABH blood type antigens and use it as a source of energy. (Pathak and Palan, 2005)

1.2.1.8 ABO Sub Groups:

Red blood cells for some A or B individuals that react moderately, weakly or not at all with standard anti-A or anti-B sera are termed subgroups, B subgroups are rare and less frequent than A subgroups. Mutations in the transferase genes that cause reduced enzyme efficiency result in a reduced numbers of antigens and altered branching structure responsible for subgroup phenotypes. Approximately 80% of group A individuals are A_1, while A_2 were 20% . Which is the primary A subgroups, while subgroups A_3, A_{gel} and A_x are much less frequent encountered. The difference between A_1 and A_2 is both quantitative (fewer A antigens on A_2 than A_1) and qualitative (structure differences), because of structural differences, A_2 individuals can form anti-A_1 (1 - 8% of A_2 individuals and 30% of A_2B individuals have anti-A_1). (Shaz *et al*., 2013)

1.2.1.9 The Rhesus-Hr Blood Group System:

Rh-Hr blood group system is probably the most complex of all red cell blood group system. Levine and Stetson (1939) reported that an antibody in the mother of still born fetus who suffered a hemolytic reaction to the transfusion of her husband's blood. In 1940, Landsteiner and Wienner injecting blood from the monkey Maccacus rhesus into rabbits and guinea pigs. Discovered that the resulting antibodies agglutinated both the monkey's red cells and 85% of human donors, those donors whose red cells were agglutinated by antibodies (AB) called Rh+ve, and the remaining 15% were called Rh-ve. (Bryant,1994)

1.2.1.10 Rhesus Antibodies:

Human RBCs are classified as Rh positive (+ve), if Rh antigen is on their membrane or Rh negative (-ve), if the Rh antigen is absent. The presence or absence of Rh

antigen (also called D antigen) is determined by testing the RBCs with Anti-Rh0 (also called anti- D). (Elegent, 2009)

The majority of Rh antibodies are IgG and usually IgG_1, IgG_3 or combination of these subclasses. IgM antibodies may be found simultaneously with IgG Rh antibodies and may reflect a rather broad immune response on the part of antibody marker, where's these IgM antibodies are most often detectable in a saline test serum, IgG Rh antibodies are most often detectable at antiglobulin phase of testing. Detection of IgG Rh antibodies is often enhanced by enzyme test method. It is interesting to note that although the majority of Rh antibodies are IgG_1 and IgG_3 (activate complement), Rh antibodies rarely activate complement. Exceptional examples of Rh antibodies (anti-D and anti c).(Rudmann, 2005)

1.2.1.11 Rhesus Nomenclatures:

Three different systems of nomenclatures have been developed to describe the genes and antigens of Rhesus blood group system antigens. The Wienner system, the Fisher – Race system and the Rosenfield numeric system. Wienner proposed that the Rh antigens were products of a single gene. The Fisher-Race nomenclature was based on the theory that reactions observed with various Rh anti sera could be explained by three pairs of allelic genes Cc, Dd, Ee. Genetic analysis doesn't support either of these models. However, both the Weinner notation and the Fisher-Race nomenclature remain widely used today because of familiarity. In 1962, Rosenfield *et al.* proposed a system of nomenclature that was based on serologic finding. The symbols were used to convey pheno typing information rather than genetic information. In this system, the antigens are numerically named in order of their discovery or assignment to the Rh blood group system. (Greer *et al.*, 2009)

1.2.1.12 The Du Phenotype:

In 1946, Stratton described a phenotype, which he called Du. D antigen present only *in vitro* tests more sensitive than those in routine used at that time were used. Over the years, through no fault of original investigator, this phenotype has caused great confusion for both practicing physicians and blood bankers. There are a number of causes for this confusion. First, although RBCs of the Du phenotype are D+, a minority of them come from individuals whose RBCs lack certain D epitope, which means that such persons can produce anti-D (to epitopes that their RBCs lack), if exposed to normal D+ red cells (i.e., all epitopes of D present). Second, because red cells of Du phenotype are D+, they can stimulate the production of anti-D if transfused into Rh (D-ve) persons. These finding led to the introduction of double standard by which potential transfusion recipients and pregnant women with Du phenotype were regarded as Rh –ve, where donors of that phenotype were called Rh +ve. Third, and adding considerably to the confusion outlined, definition of Du phenotype is very much technique dependant. *Invitro* determination of Du phenotype combined by realization that only a minority of Du phenotype can form anti-D and most Du donors whose RBCs are immunogenic in D- recipient are now typed as D+. In currently designation a weak D+ patient RBCs do not react in direct typing test. (Garratty, 1994)

1.2.2.1 Heart:

The heart pump oxygenated blood to the body and deoxygenated blood to the lungs. In the human heart there is one atrium and one ventricle for each circulation, and with both a systemic and a pulmonary circulation, there are four chambers in total: left atrium, left ventricle, right atrium and right ventricle. The right atrium is the upper chamber of the right side of the heart. The blood that is returned to the right atrium is deoxygenated (poor in oxygen) and passed into the right ventricle to be pumped through the pulmonary artery to the lungs for re-oxygenation and removal of carbon dioxide. The left atrium receives newly oxygenated blood from the lungs as well as the

pulmonary vein which is passed into the strong left ventricle to be pumped through the aorta to the different organs of the body. The coronary circulation system provides a blood supply to the heart muscle itself. The coronary circulation begins near the origin of the aorta by two arteries: the right coronary artery and left coronary artery. After nourishing the heart muscle, blood returns through the coronary veins into the coronary sinus and from this one into the right atrium. Back flow of blood through its opening during atrial systole is prevented by the Thebesian valve. (Guyton and Hall, 2000)

1.2.2.2 Functions of Cardiovascular System

Cardiovascular system has three major functions: transportation of materials, protection from pathogens and regulation of body's homeostasis.

-Transportation: The cardiovascular transport blood to almost all of the body's tissues. The blood deliver essential nutrients and oxygen and removes wastes and carbon dioxide to be processed or removed from the body. Hormones are transported throughout the body via the blood liquid plasma.(Taylor, 2012)

-Protection: The cardiovascular system protect the body through its white blood cells. White blood cells clean up cellular debris and fight pathogens that have entered the body. Platelets and red blood cells form scabs to heal wounds and prevent pathogens from entering the body and liquids from leaking out. Blood also carries antibodies that provide specific immunity to pathogens that body has previously been exposed to or has been vaccinated against.(Smith and Fernhall, 2011)

-Regulation: The cardiovascular system is instrumental in the body ability to maintain homeostasis control of several internal conditions. Blood vessels help maintain a stable body temperature by controlling the blood flow to the surface of the skin. Blood vessels near the skin's surface open during times of overheating to allow hot blood to dump its heat into the body's surroundings. In case of hypothermia, these blood vessels constrict to keep blood flowing only to vital organs in the body's core. Blood

also helps balance the body's pH due to presence of bicarbonate ions, which act as buffer solution. Finally, the albumins in blood plasma help to balance the osmotic concentration of the body's cells by maintain an isotonic environment. (Taylor, 2012)

1.2.2.3 Types of Cardiovascular Diseases:

Area class of diseases that involve heart or blood vessels. Cardiovascular diseases includes coronary artery diseases (CAD) such as angina and myocardial infarction (commonly known as heart attack). Other cardiovascular diseases are stroke, hypertensive heart disease, rheumatic heart disease, cardiomyopathy, heart arrhythmia, congenital heart disease, valvular heart disease, carditis, aortic aneurysms, peripheral artery disease and venous thrombosis.(Mendis *et al*., 2011 ; G.B.D., 2013)

Many cardiovascular diseases involving the blood vessels. They are known as vascular diseases.

-Coronary artery disease (known as coronary heart disease and ischemic heart disease).

-Peripheral arterial disease (disease of blood vessels that supply blood to the arms and legs).

-Cerebrovascular disease (disease of blood vessels that supply blood to the brain-includes stroke).

-Renal artery stenosis.

-Aortic aneurysm.

There are also many cardiovascular diseases that involve the heart.

-Cardiomyopathy (disease of cardiac muscle).

-Hypertensive heart disease (secondary to high blood pressure or hypertension).

-Heart failure.

-Pulmonary heart disease (a failure at the right side of the heart with respiratory system involvement).

-Cardiac dysrhymias (abnormalities of heart rhythm).

Inflammatory heart disease

-Endocarditis (inflammation of inner layer of the heart, the endocardium).

-Inflammatory cardiomegaly.

-Myocarditis (inflammation of myocardium muscle of the heart).

-Valvular heart disease.

-Congenital heart disease.

-Rheumatic heart disease (heart muscles and valves damage due to rheumatic fever caused by Streptococcus pyogenes, a group A streptococcal infection). (Mendis *et al*., 2011 ; G.B.D., 2013)

1.2.2.4 Risk Factors :

There are several risk factors of heart diseases: age, gender, tobacco use, physical inactivity, excessive alcohol consumption, unhealthy diet, obesity, family history of cardiovascular disease, raised blood pressure (hypertension), raised blood sugar (diabetes mellitus), raised blood cholesterol (hyperlipidemia), psychosocial factors, poverty and low educational status as well as air pollution.(Kelly and Fuster, 2010; Finks *et al.*, 2012)

Each risk factor varies between different communities or ethnic groups, the overall contribution of these risk factors is very consistent. Some of these risk factors, such as age, gender or family history, are immutable. However, many important cardiovascular risk factors are modifiable by lifestyle change, social change, drug

treatment and prevention of hypertension, hyperlipidemia and diabetes.(Howard and Wylie-Rosett, 2002)

1.2.2.5 Coronary Artery Disease:

Coronary artery disease is caused by atherosclerosis of coronary artery that leads to a restriction of blood flow to the heart. Atherosclerosis is a process that develops slowly over time. Typically, atherosclerosis begins in person's teenage year or earlier, and the disease worsens quietly for decades. As people age, their atherosclerosis becomes more likely to involve the arteries of the heart and become coronary artery disease. Atherosclerosis is a chronic condition that narrow the arteries by building fat filled bulges in the artery wall. These bulges are called atherosclerotic plaques, or simply plaques. In some people, the plaques eventually break open and the content cause blood clots. If these clots swept into the blood stream, they can lodge in the smaller arteries and completely block blood flow beyond that point. The heart muscle is constantly active, and it requires a continuous blood supply, when a heart artery is blocked suddenly, the heart muscle it supplies stops working within few minute. If blood supply remain blocked for half hour or more, heart muscle cells will begin to die. (Katz and Ness, 2015)

1.2.2.6 Artificial Heart Valve:

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

Valves are integral to the normal physiological functioning of human heart. Natural heart valves are evolved to form 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 variety of pathological causes.

Some pathologies may require complete surgical replacement of the natural heart valve with heart valve prosthesis. The introduction of valve replacement surgery in the early 1960's has dramatically improved the outcome of patients with valvular heart disease. Approximately 90 000 valve substitutes are now implanted in the United States and 280 000 worldwide each year. (Pibarot and Dumensil, 2009)

1.2.2.7 Heart Failure:

Heart failure means that the heart isn't pumping as well as it should be. Body depends on the heart pumping action to deliver oxygen and nutrient-rich blood to the body's cells. When the cells are nourished properly, the body can function normally. With heart failure, the weakened heart can't supply the cells with enough blood. This results in fatigue and shortness of breath and some people have coughing. Everyday activities such as walking, climbing stairs or carrying groceries can become very difficult. It is a serious condition and usually there's no cure. But many people with heart failure lead a full, enjoyable life when the condition is managed with heart failure medications and healthy lifestyle changes. (American Heart Association, 2015)

1.2.2.8 Congenital Heart Defect:

Congenital heart defect (CHD)**,** also known as congenital heart anomaly or congenital heart disease, 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. (Mendis *et al.*, 2011)

The causes of congenital heart defect is often unknown. Certain cases may be due to infection during pregnancy such as, rubella, use of certain medications or drugs such as alcohol or tobacco, parents being closely related, or poor nutritional status or

obesity in the mother. Having a parent with congenital heart defect is also a risk factor. A number of genetic conditions are associated with heart defect including Dawn Syndrome, Turner Syndrome and Marfan Syndrome. (Dean *et al.*, 2014)

1.2.2.9 Myocardial Infarction:

Myocardial infarction (MI) or acute myocardial infarction (AMI), commonly known as heart attack, occurs when blood flow stops to a part of the heart, causing damage to the heart muscle. The most common symptoms is chest pain or discomfort which may travel into the shoulder, arm, back, neck, or jaw. Often it is in center or left side of the chest and lasts for more than a few minutes. The discomfort may occasionally feel like heartburn. Other symptoms may include shortness of breath, nausea, feeling faint, a cold sweat, or feeling tired. About 30% of people have atypical symptoms, with women more likely than men to present atypically. Among those over 75 years old, about 5% have had an MI with little or no history of symptoms. (Steg *et al*.,2011; Coventry *et al*.,2011;Valensi *et al.*, 2011)

1.3 Previous Studies:

In Iran, (Geggel, 2015), found people whose blood type is A, B or AB have an increased risk of heart disease and shorter life spans than people who have type O blood group, the researchers follows about 50,000 middle age and elderly people in northeastern Iran for an average of seven years, found that people with non-O blood types were 9 percent more likely to die during the study for any health-related reason, and 15 percent more likely die for cardiovascular disease compared with O blood type and found people with non-O blood types had a 55 percent increased risk of gastric cancer compared with O blood types. In USA, (Zhang *et al.,* 2012), found there is substantial historical association between non-O blood group status and an increase in some cardiovascular disorders, the widespread use of genome-wide association studies (GWASs) over last 5 years has spurred an enormous acceleration in discoveries across the entire spectrum of cardiovascular disease, recent GWASs have confirmed ABO as a locus for venous thrmboembolism (VTE), myocardial infarction (MI) and multiple cardiovascular disease. In Italy, (Carpeggiani *et al.,* 2010), found in the study of ABO blood group alleles:A blood group is a risk factor for coronary artery disease, An angiographic study. A significant association was found non-O blood group and ischemic heart disease and presence of coronary atherosclerosis. Higher prevalence of A and B alleles in patients with myocardial infarction. In Pakistan, (Queshi , 2003), a descriptive cross-sectional study of the frequency of ABO blood groups among the diabetes mellitus type 2 patients, found group O significantly lower in diabetes mellitus type 2 as compared to general population.

1.4 Rational:

ABO blood group and Rh factor systems is now used for many clinical purposes. Many studies associate ABO blood group and Rh factor and some diseases such as diabetes, renal failure and cardiovascular diseases. Theories suggest that there was possible association of ABO blood group and Rh factor systems with different types of cardiovascular disease. In Sudan , there were no base line data , so results of this research could be used as data base for further future studies.

1.5 Objectives:

1.5.1 General Objectives:

To associate between ABO blood group and Rh factor with cardiovascular diseases in Sudanese patients.

1.5.2 Specific Objectives:

- To identify ABO blood group and Rh factor in patients with cardiovascular diseases and apparently healthy individuals..

- To determine the frequency of distribution of ABO blood group and Rh factor in patient with cardiovascular diseases according to type of disease phenotype.

- To detect possible association between ABO blood group and Rh factor in the study groups.

CHAPTER TWO

MATERIALS AND METHODS

Chapter Two

Materials and Methods

2.1 Study design:

This was prospective study conducted in Khartoum State during the period of February to May 2016 in Sudanese patients with cardiovascular diseases .

2.2 Study population:

Two hundred patients with cardiovascular diseases and one hundred healthy control both sexes were included .

2.3 Inclusion criteria:

Patients who were diagnosed with cardiovascular diseases attended to Sudan Heart Center in Khartoum state were included in this study. Control samples from healthy individuals free from any cardiac problems.

2.4 Exclusion criteria:

Healthy Individuals or Patients didn't have cardiovascular disease were excluded from case study. Cardiovascular patients were excluded from control sample.

2.5 Data collection:

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

2.6 Methods:

2.6.1 Blood collection:

Two and half m1 of venous blood was drawn after make sterilization by 70% alcohol use 20 or 21 G needle with limited occlusion of the arm by the tourniquet. The blood was collected in K2 EDTA (Potassium Ethylene Di amine Tetra Acetic) and mixed gently. (Kathen *et al*., 1998).

2.6.2 ABO slide agglutination test

2.6.2.1 Principle:

When red cells were mixed with various reagents of anti sera (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) .

2.6.2.2 Procedure:

1.On the section of slide labeled anti- A one drop of antibody A was placed .

2.On the section of slide labeled anti- B one drop of antibody B was placed .

3. One drop of cells was placed in each antibody containing circle .

4. Mentioned solution was mixed carefully with a separate applicator stick .

5. The slide slowly was tilted for one minute, then agglutination was observed .

6.Result was read and recorded .

2.6.2.3 Interpretation:

Agglutination (clumping) of the red blood cells is positive, while no agglutination is negative. It's critical to read the results immediately as false positive can occur when the mixture begins to dry on the slide .

2.6.2.4 Controls:

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

2.6.3 Rh (D) red blood cell typing:

2.6.3.1. Principle

The red cells are mixed with anti-D reagent. The test is used to measure visual agglutination or lack of agglutination . Agglutination is refers to the clumping of cells in the presence of antibody .(Westhoff, 2008). If test of Rh typing is negative, Du typing is should be performed .

2.6.4 DU Method (The indirect anti globulin):

2.6.4.1. Principle

The indirect anti globulin 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 .

.2.6.41The technique of D" method:

1-Two drop of mixture (IgG and IgM) anti- D was placed in l0x75mm test tube .

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

3-Mix 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 3400 rpm for 15 seconds .

8-The final results were read and recorded (Walker *et al*., 1999) .

2.6.4.2Interpretation:

If the agglutination is present in the test tube, the result is reported as Du positive (+ve). If agglutination is NOT present in test tube, the result is reported as Du negative (-ve).

2.7 Ethical consideration:

Study was approved by Ethical and Scientific Committee, Medical Laboratory Science, Sudan University of Science and Technology.

Participant were verbally informed in their simple language about the research and its benefits method of sample collection, after their approval.

2.8 Statistical Analysis:

Data was statistically checked and analyzed using Statistical Package of Social Science (SPSS) program version 20, chi- square test was done and *P-Value* was determined.

CHAPTER THREE

RESULTS

RESULTS

3.1 RESULTS:

Two hundred volunteers with cardiac problems and one hundred healthy individuals free from any cardiac problems as control were included in this study.

In the case group males were represented about 110/200 (55%) compared with females 90/200 (45%) ,while in control group males were represented about 57/100 (57%) compared with females 43/100 (43%).

Regard to ABO blood types in case group, the most frequent ABO blood type was O 103/200 (51.5%) followed by A 66/200 (33%), B 26/200 (13%) and AB 5/200 (2.5%) respectively as well as in control group which showed that O 46/100 (46%), A 39/100 (39%), B 9/100 (9%) and AB 6/100(6%).

The Rhesus factor showed in case group represented that Rh positive (+ve) was common 181/200 (90.5%), while the rest was 19/200 (9.5%) Rh negative(-ve) which is similar to control group Rh positive(+ve) was 90/100 (90%) and Rh negative (-ve) was 10/100 (10%).Table(3-1)

Table (3-1) Distribution of gender, ABO groups and Rh factors in the study groups:

variable		case	control
Gender	Male	110/200, 55%	57/100, 57%
	Female	90/200, 45%	43/100, 43%
ABO groups	O	51.5%	46%
	A	33%	39%
	B	13%	9%
	AB	2.5%	6%
Rh factors	Positive (+ve)	90.50%	90%
	Negative (-ve)	9.5%	10%

In cardiovascular patients males represented 110/200 (55%) while females represented about 90/200 (45%). Table (3-2)

Table (3-2) Distribution of gender in cardiovascular patients:

Sex	Frequency	Percent%
male	110	55.0
female	90	45.0
Total	200	100.0

The most common heart disease was valvular heart disease (VHD) 66/200 (33%) followed by vascular heart disease 62/200 (31%) , then myocardial infarction (MI) 50/200 (25%) and least frequent congenital heart disease (CHD) 22/200 (11%). Table (3-3)

Table (3-3) Frequency of different types of cardiovascular diseases in the study group:

Cardiac diseases	Frequency	Percent%
valvular disease	66	33.0
vascular disease	62	31.0
myocardial infarction	50	25.0
congenital heart disease	22	11.0
Total	200	100.0

Regard to ABO blood group in cardiovascular patients O showed high frequent compared with other ABO groups. Table (3-4)

Our results showed that Rhesus positive was common with181/200 (90.5%) while Rhesus negative was19/200 (9.5%). Table (3-5)

Table (3-4) Distribution of ABO blood group in the cardiovascular patients:

Blood group	Frequency	Percent%
O	103	51.5
A	6	33.0
B	26	13.0
AB	5	2.5
Total	200	100.0

Table (3-5) Distribution of Rhesus factors in the cardiovascular patients:

Rhesus group	Frequency	Percent%
positive	181	90.5
negative	19	9.5
Total	200	100.0

Table (3-6) Distribution of ABO blood group in different types of cardiovascular diseases:

Disease phenotype	Blood group				Total
	A	B	AB	O	
Valvular heart disease	18 9%	6 3%	0 0%	42 21%	66 33%
Vascular disease	23 11.5%	9 4.5%	2 1%	28 14%	62 31%
Myocardial infarction	16 8%	5 2.5 %	3 1.5%	26 13%	50 25%
Congenital heart disease	9 4.5%	6 3%	0 0 %	7 3.5%	22 11%
Total	66 33 %	26 13%	5 2.5%	103 51.5%	200 100%

According to distribution of ABO blood group in different disease phenotypes , there was no association between ABO and cardiovascular diseases, the *P-Value* **0.094**

Table (3-7) Distribution of Rhesus factor in different types of cardiovascular diseases:

Cardiac disease	Rhesus group		Total
	Positive	Negative	
Valvular heart disease	61 30.5%	5 2.5%	66 33%
Vascular heart disease	55 27.5%	7 3.50%	62 31%
Myocardial infarction	44 22%	6 3%	50 25%
Congenital heart disease	21 10.5%	1 0.5%	22 11%
Total	181 90.5%	19 9.5%	200 100%

According to distribution of Rhesus factor in different disease phenotypes , there was no association between Rhesus factor and cardiovascular diseases, the *P-Value* **0.681**

Table (3-8) Distribution of different types of cardiovascular diseases according to gender:

Disease Phenotype	sex male	sex female	Total
Valvular disease	29 14.5 %	37 18.5%	66 33%
Vascular disease	40 20%	22 11%	62 31%
Myocardial infarction	29 14.5%	21 10.5%	50 25%
Congenital heart disease	12 8 %	10 7%	22 11%
Total	110 55%	90 45%	200 %100

1

According to distribution of gender in the cardiovascular diseases , there was no association between gender and cardiovascular diseases , the *P-Value* **0.126**

Table (3-9) Distribution of ABO blood group in case and control group:

Blood groups	Cardiovascular diseases(case)		Non cardiovascular diseases(control)	
	No	Percent %	No	Percent %
O	103	51.5	46	46
A	66	33	39	39
B	26	13	9	9
AB	5	2.5	6	6
Total	200	100	100	100

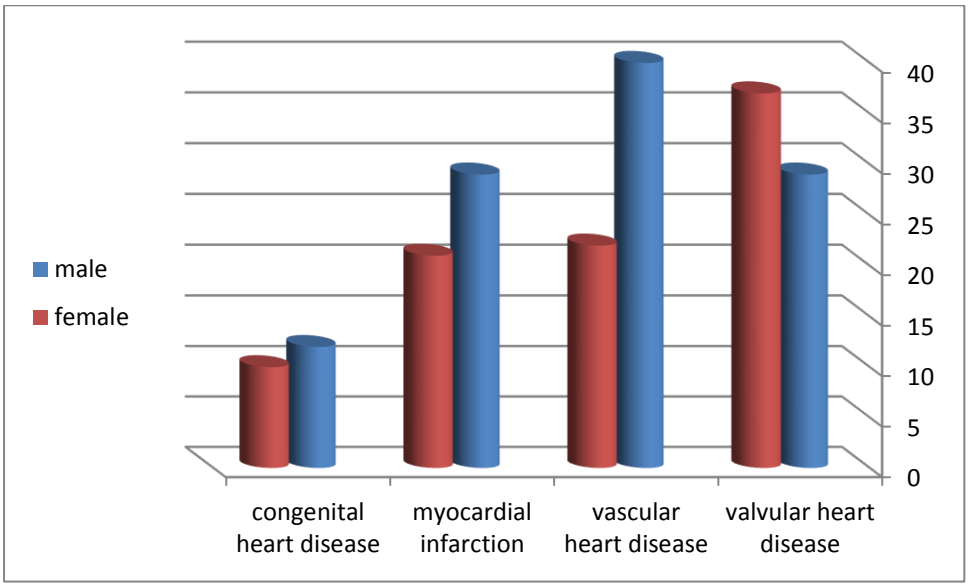

Figure (3-1): Distribution of different types of cardiac disease according to sex

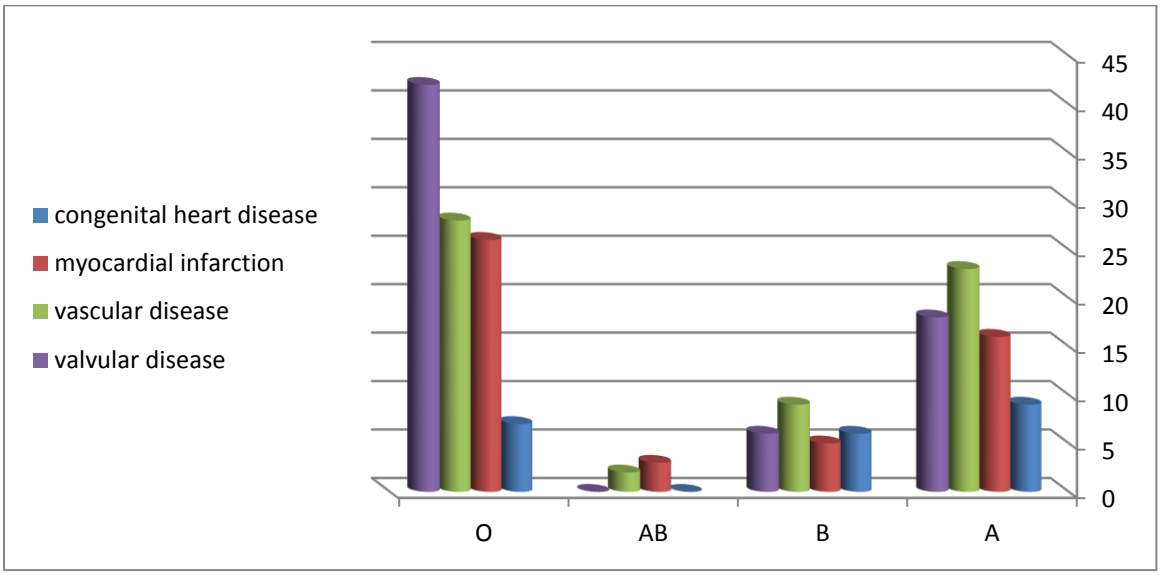

Figure (3-2): Distribution of ABO blood group in cardiac patients

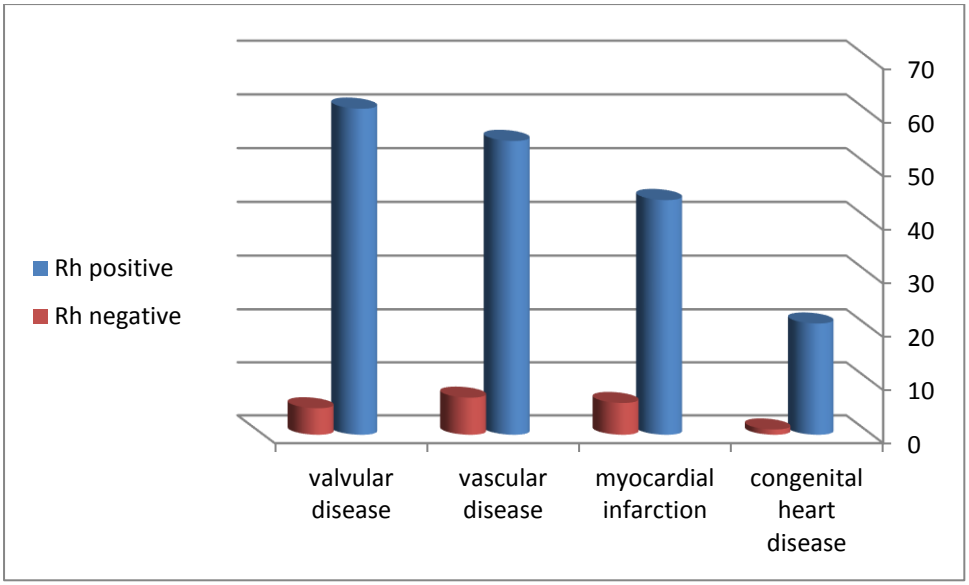

Figure (3-3): Distribution of Rhesus factor in cardiac patients

CHAPTER FOUR

DISCUSSION

4.1 Discussion :

In this prospective study, the case group was showed male: female ratio 1: 1.2 in case group and 1: 1.3 in control one, agree with (Mosca *et al*,2011), in the study of sex differences in cardiovascular diseases, they found the prevalence of coronary heart disease is higher in males within each age stratum until 75 years of age.

The frequency of ABO blood types showed : O Blood group was most common in both all study group followed by A, B and AB. This was agree with(Shahata *et al*, 2012), who study ABO blood group and Rhesus antigens among blood donors attending the Central Blood Bank, Sudan, found in the cross-sectional study the frequency percentage were O , A, B and AB.

The results of Rh factor showed that Rh positive (+v) was higher frequency compared with Rh negative (-ve) in both study volunteers, this agree with (Tesfaye *et al.*, 2015), when studied the frequency distribution of ABO and Rhesus (D) blood group alleles in Silte Zone, Ethiopia, found the O blood type is predominant and also said Rh positive was (92.06%) and Rh negative was (7.94%).

In the case study group the distribution of disease phenotype found that the most common was valvular heart disease(VHD) followed by vascular heart disease, then myocardial infarction(MI) and the least frequent was congenital heart disease (CHD). (The Heart Foundation, 2015), they concluded that coronary heart disease is the most common type of heart diseases and killing nearly 380,000 people annually. Which was disagree with this study .

Vascular heart disease was most common disease in males compared with valvular heart disease which was common in females.

The present study showed O blood type was appear as possible risk factor in the valvular heart disease.

Studies done in different countries, (Franchini and Lippi, 2015) the study of relationship between the ABO blood group , cardiovascular disease and cancer , found non-O blood group carries an approximately two-fold increased risk of venous thrombosis, which is disagree with this study.(Capuzzo *et al*., 2016) determined from cardiorisk program, that in relationship between ABO blood group and cardiovascular disease, they found non-O blood type had a significantly increased incidence of cardiovascular events compared with O blood types subjects. (Abdollahi *et al,* 2009) in the study was designed to investigate the association between ABO blood groups and cardiovascular disease, they said that the subjects with blood group A had more family of cardiovascular disease, which is disagree with finding in this study.

4.2 Conclusion:

- The common ABO blood group were O and the common Rhesus factor were Rhesus positive in study volunteers.

- High prevalence of valvular heart diseases .

- There was no association between ABO blood group and cardiovascular diseases.

4.3 Recommendations:

- Minor blood grouping tests should be done to patients with cardiovascular diseases.

- More researches using large sample size should be done to find possible association between ABO blood group and Rhesus factor with cardiovascular diseases.

References

References

Abdollahi, A.A., Qorbani, M., Salehi, A. & Mansourian, M. (2009). ABO Blood Group Distribution and Cardiovascular Major Risk Factors in Healthy Population, *Iranian Journal of Public Health,* 38**(3)**:123-126.

American Heart Association,(2015). About heart failure, what is heart failure.

Bertazzo, S., Gentlman, E., Cloyd, K.L., Chester, A.H., Yacoub, M.H. & Stevens, M.M. (2013). Nano-analytical electron microscopy reveals fundamental insight into human cardiovascular tissue calcification. *Nature Materials* 12**(6):**576-83.

Blaney, K.D. & Howard, P.R. (2013). Basic and Applied concepts of Blood Banking and Transfusion Practice, 3rd edition, ISBN: 9780323086639.

Bryant, N.J. (1994). An Introduction of Immunohematology, 3rd edition , ISBN:072163883X, 9780721638836.

Capuzzo,E., Bonfanti, C. & Franchini, M. (2016).The relationship between ABO blood group and cardiovascular disease: results from the cardiorisk program, *Ann Transl Med*,4**(10)**: 189.

Carpeggiani, C., Coceani, M., Landi, P., Michelessi, C. & Abbate, A.L. (2010).ABO blood group alleles, an angiographic study. *Atherosclerosis* 211461-466 .

Coventry, L.L., Finn, J. & Bremner, A.P. (2011).Sex differences in symptoms presentation in acute myocardial infarction, a systemic review and met-analysis "Heart and Lung". *The Journal of critical care*, 40**(6):**477.

Dean,L. (2005). Blood Groups and Red Cell Antigens, Bethesda (MD): NCBL National Centre of Biotechnology Information. NCBL Book Sheltpublish.

Dean, S.V., Lassi, Z.S., Imam, A.M. &Bhutta, Z.A. (2014). Preconception care: nutritional risks and interventions, doi:1742-4755-11-s3-s3 PMID:25415364.

Elegent, K.D. (2009). Immunology: Understanding the immune system, 2nd edition, ISBN:978-0-470-08157-0.

Estridge, B. H., Reynold, A.P. & Walters, N. J. (2000). Basic Medical Laboratory Techniques, 4th edition, ISBN:0-7668-1206-5 .

Farhud, D. D. & Yegenah, M.Z. (2013). A Brief history of human blood groups, *IranianJournalofPublicHealth* , PMCID:PMC 3595629.

Finks, S.W., Airee, A., Chow, S.L., Macauley, T.E., Moranville, M.P., Trujillo, T.C. & Rogers, K.C. (2012). Key articles of dietary intervention that influence cardiovascular mortality, 32**(4)** :e 54-87.

Franchini,M. & Lippi, G. (2015). The intriguing relationship between the ABO blood group , cardiovascular disease and cancer. *BiMedCentral Medicine BMC, 13:7* Doi:10.1186/s1296-014-0250-y.

Garratty, G. (1994). Immunology of Transfusion Medicine, ISBN:0-8247-9122-3.

Geggel, L., writer, S (2015), Your Blood Type May Put you at Risk of Heart Diseases, www.livescience.com

Global Burden of Diseases study(G.B.D.) (2013). Mortality and causes of death, collaborators(2014), "Global, Regional and National age-sex specific all causes and cause specific mortality for 240 causes of death; 1990-2013, a systemic analysis for Global Burden of Diseases study.*Lancet*,385(**9963:117-71)** PMID:25530442 , 1**(4)**:369-377.

Greer, J. P., Foester, J., Rodgers, G.M., Paraskevas, F., Glader, B., Arber, D.A., Robert , T. & Means Jr. (2009). Wintrobe's Clinical Hematology, volume one, 12th edition, ISBN-13:978-0-7817-6507-7 , ISBN-10:0-7817-6507-2.

Gutman, G.A. (2011). Immunology core notes, Medical Immunology 544, School of Medicine, University of California, IRVINE.

Guyton, A.C. & Hall, J.E. (2000). Guyton Text Book of Medical Physiology, 10th edition, ISBN-13:9780721686776.

Howard, B.V. & Wylie-Rosett, J. (2002). Surgery and Cardiovascular diseases, A statement for health care professionals from committee council on nutrition, physical activity and metabolism of the American Heart Association.

Kathen, E.B., Barbara, E.D. & Lincon, P.J. (1998). Blood group serology, 6th edition, London, UK, 3;39:88

Katz, M.J. & Ness, S.M. (2015). Coronary artery disease (CAD), WILD IRIS MEDICAL EDUCATION.INC, **wildirismedicaleducation.com**

Kelly, B.B. & Fuster, V. (2010). Promoting Cardiovascular Health in Developing World, A critical challenge to achieve global health.

Krans, B. (2015). Blood Typing, medically reviewed by Deborah Weatherspoon PhD, MSN, RN, CRNA, www.healthline.com

Mendis, S., Puska, P. & Norving, B. (2011). Global Atlas on Cardiovascular Prevention and Control , WHO in collaboration with the World Heart Federation and World Stroke Organization, ISBN:978-92-4-156437-3.

Mosca, L., Barrett-Connor, E. & Wanger, N.K. (2011). Sex/Gender differences in cardiovascular disease prevention, circulation Volume 124, Issue 19, doi: 10.101161/CIRCULATIONAHA.110.968792. sciencevolunteer.com

Pathak, S. & Palan, U. (2005). Immunology: Essential and Fundamental, 2nd edition, ISBN:1-57808-378-8.

Pibarot, P. & Dumensil, J.G. (2009). Prostatic Heart Valves: Selection of the Optimal Prosthesis and Long-Term Management, volume 119, issue7, doi:10.1161/*CIRCULATIONAHA*:108.778886 PMID:19237674.

Queshi, B. MA. (2003). Frequency of ABO blood groups among the diabetes mellitus type2 patients, descriptive cross-sectional study, PMID:12921683.

Rudmann, S.V. (2005). Textbook of Blood Banking and Transfusion Medicine, 2nd edition, ISBN:0-7216-0384-X.

Shahata,W.M., Khalil, H.B., Abass, A.E & Adam, I. (2012).Blood group and Rhesus antigens among Blood donors attending the Central Blood Bank, Sudan.*Sudan JMC* 7**(4)**:245-248.

Shaz, B.H., Hillyer, C.D., Roshal, M. & Abrams, C. (2013). Transfusion Medicine and Hemostasis: Clinical and Laboratory Aspects, 2nd edition, e BOOK ISBN:9780123977885 , Print BOOK ISBN:9780123971647.

Smith, D.L. &Fernhall, B. (2011).Advanced Cardiovascular Exercise Physiology. ISBN-10: 0-7360-7392-2 ISBN-13: 978-0-7360-7392-9.

Steg, P.G., James, S.K., Atar, D., Badano, L.P., Borger,M.A., Di Mario, C., Dickstin, K., Ducrocq, G., Fernandez-Aviles, F., Gershlick, A.H., Giannuzzi, P., Halvorsem, S., Huber, K., Juni, P., Kastrati, A., Knuuti, J., Lenzen, M.J., Mahaffey, K.W.,

Valgimili, M., Widimsky, P. &Zahger, D. (2012). ESC Guidelines for the management of acute Myocardial Infarction in patients presenting with ST-Segment elevation .*EuropeanHeartJournal*, 33**(20):**2569-619 PMID:22922416

Taylor, T. (2012). Anatomy and Physiology of Cardiac System ,www.InnerBody.com

Tesfaye, K., Petros, Y. & Andagie, M. (2015). Frequency distribution of ABO and Rhesus (D) blood group alleles in Silte Zone, Ethiopia, *Egyptian Journal of Medical Human Genetics*, vol 16**(1),** doi:10.1016/j.ejmhg. ScienceDirect.com

The Heart Foundation,(2015). Heart disease facts, Heart Disease; Scope and Impact.

Valensi, P., Lorgis, L. & Cottin, Y. (2011).Prevalence , incidence, predictive factors and progresses of silent myocardial infarction, doi:10.1016/j.acvd.2010.11.013 PMID:21497307.

Walker, R.H., Hoppe, P.A. & Judd, W.J. (1999). Technical Manual, 3rd edition, American Association of Blood Banks(**AABB**).*Arlington*, 197;223**.**

Wassen, A.G., Iqbal, M., Awab Khan, O. & Tahir, M. (2012). Association of Diabetes Mellitus with ABO and Rh blood groups, *Ann,Pak,Inst,Med,Sci*, 8**(2)**:134-136 .

Westhoff, C. (2008).ABO, H, and Lewis blood groups and structurally related antigens and methods. Roback J., Coombs MR., Grossman B., Hillyer C., eds. Technical Manual , 16th edition, Bethesda, Md: *American Association of Blood Banks* (**AABB**); 2008.361-97.

Zhang, H., Mooney, C.J. & Reilly, M.P. (2012). ABO blood groups and Cardiovascular disease, Article ID 641917, *International Journal of Vascular Medicine*, doi:10.1155/2012/641017 PMID:23133757.

APPENDIXES

Appendix 1

General equipments and reagents:

- Syringes
- Cotton and gloves
- 70% alcohol
- EDTA containers
- Slides
- Antibody A
- Antibody B
- Applicator sticks
- Pipettes

Du technique:

Requirements:

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

Appendix 2

Sudan University of Science and Technology

Collage of Graduate Studies

Medical Laboratory Science

Hematology Department

Questionnaire about ABO blood group and Rhesus factor in Sudanese patients with cardiovascular diseases

Sample No. :……..

Pt. Name:……

Gender:……

Type of cardiovascular disease:…….

Other diseases: (if found)……...

Laboratory Results:

ABO group:…….

Rhesus factor:……..

Date: / /

Appendix 3

بسم الله الرحمن الرحيم

جامعة السودان للعلوم والتكنولوجيا

كلية الدراسات العليا-برنامج الماجستير-مختبرات طبية

تخصص علم الدم ومبحث المناعة

<u>إقرار موافقة</u>

الاسم:.....

سوف يتم اخذ عينة دم من الوريد بواسطة حقنة طعن طبية معقمه وذلك بعد مسح مكان اخذ العينة بمسحه طبية مطهره. كل الأدوات المستخدمة معقمة ومتبع فيها جميع وسائل السلامة المعملية المتبعة.

وأنا اقر بان هذه العينات سوف يتم تحليلها فقط لأغراض البحث.

أوافق أنا المذكور أعلاه بأخذ العينة لإجراء الدراسة.

الاسم:.....

التوقيع:.....